SPRINGER
REFERENCE

Laura Nuño de la Rosa
Gerd B. Müller
Editors

Evolutionary Developmental Biology

A Reference Guide

Springer

Evolutionary Developmental Biology

Laura Nuño de la Rosa • Gerd B. Müller
Editors

Evolutionary Developmental Biology

A Reference Guide

With 172 Figures and 7 Tables

Springer

Editors
Laura Nuño de la Rosa
Department of Logic and Theoretical Philosophy
Complutense University of Madrid
Madrid, Spain

Gerd B. Müller
Department of Theoretical Biology
University of Vienna
Vienna, Austria

Konrad Lorenz Institute for Evolution and Cognition Research
KLI Institute
Klosterneuburg, Austria

ISBN 978-3-319-32977-2 ISBN 978-3-319-32979-6 (eBook)
ISBN 978-3-319-32978-9 (print and electronic bundle)
https://doi.org/10.1007/978-3-319-32979-6

This Springer imprint is published by the registered company Springer Nature Switzerland AG.
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Evolutionary developmental biology is one of the most fascinating fields in the biosciences. Its goal is to provide a comprehensive understanding of the principles underlying the origination of all life forms on Earth. This is to be achieved through the study of the evolution of the processes that create organismal structures and at the same time impinge on the processes that govern change in populations. Adequate methodologies for the comparative and experimental study of development in evolutionary contexts have only become available during the last quarter of the twentieth century. But whereas the field of evo-devo, as it has come to be known, has rapidly expanded and diversified, including advanced molecular, cell biological, and experimental techniques, its distinct conceptual and methodological foundations and its significant theoretical achievements are not always fully perceived. For this reason, the present Reference Guide provides an accessible synopsis of the field's basic themes through a highly interconnected collection of chapters that offer a comprehensive introduction to the universe of evo-devo.

The establishment of this Reference Guide was very much a collective enterprise based on the input of many colleagues and institutions. We would like to thank the section editors Ehab Abouheif, Sergio Balari, Shigeru Kuratani, Alan Love, Philipp Mitteröcker, Dan Nicholson, Mihaela Pavličev, and Charles Scutt for their extensive efforts and dedication to curating the 84 chapters of this collection. We also thank each of the 127 contributors, all renowned experts in their respective fields, for their willingness to participate in this vast endeavor and for the great care they took in composing their chapters. We are equally grateful to Doug Erwin and Manfred Laubichler who accompanied earlier phases of the project.

This undertaking would not have happened without the encouragement from the experienced members of Springer's editorial staff, Daniela Graf, Melanie Thanner, Veronika Mang, and Andrew Spencer, as well as the continued assistance provided by Springer throughout the development and evolution of this venture. We are very grateful for all their input. Finally, we wish to acknowledge the KLI Institute fellowship to Laura Nuño de la Rosa that helped to ignite this work, as well as

two fellowships by the Spanish Ministry of Economy and Competitiveness (FJCI-2014-22685 and IJCI-2017-34092) that allowed her to continue the project over the last 4 years. We hope that our compendium will provide a useful starting point for further inquiries into the captivating science of evo-devo.

February 2021

Laura Nuño de la Rosa
Gerd B. Müller

Contents

Volume 1

About the Editors

Laura Nuño de la Rosa, Ph.D., is a philosopher of science working on the history and philosophy of evolutionary biology, with a special emphasis on evo-devo. Graduated in Humanities, in 2010 she completed a master's degree in biophysics from the Autonomous University of Madrid. In 2012, she obtained a Ph.D. in philosophy, with a thesis on the problem of organismal form, jointly from Complutense University of Madrid and Pantheon-Sorbonne University. After enjoying postdoc positions at the KLI Institute and the University of the Basque Country, Dr. Nuño de la Rosa joined the Department of Logic and Theoretical Philosophy at the Complutense University in 2015.

Gerd B. Müller, M.D., Ph.D., is professor emeritus at the University of Vienna, Austria, where he headed the Department of Theoretical Biology. He is president of the Konrad Lorenz Institute for Evolution and Cognition Research in Klosterneuburg, Austria, and president of the European Society for Evolutionary Developmental Biology. His research interests include developmental patterning, evolutionary innovation, evo-devo, and evolutionary theory.

About the Section Editors

Part II: Key Concepts in Evo-Devo
Gerd B. Müller Department of Theoretical Biology, University of Vienna, Vienna, Austria

Konrad Lorenz Institute for Evolution and Cognition Research, KLI Institute, Klosterneuburg, Austria

Part III: History of Evo-Devo
Daniel Nicholson Department of Philosophy, George Mason University, Fairfax, Virginia, USA

Part IV: Philosophy of Evo-Devo
Alan C. Love Department of Philosophy, Minnesota Center for Philosophy of Science, University of Minnesota, Minneapolis, MN, USA

Part V: Evo-Devo of Basic Mechanisms
Laura Nuño de la Rosa Department of Logic and Theoretical Philosophy, Complutense University of Madrid, Madrid, Spain

Gerd B. Müller Department of Theoretical Biology, University of Vienna, Vienna, Austria

Konrad Lorenz Institute for Evolution and Cognition Research, KLI Institute, Klosterneuburg, Austria

Part VI: Plant Evo-Devo
Charles P. Scutt Laboratoire Reproduction et Développement des Plantes, Univ Lyon, ENS de Lyon, UCB Lyon 1, CNRS, INRA, Lyon, France

Part VII: Invertebrate Evo-Devo
Ehab Abouheif Department of Biology, McGill University, MontrealQC, Canada

Part VIII: Vertebrate Evo-Devo

Shigeru Kuratani Laboratory for Evolutionary Morphology, RIKEN Center for Biosystems Dynamics Research (BDR), Kobe, Japan

Evolutionary Morphology Laboratory, RIKEN Cluster for Pioneering Research (CPR), Kobe, Japan

Part IX: Modeling Approaches to Evo-Devo

Philipp Mitteröcker Department of Evolutionary Biology, University of Vienna, Vienna, Austria

Part X: Evo-Devo and Population Genetics

Mihaela Pavličev Department of Evolutionary Biology, Unit for Theoretical Biology, University of Vienna, Vienna, Austria

Part XI: Extensions of Evo-Devo

Sergio Balari Universitat Autònoma de Barcelona and Centre de Linguística Teòrica, Barcelona, Spain

Contributors

Gembu Abe Laboratory of Organ Morphogenesis, Department of Ecological Developmental Adaptability Life Sciences, Graduate School of Life Sciences, Tohoku University, Sendai, Japan

Ehab Abouheif Department of Biology, McGill University, Montreal, QC, Canada

Ana M. R. Almeida Department of Biology, California State University East Bay, Hayward, CA, USA

David M. Althoff Department of Biology, Syracuse University, Syracuse, NY, USA

Archbold Biological Station, Venus, FL, USA

Gro V. Amdam School of Life Sciences, Arizona State University, Tempe, AZ, USA

Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, Aas, Norway

Ron Amundson Philosophy Department, University of Hawaii at Hilo, Hilo, HI, USA

Christopher J. Austin Faculty of Philosophy, Radcliffe Humanities, University of Oxford, Oxford, UK

Jan Baedke Department of Philosophy I, Ruhr University Bochum, Bochum, Germany

Sergio Balari Universitat Autònoma de Barcelona and Centre de Linguística Teòrica, Barcelona, Spain

Jimena Barbeito-Andrés Institute for Studies in Neurosciences and Complex Systems (ENyS – Unidad Ejecutora de Estudios en Neurociencias y Sistemas Complejos) CONICET-UNAJ-HEC, Florencio Varela, Buenos Aires, Argentina

Ryan D. Bickel Department of Biology, University of Rochester, Rochester, NY, USA

Ronald M. Bonett Department of Biological Science, College of Engineering and Natural Sciences, University of Tulsa, Tulsa, OK, USA

Simon Bourdareau UMR 8227 Integrative Biology of Marine Models, Algal Genetics Group, Station Biologique de Roscoff, CS 90074, Sorbonne Universités, UPMC University Paris 06, CNRS, Roscoff, France

Ingo Brigandt Department of Philosophy, University of Alberta, Edmonton, AB, Canada

Jennifer A. Brisson Department of Biology, University of Rochester, Rochester, NY, USA

Rachael L. Brown School of Philosophy, Research School of Social Sciences, The Australian National University, Canberra, ACT, Australia

Lynette R. Brownfield Department of Biochemistry, University of Otago, Dunedin, New Zealand

Miguel Brun-Usan Genomics, Bioinformatics and Evolution, Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona, Barcelona, Spain

Ann C. Burke Biology Department, Wesleyan University, Middletown, CT, USA

Brett Calcott Department of Philosophy, University of Sydney, Sydney, NSW, Australia

Mauro Centritto Tree and Timber Institute, National Research Council (CNR – IVALSA), Florence, Italy

Wei-Ling Chang Integrative Stem Cell Center, China Medical University Hospital, China Medical University Taichung, Taichung, Taiwan

Mathieu Charbonneau Science Studies Program, Departments of Philosophy and Cognitive Science, Central European University, Budapest, Hungary

Ariel D. Chipman The Department of Ecology, Evolution and Behavior, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel

Cheng-Ming Chuong Department of Pathology, Keck School of Medicine of University of Southern California, Los Angeles, CA, USA

Hélène L. Citerne GQE – Le Moulon, INRA, Univ. Paris-Sud, CNRS, AgroParisTech, Université Paris-Saclay, Gif-sur-Yvette, France

J. Mark Cock UMR 8227 Integrative Biology of Marine Models, Algal Genetics Group, Station Biologique de Roscoff, CS 90074, Sorbonne Universités, UPMC University Paris 06, CNRS, Roscoff, France

Susana M. Coelho UMR 8227 Integrative Biology of Marine Models, Algal Genetics Group, Station Biologique de Roscoff, CS 90074, Sorbonne Universités, UPMC University Paris 06, CNRS, Roscoff, France

Yoan Coudert Laboratoire Reproduction et Développement des Plantes, Ecole Normale Supérieure de Lyon, Lyon, France

Andrew G. Cridge Laboratory for Evolution and Development, Genetics Otago and Department of Biochemistry, University of Otago, Dunedin, New Zealand

Flavio D'Abramo Max Planck Institute for the History of Science (MPIWG), Berlin, Germany

Catherine Damerval GQE – Le Moulon, INRA, Univ. Paris-Sud, CNRS, AgroParisTech, Université Paris-Saclay, Gif-sur-Yvette, France

Gregory K. Davis Department of Biology, Bryn Mawr College, Bryn Mawr, PA, USA

Peter K. Dearden Laboratory for Evolution and Development, Genetics Otago and Department of Biochemistry, University of Otago, Dunedin, New Zealand

James DiFrisco Institute of Philosophy, KU Leuven, Leuven, Belgium

Adam G. Dolezal Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA, USA

Alexander V. Ereskovsky Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), Aix Marseille University, CNRS, IRD, Avignon University, Station Marine d'Endoume, Marseille, France

Department Embryology, Faculty of Biology, Saint-Petersburg State University, Saint-Petersburg, Russia

Koltzov Institute of Developmental Biology of Russian Academy of Sciences, Moscow, Russia

Markus I. Eronen Faculty of Philosophy, University of Groningen, Groningen, The Netherlands

Borja Esteve-Altava Institut de Biologia Evolutiva (UPF-CSIC), Universitat Pompeu Fabra, Barcelona, Spain

Arantza Etxeberria IAS-Research Center for Life, Mind, and Society, Department of Logic and Philosophy of Science, University of the Basque Country UPV/EHU, Donostia-San Sebastián, Spain

Flavia Fabris Konrad Lorenz Institute for Evolution and Cognition Research, Klosterneuburg, Austria

Egenis – The Centre for the Study of Life Science, University of Exeter, Exeter, UK

Cécile Fruchard Laboratoire de Biométrie et Biologie Evolutive (UMR 5558), Université Lyon 1, CNRS, Villeurbanne, France

Frietson Galis Naturalis Biodiversity Center, Leiden, The Netherlands

Sylvain Gerber Institut de Systématique, Évolution, Biodiversité, UMR 7205 MNHN-CNRS-EPHE-UPMC-Sorbonne Universités, Muséum National d'Histoire Naturelle, Paris, France

Paula N. Gonzalez Institute for Studies in Neurosciences and Complex Systems (ENyS – Unidad Ejecutora de Estudios en Neurociencias y Sistemas Complejos) CONICET-UNAJ-HEC, Florencio Varela, Buenos Aires, Argentina

Eric S. Haag Department of Biology, University of Maryland, College Park, MD, USA

David Haig Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, USA

Thomas F. Hansen Department of Biology, CEES and EVOGENE, University of Oslo, Oslo, Norway

Carolin Haug Department Biology II, Functional Morphology Group, LMU Munich, Planegg-Martinsried, Germany

Joachim T. Haug Department Biology II, Functional Morphology Group, LMU Munich, Planegg-Martinsried, Germany

Matthew Haworth Tree and Timber Institute, National Research Council (CNR – IVALSA), Florence, Italy

Svenja Heesch UMR 8227 Integrative Biology of Marine Models, Algal Genetics Group, Station Biologique de Roscoff, CS 90074, Sorbonne Universités, UPMC University Paris 06, CNRS, Roscoff, France

Jonathan Q. Henry Department of Cell and Developmental Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Tatsuya Hirasawa Department of Earth and Planetary Science, Graduate School of Science, The University of Tokyo, Tokyo, Japan

Uwe Hoßfeld Arbeitsgruppe Biologiedidaktik, Institut für Zoologie und Evolutionsforschung, Fakultät für Biowissenschaften, Friedrich-Schiller-Universität Jena, Jena, Germany

Melanie J. Hopkins Division of Paleontology, American Museum of Natural History, New York, NY, USA

David Houle Department of Biological Science, Florida State University, Tallahassee, FL, USA

Kate E. Ihle Department of Evolutionary Biology and Environmental Science, University of Zurich, Zurich, Switzerland

Florian Jabbour Muséum national d'Histoire naturelle, Institut de Systématique, Evolution, Biodiversité, UMR 7205 ISYEB MNHN/CNRS/UPMC/EPHE, Sorbonne Universités, Paris, France

Luke T. Jones Department of Biological Science, Florida State University, Tallahassee, FL, USA

Marie I. Kaiser Department of Philosophy, Bielefeld University, Bielefeld, Germany

Hidehiro Kudoh Laboratory of Organ Morphogenesis, Department of Ecological Developmental Adaptability Life Sciences, Graduate School of Life Sciences, Tohoku University, Sendai, Japan

Shigeru Kuratani Laboratory for Evolutionary Morphology, RIKEN Center for Biosystems Dynamics Research (BDR), Kobe, Japan

Evolutionary Morphology Laboratory, RIKEN Cluster for Pioneering Research (CPR), Kobe, Japan

Rie Kusakabe Laboratory for Evolutionary Morphology, RIKEN Center for Biosystems Dynamics Research (BDR), Kobe, Japan

Paul Lasko Department of Biology, McGill University, Montreal, QC, Canada

Mingxing Lei Integrative Stem Cell Center, China Medical University Hospital, China Medical University Taichung, Taichung, Taiwan

Maryna P. Lesoway Department of Cell and Developmental Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Georgy S. Levit Institute for Biology, Kassel University, Kassel, Germany

Biology Education Research Group ("Bienenhaus"), Friedrich-Schiller University Jena, Jena, Germany

Department of Social Sciences and Humanities, ITMO National Research University, St. Petersburg, Russia

Arnon Levy The Hebrew University of Jerusalem, Jerusalem, Israel

Guillermo Lorenzo Universidad de Oviedo, Oviedo, Spain

Alan C. Love Department of Philosophy, Minnesota Center for Philosophy of Science, University of Minnesota, Minneapolis, MN, USA

Gabriel A. B. Marais Laboratoire de Biométrie et Biologie Evolutive (UMR 5558), Université Lyon 1, CNRS, Villeurbanne, France

Jeffrey M. Marcus Department of Biological Sciences, University of Manitoba, Winnipeg, MB, Canada

Giovanni Marino Tree and Timber Institute, National Research Council (CNR – IVALSA), Florence, Italy

Miquel Marin-Riera Center of Excellence in Experimental and Computational Biology, Institute of Biotechnology, University of Helsinki, Helsinki, Finland

Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Barcelona, Spain

George R. McGhee Department of Earth and Planetary Sciences, Rutgers University, New Brunswick, NJ, USA

Daniel W. McShea Biology Department, Duke University, Durham, NC, USA

Johan A. J. Metz Naturalis Biodiversity Center, Leiden, The Netherlands
International Institute for Applied Systems Analysis, Laxenburg, Austria
Mathematical Institute, University of Leiden, Leiden, The Netherlands

Laure Mignerot UMR 8227 Integrative Biology of Marine Models, Algal Genetics Group, Station Biologique de Roscoff, CS 90074, Sorbonne Universités, UPMC University Paris 06, CNRS, Roscoff, France

Alessandro Minelli University of Padova, Padova, Italy

Philipp Mitteröcker Department of Evolutionary Biology, University of Vienna, Vienna, Austria

Armin P. Moczek Department of Biology, Indiana University, Bloomington, IN, USA

Gerd B. Müller Department of Theoretical Biology, University of Vienna, Vienna, Austria
Konrad Lorenz Institute for Evolution and Cognition Research, KLI Institute, Klosterneuburg, Austria

Corinne E. Myers Department of Earth and Planetary Sciences, University of New Mexico, Albuquerque, NM, USA

Sophie Nadot Laboratoire Ecologie, Systématique et Evolution, UMR 8079 Université Paris-Sud/CNRS/AgroParisTech, Orsay, France

Stuart A. Newman New York Medical College, Valhalla, NY, USA

Laura Nuño de la Rosa Department of Logic and Theoretical Philosophy, Complutense University of Madrid, Madrid, Spain

Annalise B. Paaby School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, USA

Mihaela Pavličev Department of Evolutionary Biology, Unit for Theoretical Biology, University of Vienna, Vienna, Austria

Akira F. Peters Bezhin Rosko, Santec, France

Annemie Ploeger Department of Psychology, University of Amsterdam, Amsterdam, The Netherlands

Arthur Porto Centre for Ecological and Evolutionary Synthesis, University of Oslo, Oslo, Norway

Nico Posnien Abteilung für Entwicklungsbiologie, Johann-Friedrich-Blumenbach-Institut für Zoologie und Anthropologie, Universität Göttingen, Georg August University, Göttingen, Germany

Jani Raerinne Helsinki Collegium for Advanced Studies, University of Helsinki, Helsinki, Finland

Claire Ramsay Department of Biology, McGill University, Montreal, QC, Canada

Diego Rasskin-Gutman Theoretical Biology Research Group, Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Valencia, Spain

Robert C. Richardson Department of Philosophy, University of Cincinnati, Cincinnati, OH, USA

Giulia Rispoli Max Planck Institute for the History of Science (MPIWG), Berlin, Germany

Gar W. Rothwell Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR, USA

Department of Environmental and Plant Biology, Ohio University, Athens, OH, USA

Florian Rümpler Department of Genetics, Friedrich Schiller University Jena, Jena, Germany

Isaac Salazar-Ciudad Genomics, Bioinformatics and Evolution, Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona, Barcelona, Spain

Evo-Devo Helsinki Community, Center of Excellence in Experimental and Computational Biology, Institute of Biotechnology, University of Helsinki, Helsinki, Finland

Erin E. Saupe Department of Earth Sciences, University of Oxford, Oxford, UK

Daniel B. Schwab Department of Biology, Indiana University, Bloomington, IN, USA

Charles P. Scutt Laboratoire Reproduction et Développement des Plantes, Univ Lyon, ENS de Lyon, UCB Lyon 1, CNRS, INRA, Lyon, France

Kari A. Segraves Department of Biology, Syracuse University, Syracuse, NY, USA

Archbold Biological Station, Venus, FL, USA

Chelsea D. Specht School of Integrative Plant Sciences, Section of Plant Biology and the Bailey Hortorium, Cornell University, Ithaca, NY, USA

Sonia E. Sultan Biology Department, Wesleyan University, Middletown, CT, USA

Kuni Tagawa Marine Biological Laboratory, Graduate School of Integrated Sciences for Life, Hiroshima University, Onomichi, Hiroshima, Japan

Koji Tamura Laboratory of Organ Morphogenesis, Department of Ecological Developmental Adaptability Life Sciences, Graduate School of Life Sciences, Tohoku University, Sendai, Japan

Yoshitaka Tanaka Laboratory of Organ Morphogenesis, Department of Ecological Developmental Adaptability Life Sciences, Graduate School of Life Sciences, Tohoku University, Sendai, Japan

Kirsten H. W. J. ten Tusscher Theoretical Biology Group, Department of Biology, Utrecht University, Utrecht, The Netherlands

Nicholas D. Testa School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, USA

Günter Theißen Department of Genetics, Friedrich Schiller University Jena, Jena, Germany

John N. Thompson Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, Santa Cruz, CA, USA

Alexandru M. F. Tomescu Department of Biological Sciences, Humboldt State University, Arcata, CA, USA

John R. True Department of Ecology and Evolution, College of Arts and Sciences, Stony Brook University, Stony Brook, NY, USA

Federica Turriziani Colonna Center for Biology and Society, Arizona State University, Tempe, AZ, USA

Renske M. A. Vroomans Theoretical Biology Group, Department of Biology, Utrecht University, Utrecht, The Netherlands

Günter P. Wagner Department of Ecology and Evolutionary Biology, Systems Biology Institute, Yale University, New Haven, CT, USA

Denis M. Walsh Department of Philosophy, Institute for the History and Philosophy of Science and Technology, Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, Canada

Richard A. Watson Electronics and Computer Science, University of Southampton, Southampton, UK

Elizabeth Watts Arbeitsgruppe Biologiedidaktik, Institut für Zoologie und Evolutionsforschung, Fakultät für Biowissenschaften, Friedrich-Schiller-Universität Jena, Jena, Germany

Ping Wu Department of Pathology, Keck School of Medicine of University of Southern California, Los Angeles, CA, USA

Sayuri Yonei-Tamura Laboratory of Organ Morphogenesis, Department of Ecological Developmental Adaptability Life Sciences, Graduate School of Life Sciences, Tohoku University, Sendai, Japan

Yawen Zou Center for Biology and Society, Arizona State University, Tempe, AZ, USA

The Chinese University of Hong Kong, Shenzhen, Shenzhen/Guangdong Province, P. R. China

Part I

Introduction

A Reference Guide to Evo-Devo

Laura Nuño de la Rosa and Gerd B. Müller

Abstract

Evo-devo is a principal research area in present-day evolutionary biology. This introductory chapter characterizes the evo-devo research program, recapitulates its origins, and lays out the purpose and structure of the reference guide. Volume one is dedicated to the key concepts, the history, and the philosophy of evo-devo. Volume two concentrates on selected topics of empirical research in the evo-devo of plants, invertebrates, and vertebrates. Volume three is focused on the modelling of evo-devo, its relation to population thinking, and further extensions into adjoining disciplines. The sections are arranged in a way that reflects the progression of the field and facilitates the guide's uses for the in-depth study and teaching of evo-devo.

Keywords

Evolutionary Developmental Biology · History of Biology · Philosophy of Biology · Evolutionary Theory

L. Nuño de la Rosa (✉)
Department of Logic and Theoretical Philosophy, Complutense University of Madrid, Madrid, Spain
e-mail: lauranun@ucm.es

G. B. Müller
Department of Theoretical Biology, University of Vienna, Vienna, Austria

Konrad Lorenz Institute for Evolution and Cognition Research, KLI Institute, Klosterneuburg, Austria
e-mail: gerhard.mueller@univie.ac.at

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_194

Evolutionary developmental biology, commonly known by its shorthand "evo-devo," denotes the science that combines evolutionary with developmental research. The formation of this distinct field of study has been the result of a significant change in the research practices and theoretical conceptualization of organismal biology during the last decades. Until the late 1970s and early 1980s, evolutionary biology and developmental biology were nearly exclusively studied via independent research programs, the former focused on the transformations of species and populations over geological time and the latter on the transformations that occur during individual development. To bring both strands of investigation together was an almost heretical project, eyed suspiciously by those who feared a return of typology and recapitulationism. A major worry, for instance, was whether the "how questions" and the "why questions" that govern biological inquiry, often perceived as the study of evolution's "proximate" and "ultimate" causes, could be merged in meaningful ways. But from the mid-1980s onward, methodological as well as conceptual progress enabled the formulation of a combined approach that united a large number of subfields under a joint epistemological quest: understanding how organismal development evolves and in which ways its intrinsic potentialities guide organismal evolution. Unlike earlier phases of evolutionary biology, which were largely based on abstract correlations of population-level processes with phenotypic change, the newly emerging approach for the first time permitted the study of organismal change by experimental analyses of the generative processes that effectuate the transformation of phenotypes. This approach was soon termed *evolutionary developmental biology* or *evo-devo*. At its core stands the investigation of the dynamical interactions between genes, cells, and tissues in evolving developmental systems and their effects on the evolvability of populations.

Today, we are very accustomed to the well-established discipline of evo-devo with its own set of questions and methods, but it is generally underappreciated how much its advent represented a change in evolutionary science, prompting new questions, new research approaches, new theoretical concepts, and new predictions. The success of this integrated project of evo-devo soon generated features of institutionalization, such as dedicated research positions, professional societies and conferences, specialized journals, books, and other forms of academic dissemination. All represent characteristic attributes of a mature discipline.

Over the last decades, these developments were accompanied by a dramatic expansion of the field. Evo-devo now spans such diverse areas as molecular genetics, cell biology, biophysics, quantitative analysis, and modeling, as well as the study of ecological, behavioral, and even cultural influences on development and evolution – to name but some of the major ones. The profoundly interdisciplinary character of evo-devo overcomes traditional disciplinary boundaries, attracting attention from multiple nonbiological domains, including the history and philosophy of science. Many who are new to the field may find it difficult to grasp the underlying tenets and principal concepts, the genuine findings, and the epistemological characteristics of the evo-devo approach. For others who already work in one of the areas of evo-devo, it may be demanding to follow the rapid expansion of the entire field, such as the accumulating amount of comparative data on plant and animal development brought about by new molecular and quantitative methods. For this reason, the present collection intends to

bring together in one place an integrated set of reflections on major current issues in evo-devo as well as on promising expansions into neighboring areas.

This is not the first attempt at a comprehensive characterization of the evo-devo field. Since Gould's (1977) seminal *Ontogeny and Phylogeny*, a large number of compendia on evolutionary developmental biology have appeared. Classical volumes include *Evolution and Development*, edited by John Bonner (1982); *Embryos, Genes, and Evolution* by Raff and Kaufman (1983); *The shape of life: genes, development, and the evolution of animal shape*, by Rudolf Raff (1996); *Cells, Embryos, and Evolution* by Gerhart and Kirschner (1997); and Brian Hall's (1992) *Evolutionary Developmental Biology*, the first textbook on evo-devo. Its updated 1999 edition still constitutes a standard reference for any researcher, student, or lecturer interested in the subject. Present-century treatments of different aspects of the evo-devo enterprise are found in Wilkins (2002), Hall and Olson (2003), Minelli (2003), Müller and Newman (2003), West-Eberhard (2003), Carroll (2005), Carroll et al. (2001), and Minelli and Fusco (2008). More recent works include Streelman's (2013) compendium on advances in evo-devo, Wagner's (2014) volume on homology and innovation, and Fusco's (2019) collection of essays. Already early on in the development of the evo-devo discipline, major works devoted to the history (Amundson 2005; Laubichler and Maienschein 2007; Love 2014) and the philosophy (Samson and Brandon 2007) of evo-devo started to appear. Further volumes are devoted to the interrelations of evo-devo with the environmental and ecological sciences (Hall et al. 2003; Gilbert and Epel 2009). Since this reference guide was first conceived in 2016, the field has grown further, and treatments of the evo-devo of entire kingdoms, as in the case of plants (Minelli 2018), and of representatives of numerous animal phyla have appeared, such as the six edited volumes on the evo-devo of invertebrates (Wanninger 2015), of crustacea (Scholtz 2004), and of fishes (Johanson et al. 2018). The bulk of these works concentrate on advances in the mechanistic, comparative, and experimental aspects of the extant development of selected groups of organisms. As a complement, the present reference guide explicitly aims to unite the conceptual and the empirical strands of the evo-devo project. This makes the compilation suitable, as a whole or by use of selected sections or chapters, for undergraduate or graduate course material.

The evo-devo reference guide is targeted at a wide audience of researchers, teachers, students, and practitioners from different domains of science, such as evolutionary biology, paleontology, phylogenetics, developmental and cell biology, theoretical biology, philosophy of biology, or history of biology. With that aim in mind, the content of this reference work is presented in a way that is accessible to scholars from a diverse range of backgrounds. The level of presentation is midway between that for a beginning graduate student and that for a professional evolutionary biologist – in other words, the kind of article you'd like to find when looking up a term you haven't previously heard of. Due to the condensed format of the individual chapters, a careful weighing of conciseness against level of detail was necessary, as well as a strict limitation on the number of cited references to a selection of essential works on each topic, both from classical literature and ongoing scientific work. The electronic version of the guide can be readily updated as topics evolve and new

information becomes available. In that version, a sophisticated search capability is provided, including extensive cross-referencing throughout all entries that takes the reader directly to a chapter of interest or to other publications through hyperlinks.

The printed version of the reference guide is organized in three volumes, each composed of several sections edited by renowned specialists in each research area. Volume one is dedicated to the key concepts (edited by Gerd Müller), the history (Dan Nicholson), and the philosophy of evo-devo (Alan Love). Volume two concentrates on selected topics of empirical research in the evo-devo of plants (Charles Scutt), invertebrates (Ehab Abouheif), and vertebrates (Shigeru Kuratani). Volume three is focused on the modeling of evo-devo (Philipp Mitteroecker), its relation to population thinking (Mihaela Pavlicev), and further extensions into adjoining disciplines (Sergio Balari). Following is a more detailed overview of the chapters that compose the sections of the three volumes.

Volume one begins with a section on "Key Concepts in Evo-Devo." These include elaborated versions of classical concepts, such as evolutionary changes in developmental timing (▶ "Heterochrony"), the facilitation of evolution through the (re-)use of developmental traits (▶ "Developmental Exaptation"), as well as the evolutionary consequences of developmental interdependencies (▶ "Concept of Burden in Evo-Devo"), and the limitations development imposes on the generation of variation (▶ "A Macroevolutionary Perspective on Developmental Constraints in Animals"). Another set of chapters covers the evo-devo implications of homology (▶ "Developmental Homology"), the related problem of the origin of novel traits (▶ "Developmental Innovation and Phenotypic Novelty"), and the role of changing developmental pathways leading to the same phenotype (▶ "Developmental System Drift"). Further chapters address the principle of development's material identity (▶ "Inherency"), the phylotypic model of evolving development (▶ "The Developmental Hourglass in the Evolution of Embryogenesis"), and the capacity of developmental systems to further evolve (▶ "Evolvability"). A final group of chapters reflects on the macroevolutionary implications of evo-devo, including explanations of characteristic phenotypic patterns (▶ "Macroevolution"), the origins of organismal complexity (▶ "Evolution of Complexity"), convergent evolution (▶ "Convergence"), and coevolutionary diversification (▶ "Coevolution and Macroevolution"). Taken together, these chapters provide an introduction to the specific conceptual background of the evo-devo enterprise.

Many of these ideas have roots in pre-evo-devo thought on the intersection of development and evolution. The "History of Evo-Devo" section includes a chapter on the origins of the complex relations between theories of development, inheritance, and evolution (▶ "Theories of Inheritance: The Evolution and Development of Form") followed by a selection of chapters covering the life and work of key figures in the history of evo-devo. Although evo-devo has antecedents prior to Darwin, our selection is restricted to biologists who developed their work after the widespread acceptance of evolution that followed the publication of the *Origin*. They include little-acknowledged intellectual ancestors like Alexander Kowalevsky (▶ "Alexander Onufrievich Kowalevsky (1840–1901)"), or Valentin Haecker (▶ "Valentin Haecker (1864–1927)"), and outsiders of the Modern Synthesis, such

as Richard Goldschmidt (▶ "Richard Goldschmidt (1878–1958)"), Sergey Chetverikov (▶ "Sergey S. Chetverikov (1880–1959)"), Ivan Schmalhausen (▶ "Ivan I. Schmalhausen (1884–1963)"), Gavin de Beer (▶ "Gavin de Beer (1899–1972)"), and Conrad H. Waddington (▶ "Conrad Hal Waddington (1905–1975)"). More recent and well-acknowledged contributors, such as John Bonner (▶ "John Tyler Bonner (1920–2019)"), Stephen Jay Gould (▶ "Stephen Jay Gould (1941–2002)"), and Pere Alberch (▶ "Pere Alberch (1954–1998)"), are also covered.

The conceptual innovations provided by evo-devo have attracted increasing attention from philosophers of biology, who have moved from the classical issues prompted by the topics of the Modern Synthesis to examinations of the epistemic and metaphysical impact of new conceptual and empirical developments in evolutionary biology. The section "Philosophy of Evo-Devo" gathers a series of chapters on philosophical perspectives on evo-devo. This includes treatments of the impact of evo-devo on classical themes in philosophy of science such as explanation (▶ "Explanation in Evo-Devo"), generalization (▶ "Generalization in Evo-Devo"), the concept of mechanism (▶ "Mechanisms in Evo-Devo"), as well as the explanatory functions of models and computer simulations (▶ "Modeling and Simulation in Evo-Devo"), and interdisciplinary relations (▶ "Interdisciplinarity in Evo-Devo"). These chapters are followed by a group of entries reflecting on the consequences of evo-devo on philosophical debates about evolutionary biology, such as the classical distinction between proximate and ultimate causation (▶ "Proximate Versus Ultimate Causation and Evo-Devo"), the issues raised by accounting for the evolution of complexity (▶ "Complexity in Evo-Devo") at different levels of organization (▶ "Levels of Organization in Evo-Devo"), the relationship between form and function (▶ "Form and Function in Evo-Devo"), the nature and epistemic role of dispositional (▶ "Dispositional Properties in Evo-Devo") and typological (▶ "Typology and Natural Kinds in Evo-Devo") notions, and the status of teleological explanations (▶ "Teleology in Evo-Devo").

Volume two offers a selection of case studies in the evolution and development of plants and animals. Whereas the field of empirical investigations of development that have an implicit relation with evolution is vast, this volume concentrates on representative studies that were conducted under explicit evo-devo goals. The four sections constituting this volume show that current evo-devo has ceased to be a plea for filling a gap in evolutionary theory, but consists of empirical work on a rapidly increasing range of taxa that exceed the traditional model organisms. The section "Evo-Devo of Basic Mechanisms" includes a short collection of chapters on topics cutting across taxa, such as the evolution and development of cell types (▶ "Devo-Evo of Cell Types"), cleavage (▶ "The Evolution of Cleavage in Metazoans"), and segmentation (▶ "The Evolution and Development of Segmented Body Plans"), as well as on general mechanisms of pattern formation, morphogenesis, and evolution (▶ "Mechanisms of Pattern Formation, Morphogenesis, and Evolution"). This introductory part is followed by three specific sections devoted respectively to the evo-devo of plants, invertebrates, and vertebrates.

Plants have traditionally been underrepresented in evo-devo research, but currently they constitute one of its most rapidly expanding areas. Central themes in this domain are gathered in the section "Plant Evo-Devo." The chapters combine general reflections on conceptual and methodological approaches to plant evo-devo, from the contributions of the fossil record (▶ "Structural Fingerprints of Development at the Intersection of Evo-Devo and the Fossil Record") to the importance of undertaking a process-based approach to understanding the evolution of development (▶ "A Process-Based Approach to the Study of Flower Morphological Variation"), with state-of-the-art inquiries into key episodes in plant evolution. Major innovations in plant evo-devo include the evolution of photosynthesis (▶ "The Impact of Atmospheric Composition on the Evolutionary Development of Stomatal Control and Biochemistry of Photosynthesis over the Past 450 Ma"), the evolution of generations in life cycles (▶ "Alternation of Generations in Plants and Algae"), the establishment and diversification of branching modes (▶ "The Evolution of Branching in Land Plants: Between Conservation and Diversity"), and the origin of angiosperms (▶ "The Origin of Angiosperms"). Further core events in the history of flowering plants comprise the evolution of sex determination (▶ "The Evolution of Sex Determination in Plants"), the evolution of organ identity (▶ "Evolution of Floral Organ Identity"), morphological variation in flowers (▶ "A Process-Based Approach to the Study of Flower Morphological Variation"), and the evolution of symmetry (▶ "Evolution of Symmetry in Plants").

The subsequent section collects six lessons we can draw from "Invertebrate Evo-Devo." The topics range from well-studied trait models such as butterfly wing patterns (▶ "Evo-Devo of Butterfly Wing Patterns") and snail shells (▶ "Twisted Shells, Spiral Cells, and Asymmetries: Evo-Devo Lessons Learned from Gastropods"), to organisms considered obscure until recently, such as hemichordates (▶ "Evo-Devo Lessons Learned from Hemichordates"). This leads on to a combination of more classical studies on the evo-devo of morphological traits and new approaches to the evolution of reproductive constraints in eusocial hymenoptera (▶ "Evo-Devo Lessons from the Reproductive Division of Labor in Eusocial Hymenoptera"), environmental influences and polyphenism in honeybees (▶ "Evo-Devo Lessons Learned from Honeybees"), and bacterial endosymbiosis in aphids (▶ "Evo-Devo Lessons Learned from Aphids"). The contributions highlight the enormous potential of applying the evo-devo approach to non-model organisms and non-standard explananda, such as life cycles and reproduction.

The section on "Vertebrate Evo-Devo" joins together the evolution and development of vertebrate tissues, including the vertebrate mesoderm (▶ "Shifting the Black Box: Approaches to the Development and Evolution of the Vertebrate Mesoderm"), neck muscles (▶ "Development and Evolution of the Neck Muscles"), and skeletal tissues (▶ "Evolution of Skeletal Tissues"), with that of body parts and structures, comprising the vertebrate head (▶ "History and Current Theories of the Vertebrate Head Segmentation"), the cranium (▶ "Evolution and Development of the Vertebrate Cranium"), and major events in vertebrate evolution, such as the fin-limb transition (▶ "Evo-Devo of the Fin-to-Limb Transition") and the evolutionary origination of scales, feathers, and hairs (▶ "Evo-Devo of Scales, Feathers, and Hairs"). Whereas

many other approaches to vertebrate evo-devo do exist, the emphasis in this section is on current uses of a comparative morphological approach that combines current knowledge on molecular developmental biology with cell- and tissue-level explanations of evolutionary change.

Volume three concentrates on quantification and modeling in evo-devo as well as its ramifications into other domains of evolutionary thought. The opening section offers an overview of "Modeling Approaches to Evo-Devo." In the last decades, evo-devo has been transformed by technical innovations in the methodologies for the imaging and quantitative analysis of phenotypic patterns and the molecular tools for the study of developmental processes. This opened new possibilities for the investigation of animal development on a broad, comparative level. Modeling approaches in evo-devo include the application of qualitative and quantitative techniques such as morphometrics (▶ "Morphometrics in Evolutionary Developmental Biology"), phenotyping (▶ "Phenotyping in Evo-Devo"), measures of disparity (▶ "Morphological Disparity"), and anatomical network analysis (▶ "Anatomical Network Analysis in Evo-Devo") to the comparison of the development and evolution of extinct and extant phenotypes. They also comprise the development of explanatory models of the evolution of developmental processes at different levels of organization, from simulations of gene regulatory networks (▶ "Modeling Evolution of Developmental Gene Regulatory Networks") to computational models at the cell and tissue levels (▶ "Computational Modeling at the Cell and Tissue Level in Evo-Devo"). Apart from providing interesting information concerning the developmental influences on evolutionary processes, these kinds of new tools enable evo-devo to join up with other quantitative domains of evolutionary biology.

The remaining two sections explore the connections of evo-devo to classical approaches in evolutionary biology such as population and quantitative genetics as well as to other biological fields both inside and outside evolutionary biology, thus expanding the current disciplinary boundaries of the field. The section "Evo-Devo and Population Genetics" discusses the distinct devo-evo approach (▶ "Developmental Evolutionary Biology (Devo-Evo)") and the potentials of microevolutionary effects in evo-devo (▶ "Micro-Evo-Devo") as two different but complementary ways of connecting evo-devo with the theoretical corpus of evolutionary genetics. This is followed by updated reviews of research areas that have played a key role in building bridges between statistical and mechanistic approaches to evolution, such as population studies on the interplay between canalization (▶ "Canalization: A Central but Controversial Concept in Evo-Devo") and plasticity (▶ "Developmental Plasticity and Evolution"), the studies of pleiotropy (▶ "Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics"), epistasis (▶ "Epistasis"), and variational approaches to evolvability (▶ "Variational Approaches to Evolvability: Short- and Long-Term Perspectives").

In the final section, "Extensions of Evo-Devo," a collection of papers reflect on the place of evo-devo in the larger context of a reformed evolutionary framework (▶ "Evo-Devo's Contributions to the Extended Evolutionary Synthesis"), its expansion into other areas of evolutionary biology, including traditional historical

disciplines such as phylogenetics (▶ "Evo-Devo and Phylogenetics") and paleontology (▶ "Methods and Practices in Paleo-Evo-Devo"), and the new syntheses derived from the integration into the evo-devo framework of the role of ecological interactions: eco-evo-devo (▶ "Eco-Evo-Devo") and niche construction theory (▶ "Evo-Devo and Niche Construction"). Further ramifications extend into related disciplines such as behavioral biology (▶ "Evo-Devo of Social Behavior"), the cognitive sciences (▶ "Evo-Devo and Cognitive Science"), and language (▶ "Evo-Devo of Language and Cognition"), as well as cultural evolution (▶ "Evo-Devo and Culture"). Its growing extensions indicate that evo-devo is not only a powerful field of integration for a multitude of biological disciplines, as it has sometimes been called, but also a distinct perspective in evolutionary theory that affords new ways of looking at well-known phenomena.

We are well aware that substantially different views exist on many of the topics covered by this collection. This is partly due to an emphasis on different problem agendas by different research traditions: how development evolves versus how development structures the evolution of organismal traits, micro versus macro evolution, reliance on experimental data in development versus evolutionary theory, hierarchical perspectives emphasizing the centrality of cell and tissue levels and of generic material properties of development versus those focusing on comparative molecular and developmental genetics, internalist views versus those stressing the radical interplay between environment and development or the role of natural selection in the evolution of development. Many more examples of how different perspectives shape different research agendas could be provided. However, this heterogeneity and lack of theoretical integration does not necessarily present a problem. Rather, evo-devo as a "trading zone" of collaboration and conflict between different styles and paradigms (Winther 2013) can inspire the advancement of the field as it did in other domains of science.

Obviously, even in a comprehensive enterprise like the present one, not everything could be covered. But we hope that through the selected references and cross-references, even those approaches that could not be treated in a dedicated chapter will become accessible more easily. Some editorial happenstances have influenced the final content. For instance, we regret that in the present print version no chapters on Haeckel and Garstang are included, obvious historical influences on the foundation of the evo-devo research field. Also, evo-devo studies on modularity and integration, as well as the notion of the genotype-phenotype map ended up with no separate entry, even though many of the chapters mention these essential domains. Other topics are missing as well, but the electronic version of the reference guide is able to grow and, with time, hopefully will include even more topics.

This compendium is testimony to the huge intellectual journey that evo-devo, a field often ridiculed at its beginnings about four decades ago, has taken to become one of the dominant research programs in present-day evolutionary biology. It has not only dramatically increased our understanding of the mechanistic detail underlying organismal change, but also has substantially modified the ways in which we think about the origin and evolution of life's forms and in which we conceptualize these processes in evolutionary theory. Inevitably, the field of evo-devo will expand

and diversify further, potentially enriching evolutionary biology and related disciplines in hitherto unforeseen ways. We hope that our reference guide will contribute to the enhancement of this process.

References

Amundson R (2005) The changing role of the embryo in evolutionary thought. Cambridge University Press, Cambridge

Bonner JT (ed) (1982) Evolution and Development. Report of the Dahlem Workshop on Evolution and Development. Springer, Berlin

Carroll SB (2005) Endless forms most beautiful: the new science of evo-devo and the making of the animal kingdom. W.W. Norton & Company, London

Carroll SB, Grenier JK, Weatherbee SD (2001, revised and expanded in 2012) From DNA to diversity: molecular genetics and the evolution of animal design. Blackwell Science Ltd, Oxford

Fusco G (ed) (2019) Perspectives on evolutionary and developmental biology. Essays for Alessandro Minelli. Padova University Press, Padova

Gerhart J, Kirschner M (1997) Cells, embryos, and evolution. Blackwell Science, Malden, MA

Gilbert SF, Epel D (2009) Ecological developmental biology. Sinauer Associates, Sunderland, MA

Gould SJ (1977) Ontogeny and phylogeny. Harvard University Press, Cambridge, MA

Hall BK (1992, revised and expanded in 1999) Evolutionary developmental biology. Kluwer Academic Publishers, Dordrecht

Hall BK, Olson WM (eds) (2003) Keywords and concepts in evolutionary developmental biology. Harvard University Press, Cambridge, MA

Hall BK, Pearson RD, Müller GB (eds) (2003) Environment, development, and evolution. MIT Press, Cambridge, MA

Johanson Z, Underwood C, Richter M (eds) (2018) Evolution and development of fishes. Cambridge University Press, Cambridge

Laubichler MD, Maienschein J (eds) (2007) From embryology to evo-devo: a history of developmental evolution. MIT Press, Cambridge, MA

Love AC (ed) (2014) Conceptual change in biology: scientific and philosophical perspectives on evolution and development. Springer, Dordecht

Minelli A (2003) The development of animal form: ontogeny, morphology, and evolution. Cambridge University Press, Cambridge

Minelli A (2018) Plant evolutionary developmental biology: the evolvability of the phenotype. Cambridge University Press, Cambridge

Minelli A, Fusco G (eds) (2008) Evolving pathways: key themes in evolutionary developmental biology. Cambridge University Press, Cambridge

Müller GB, Newman SA (eds) (2003) Origination of organismal form. Beyond the gene in development and evolution. MIT Press, Cambridge, MA

Raff RA, Kaufman TC (1983) Embryos, genes, and evolution: the developmental-genetic basis of evolutionary change. Indiana University Press, Bloomington (Indiana)

Samson R, Brandon RN (eds) (2007) Integrating evolution and development: from theory to practice. MIT Press, Cambridge, MA

Scholtz G (ed) (2004) Evolutionary developmental biology of crustacea. A. A. Balkema Publishers, Lisse

Streelman JT (ed) (2013) Advances in evolutionary developmental biology. John Wiley & Sons, Hoboken, New Jersey

West-Eberhard MJ (2003) Developmental plasticity and evolution. Oxford University Press, Oxford

Wagner GP (2014) Homology, genes, and evolutionary innovation. Princeton University Press, Princeton

Wanninger A (ed) (2015) Evolutionary developmental biology of invertebrates, 6 vols. Springer, Wien
Wilkins AS (2002) The evolution of developmental pathways. Sinauer Associates, Sunderland, MA
Winther RG (2013) Evo-devo as a trading zone. In: Love A (ed) Conceptual change in biology: scientific and philosophical perspectives on evolution and development. Springer, Dordecht

Part II

Key Concepts in Evo-Devo

Heterochrony

Ronald M. Bonett

Contents

Abstract

Heterochrony describes evolution in the timing of developmental traits and has been an important concept for explanations of phenotypic diversity. Heterochronic processes have the potential to produce tremendous diversification of forms by varying the ontogenetic timing and rate of trait development. When suites of traits are subject to the same heterochronic process, then disparate forms can be produced rapidly. The language of heterochrony has been complicated by several ad hoc additions and revisions of the original terms to suit different types of developmental patterns and processes. Furthermore, heterochronic terms have been applied to both individual traits and more vaguely to whole organisms, which has overshadowed the intricate uses of the concept and led to confused definitions. The analysis of heterochronic patterns must be well grounded in a phylogenetic or paleontological context, as interpretations are contingent upon

R. M. Bonett (✉)
Department of Biological Science, College of Engineering and Natural Sciences, University of Tulsa, Tulsa, OK, USA
e-mail: ron-bonett@utulsa.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_71

understanding the conditions of both the descendants and the ancestors. The concept of heterochrony has been primarily used in morphology, even though these principles are broadly applicable to behavioral, ecological, and physiological traits. The recognition of heterochronic patterns and processes across disciplines will more broadly integrate these concepts into biology and provide further insights into the evolution of eukaryotes. This chapter provides definitions for the various types of heterochronic patterns with examples from salamanders, which exhibit extensive ontogenetic evolution. The challenges of analyzing ontogenetic data are also discussed. The evolution of developmental timing can provide a central framework for understanding the ecological drivers and developmental mechanisms that shape phenotypic diversity.

Keywords

Acceleration · Deceleration · Hypermorphosis · Hypomorphosis · Neoteny · Paedomorphosis · Peramorphosis · Progenesis

Introduction

Heterochrony characterizes differences in the timing of development in descendants compared to their ancestors and is one of the foundational concepts in evolutionary developmental biology. It has garnered serious attention because altering the ontogenetic schedule of development can potentially produce tremendous phenotypic diversity. Furthermore, the eukaryotic tree of life is adorned with a wide array of forms that appear to have been shaped from heterochronic processes.

Heterochrony involves both changes in the relative timing in the appearance of a trait (*event heterochronies*) and/or differences in the relative amount of time for a feature to develop (*rate heterochronies*). Therefore, event heterochronies refer to evolution in a single point of development (*onset* or *offset*), whereas rate heterochronies describe differences in the degree of developmental change between at least two time points (*rate*). In either case, the ontogenetic timing of development may be advanced or delayed in descendants compared to their ancestors. Likewise, the rate of trait development during ontogeny may be *accelerated* or *decelerated*. In other words, a trait may be expressed earlier or later in development and/or may develop at a faster or slower rate.

Heterochronic mechanisms that regulate multiple traits may simultaneously shift development in a similar manner (e.g., accelerated or decelerated) and can produce abrupt phenotypic discontinuities between ancestors and descendants. This can give the misleading impression that all traits have followed a singular pattern, and, as a consequence, the development of some species has been characterized as if developmental shifts had only occurred in one direction (e.g., in "neotenic" salamanders). This has been convenient for coarse categorizations of species into developmental or ecological groupings, but this practice also obscures the language of heterochrony.

Apart from the heterochronic terms popularized by Gould (1977) and formalized by Alberch et al. (1979), several other terms have been coined to define diverse patterns of developmental evolution. These include the evolution of structural repetition (*heterometry*), evolution of structural relocation on the organism (*heterotopy*), and a myriad of other developmental modes. Webster and Zelditch (2005) point out that much confusion stems from terms conflating developmental processes, and they suggest that clarification may be achieved by matching the terms to specific modes. However, the number of potential modes of developmental evolution may be infinite (Rice 1997; Webster and Zelditch 2005). Here I focus on rate and timing modifications as described by Alberch et al. (1979) with additional qualifications provided by McKinney and McNamara (1991), Hall (1999), Webster and Zelditch (2005), and in some respects Reilly et al. (1997). The history of heterochronic inferences in biology and the change of concepts over time were recently reviewed by Hanken (2015) and will not be rehashed here.

In this chapter, heterochronic concepts are described using examples from salamanders, which include several classic cases of ontogenetic evolution. Like most amphibians, many salamanders exhibit a *biphasic* life cycle with an aquatic larval stage followed by metamorphosis into a terrestrial stage for adulthood. However, some lineages of salamanders retain at least some larval structures and aquatic ecology throughout life (*larval form paedomorphosis*). At the opposite extreme, other salamanders rapidly accelerate through (or skip) ancestral larval development and have a completely terrestrial life cycle (*direct development*). These deviations in developmental timing provide hallmark examples of major heterochronic patterns, but they also display nuances that require special consideration when applying these concepts and terms. The recognized categories of heterochrony (described below) are primarily based on changes in the timing of events and rates of somatic shape and reproductive development. However, beyond morphological development, organisms can also vary in the timing of other traits, such as shifts in behavior, ecology, or physiology. Also discussed are some of the challenges to analyzing the evolution of ontogeny as well as the importance of integrating heterochronic concepts with other disciplines of biology.

Patterns of Heterochrony

Major patterns of heterochronic change should be understood without limitation to somatic or reproductive traits (McKinney and McNamara 1991). In general, heterochrony can result in the *truncation* or *extension* of development in descendants compared to their ancestors (Fig. 1). Developmental truncation (*underdevelopment*) can result from a delayed onset of development (*postdisplacement*), an early offset of development (*hypomorphosis*, also known as "*progenesis*"), or a slowed rate of development (*deceleration*, sometimes equated with "*neoteny*"). It is important to note that the term "progenesis" has been widely used to describe the evolution

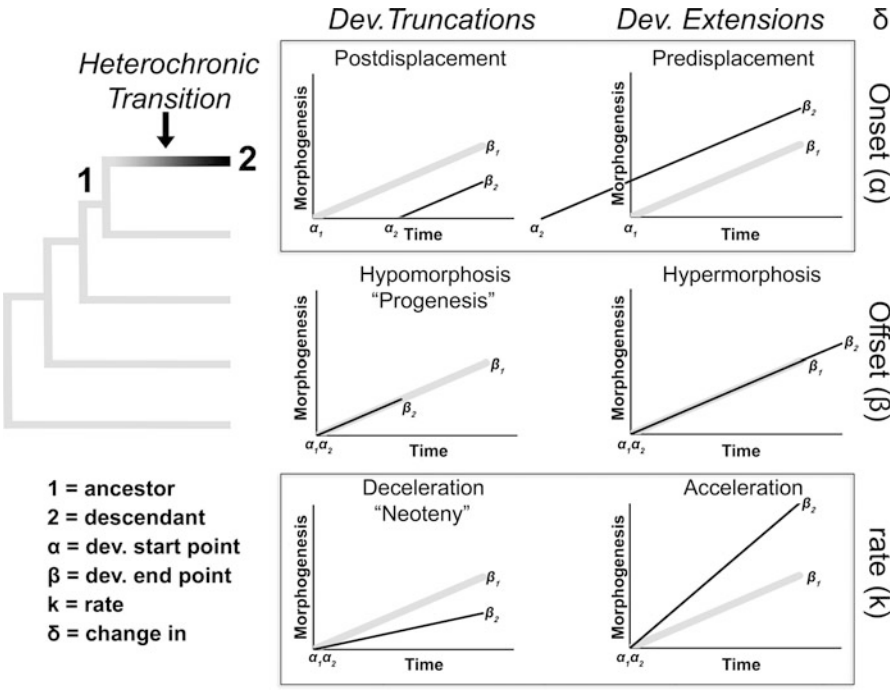


Fig. 1 Heterochronic modes for the achievement of developmental truncations and accelerations as described by Alberch et al. (1979) with modifications by Reilly et al. (1997). The phylogeny on the left shows the ancestral developmental trajectory (gray, 1) with a heterochronic transition to a derived developmental state (black, 2). Six alternative ontogenetic trajectories are plotted on the right, indicating how changes in (δ) the onset of development (post- and predisplacement), the offset of development (hypomorphosis and hypermorphosis), and the rate of development (deceleration and acceleration) can be altered in a descendant (thin black line, 2) compared to the ancestral trajectory (wide gray line, 1). The developmental starting points (α) and ending points (β) are indicated. Symbols are based on Alberch et al. (1979)

of early reproduction, while "neoteny" has often been applied to the evolution of decelerated somatic development (Gould 1977; Alberch et al. 1979; see also below). However, the historical meanings of these terms have been tumultuous, and they are also fraught with technical issues (McKinney and McNamara 1991; Reilly et al. 1997). The primary problem is that "progenic" (hypomorphic) and "neotenic" (decelerated) patterns are not restricted to changes in only reproductive and somatic tissues, respectively (McKinney and McNamara 1991). Furthermore, progenesis has been formalized to mean an early offset in development (Alberch et al. 1979; McKinney and McNamara 1991; Fig. 1). Therefore, reproductive "progenesis" (as it is commonly used) is really the early offset of the juvenile stage, which is a convoluted way of depicting early adulthood. Extended (*overdeveloped*) patterns can be achieved by an early developmental onset (*predisplacement*), a delayed developmental offset (*hypermorphosis*), or a heightened rate of development (*acceleration*).

The differences between event heterochronies (hypomorphosis, hypermorphosis, predisplacement, and postdisplacement) and rate heterochronies (acceleration and deceleration) can be a matter of perspective, so it is important to carefully define the phenomenon that is being examined. For example, the start of reproductive maturation is a single event, but the gonadal tissue itself has a developmental rate, which is the amount of time for development/differentiation from the embryonic origin in the genital ridge until maturation. In addition, in some cases the same event could indicate at the same time the start of a new developmental stage or the end of an earlier developmental stage (e.g., the onset of maturation is the offset of immaturity). In summary, many patterns of heterochronic evolution could be described either as rate heterochronies or as event heterochronies, depending on the perspective taken, including most of the examples discussed in this chapter. This flexibility is sometimes necessary in order to align heterochronic definitions with their common usage.

Heterochronic Shifts Across Life Stages

Heterochronic shifts that result in the expression of traits at different stages of the life cycle have received considerable attention, as they can have major functional consequences on the biology of the organism (Gould 1977). Heterochronic patterns that give rise to such transitions can be surmised by comparing the ontogenetic evolution of at least one trait to another that defines a stage (e.g., maturation of gonads defines adulthood). For example, heterochronic evolution of reproductive versus somatic development can involve either the retention of ancestral juvenile traits in the adults of descendants (*paedomorphosis*) or the precocial development of ancestral adult traits in the juvenile stages of descendants (*peramorphosis*). Notably, Alberch et al. (1979) specified that these terms should only apply to the ontogenetic evolution of morphology (*differentiation heterochrony*) and not differences solely based on size (*growth heterochrony*).

Both paedomorphosis and peramorphosis can evolve in multiple ways through altering the timing of somatic and/or reproductive development. With respect to the major heterochronic modes listed above, paedomorphosis can result from early maturation through reproductive acceleration or reproductive predisplacement. The commonly used term "progenesis" (hypomorphosis) could also be invoked here, but only to mean an early offset of the juvenile stage. Paedomorphosis through early reproduction can produce individuals with juvenilized traits without necessarily altering somatic development. Ryan and Semlitsch (1998) used mesocosms to demonstrate that larval form paedomorphosis in mole salamanders (*Ambystoma talpoideum*) was a product of early maturation ("predisplacement of reproduction"). They found that the earliest individuals to reach reproductive maturity maintained a larval morphology, and there was no difference in body size between paedomorphic and metamorphic adults.

An alternative pathway to paedomorphosis is through truncation of somatic development compared to the ancestral condition. In this mode, reproductive development may progress at the same rate as in the ancestors, but the deceleration

("neoteny") or postdisplacement of one or more somatic traits can result in their paedomorphosis. Based on reconstructions of ancestral states, Bonett et al. (2014a) showed that larval form paedomorphosis in a radiation of salamanders (*Eurycea*) from the Edwards Plateau of Central Texas likely resulted from truncated somatic development. This is based on the loss of metamorphosis without a significant change in the age of maturation. Bonett et al. (2014a) applied the term "neoteny" to describe this somatic truncation leading to paedomorphosis, although it could also be perceived as a permanent postdisplacement of metamorphosis. That is, a delay in the onset of metamorphosis produced paedomorphic expression of postdisplaced traits. If metamorphosis is postdisplaced until after the start of reproduction, then this usually results in (at least temporarily) a period of paedomorphosis (discussed further in the context of obligate and facultative paedomorphosis below).

Peramorphic expression of ancestral adult traits in the juveniles of descendants can result from accelerated somatic or delayed reproductive development. Without alteration of the timing of reproduction, peramorphic somatic traits can evolve through somatic acceleration or somatic predisplacement. Direct-developing salamanders in the family Plethodontidae exhibit extreme peramorphosis, with an exceptionally rapid rate of somatic development compared to ancestral biphasic salamanders (Wake and Hanken 1996; Bonett et al. 2014a). While most other salamanders with a terrestrial stage do not metamorphose until close to adulthood, direct developers typically complete transformation before hatching. Interestingly, among direct developers, peramorphosis appears to occur by different modes depending on the lineage and structure. For example, most salamanders have a throat skeleton for gape-and-suction feeding during their aquatic larval phase, which is extensively remodeled at metamorphosis into a tongue skeleton primarily for terrestrial feeding. Even though nonfunctional, the ancestral larval throat skeleton of some direct-developing salamanders (e.g., *Plethodon*) is recapitulated in the egg. These species rapidly accelerate through a larval-like throat development and metamorphosis *in ovum* (Kerney et al. 2012). By comparison, some other direct developers (e.g., *Bolitoglossa*) skip the ancestral larval throat skeleton entirely and directly develop a terrestrially adapted tongue skeleton. In other words, their throat skeletons are predisplaced and start to develop at an advanced stage compared to ancestral salamanders. This mode of predisplacement enhances the already accelerated speed of somatic development in the throat of these direct developers. Peramorphosis can also evolve through the deceleration or postdisplacement of reproductive development or a hypermorphic delay in the offset of the juvenile stage. By the delay of maturation through any of these pathways, ancestral adult traits may be expressed in the juveniles of the descendants.

The heterochronic modes described above can be applied to evolutionary developmental changes of a single trait or to suites of correlated traits that may be influenced by the same mechanism. The point is that independent traits are not necessarily required to follow a single heterochronic trajectory, and the phenotypes of descendants may represent a diversity of modified developmental

patterns. Nevertheless, for convenience, species have often been categorized under a single heterochronic term, even though the process may only apply to some of its traits. For example, completely aquatic life cycles have evolved multiple times in salamanders. Species with this ecology are often referred to as "paedomorphic" when they retain their larval form hyobranchial (throat) structure and gill openings. However, the composition of larval traits retained into adulthood varies from lineage to lineage (Table 1), a condition that has been referred to as "*differential metamorphosis*" (Wake 1966). To allow for the possibility of different developmental changes within a lineage, it is best to always specify which trait or sets of traits have undergone a particular heterochronic modification.

Whether considering a single trait or the whole organism, heterochronic patterns are potentially labile and can readily reverse course. For example, even though direct development in plethodontids is likely the result of extreme somatic acceleration, subsequently several major somatic truncations occurred in this clade. This is most apparent in major decelerations (delays) in larval development, leading to life cycle diversification within the family (Bonett et al. 2014a, discussed below). But there were also major heterochronic reversals within strictly direct-developing clades (e.g., neotropical plethodontids of the tribe Bolitoglossini). In one of the earliest comparative studies to specifically quantify patterns of heterochrony, Alberch and Alberch (1981) demonstrated that an arboreal direct-developing plethodontid (*Bolitoglossa occidentalis*) exhibits substantial somatic truncation ("paedomorphosis") compared to other generalized members of the genus. An even more extreme example of paedomorphosis is found in the miniaturized bolitoglossine genus *Thorius*, which has lost many skeletal elements leading to extensive morphological novelty (Hanken 1984).

Table 1 Several traits that are typically modified during metamorphosis are compared among salamanders to demonstrate differential metamorphosis. Fully metamorphosed representatives from the family Plethodontidae include direct-developing (dd) *Plethodon* and some *Eurycea* that are biphasic (bi). Obligately paedomorphic (pd) salamanders include some *Eurycea* and the families Amphiumidae, Cryptobranchidae, Sirenidae, and Proteidae. An X indicates that the developmental change occurs. Note the transformation of some of these traits in some "obligately paedomorphic" families (e. g., Amphiumidae and Cryptobranchidae) that exhibit differential metamorphosis

	Lost before adulthood			Develop before adulthood			
	External gills	Gill slits	Tail fin	Eyelids	Maxillary bones	Septomax bones	Prefrontal bones
Plethodon (dd)	x	x	x	x	x	x	x
Eurycea (bi)	x	x	x	x	x	x	x
Eurycea (pd)							
Amphiumidae (pd)	x				x		x
Cryptobranchidae (pd)	x				x		x
Sirenidae (pd)					x		
Proteidae (pd)							

Reconstructing Heterochronic Patterns

Deciphering heterochronic patterns first involves orienting the direction of change (e.g., advanced/accelerated or delayed/decelerated) over time for a given trait or set of traits. Therefore, understanding the developmental patterns of the most recent ancestor is as important as that of the descendant(s) (Fink 1982; Bonett et al. 2014a). Misestimation of the ancestral condition is likely to result in a completely opposite interpretation of the direction of developmental change. For this reason the ancestral condition should be based on direct evidence of the state of the parent population, trait reconstruction on a phylogeny, or paleontological evidence. For example, the ancestral condition of the family Plethodontidae was historically depicted as a salamander with a multi-year larval stage. The great diversity of plethodontids with direct development were thought to result from multiple independent cases of accelerated somatic development (repeated peramorphosis). However, phylogenetic reconstructions suggest that ancestral plethodontids had either a very short larval period or direct development. Therefore, direct development (or a brief larval period) was likely inherited from a common ancestor, and there probably were a relatively limited number of lineages with decelerated somatic development leading to species with long larval periods (Bonett et al. 2014a).

Reconstructing ancestral states requires a phylogenetic hypothesis (the tree) and data on the trait of interest for at least some representative taxa from across the phylogeny. In many cases it can be challenging to collect ontogenetic data for multiple species, particularly when the trait is a molecular characteristic (e.g., protein expression patterns). Even when data are available, ancestral states can only be reconstructed with confidence under some scenarios. If there are too many possible states for the trait (compared to the number of taxa), or if the trait is highly variable, then ancestral states can be very difficult to estimate.

Several methods have been developed for analyzing changes in the sequence of developmental events (*sequence heterochronies*) (e.g., Maxwell and Harrison 2009). These methods are primarily based on "event pairing," and one advantage is that they allow developmental sequences to be analyzed irrespective of the actual age when a trait appears. However, sequence heterochronies can be difficult to reconstruct on a phylogeny, due to the potentially large number of character states that in turn requires data from many taxa. Shape trajectories can be analyzed through geometric morphometrics of homologous landmarks. This is another way to potentially capture heterochronic changes in multiple traits (Webster and Zelditch 2005). However, there are statistical challenges to analyzing the evolution of high-dimensional data, and coarse shapes may capture multiple traits that are not necessarily evolving in the same direction.

The essence of heterochrony is the evolution of timing. When data on actual age of a developmental event are available, then age (of the event) can be treated as a continuous trait (Bonett et al. 2014a). Likewise, the evolution of developmental rate based on the amount of phenotypic change between two ontogenetic points could also be studied in a similar manner. Analyzing heterochrony this way can allow one to test for the direction of significant developmental deviations through time. It also

allows for the potential implementation of diverse models of continuous trait evolution (e.g., Brownian Motion, Ornstein-Uhlenbeck, Punctuated Equilibrium, etc.) when testing heterochronic patterns. Obtaining absolute ontogenetic ages of developmental timing across clades can be difficult, but other variables, such as organism size, may be used as a reasonable proxy for age. However, one should take caution in using size, as growth rate can be subject to heterochrony too (Klingenberg and Spence 1993).

The term *isomorphosis* has been used to describe when divergent patterns of heterochrony ultimately produce the same phenotype (Reilly et al. 1997). This could be the product of *convergent evolution* or *developmental system drift*. Convergence through isomorphosis is evident throughout the independent evolution of larval form paedomorphosis in salamanders. For example, mole salamanders have been shown to exhibit paedomorphosis through early reproduction (Ryan and Semlitsch 1998), while distantly related Central Texas *Eurycea* likely evolved larval form paedomorphosis through somatic truncation (Bonett et a. 2014a). Variable heterochronic patterns leading to similar phenotypes have also been documented among close relatives. For instance, Denoël and Joly (2000) show that larval form paedomorphosis in alpine newts (*Mesotriton alpestris*) can be derived from both reproductive acceleration (stated as "progenesis") and somatic deceleration (stated as "neoteny") among populations. Developmental system drift can also produce isomorphic patterns, whereby lineages share a directly inherited phenotype but have pathways with heterochronic divergence (see chapter ▶ "Developmental System Drift"). This is probably quite common and could be applied to any group of species that vary in the timing of life cycle events but share the same final stage of ontogeny (e.g., metamorphosis). Comparative analyses of heterochronic patterns across clades can be used to test whether ontogenetic convergence is based upon the same mechanism or whether independent or divergent mechanisms have produced similar developmental patterns. This is the foundation of understanding the repeatability and limitations of developmental diversification. Given the importance of heterochrony to understanding phenotypic evolution, analytical methods for dealing with ontogenetic data require more attention, particularly in the realm of testing how heterochrony influences patterns of trait integration and diversification.

Appropriate Scale for Heterochronic Inference

Heterochronic patterns have been recognized as ranging from comparisons of descendants and ancestors separated by tens of millions of years to abrupt atavisms observed in a single generation. In general, ancestral states can be reconstructed with a high likelihood when transition rates between states are low. For the same reason, interpreting heterochronic patterns will be more likely when the rate of pattern reversal is low. If rates of change are high, then a difference between ancient ancestors and recent descendants is not likely to be representative of a single heterochronic shift in a given direction.

Reilly et al. (1997) introduced terms to describe intraspecific heterochronic patterns (*paedotypic* and *peratypic*) that parallel the original "between species" terminology (*paedomorphosis* and *peramorphosis*). The intraspecific terms have not been widely adopted and may be unnecessary. If a trait can be polarized correctly, then the only difference between intra- and interspecific comparisons is the scale (recent vs. more distant ancestor). The reasoning for suggesting intraspecific analogues was that the original terms were meant to address the timing of developmental patterns with respect to a phylogeny. However, phylogenies are no longer considered exclusive to interspecific relationships, and it is common to reconstruct molecular-based phylogenies among populations within species. Also, the cutoff between populations and species can sometimes be arbitrary. It seems that the original definitions of *paedomorphosis* and *peramorphosis* equally apply whether comparing descendants to ancient ancestors or parents to offspring. What is most important is whether there is confidence in understanding the directionality of change.

Heterochronic Processes that Underlie Heterochronic Patterns

Heterochronic patterns can potentially shed light on the evolution of underlying developmental mechanisms. A heterochronic pattern that shows a shift in the onset of a developmental event can signify modifications to gene/protein expression, hormone sensitivity/release, cell differentiation, or cellular condensations. Ontogenetic changes in offset points may also indicate changes in gene/protein expression, hormone sensitivity/release, or cessation of cell differentiation. Rate heterochronies can be influenced by differences in rates of differentiation or morphogenesis (Hall 1999; Bonett 2016). The processes themselves may also be heterochronic in nature. For example, an inductive signal may diffuse through fewer tissue layers compared to ancestors, which is analogous to hypomorphosis or deceleration (McKinney and McNamara 1991). It is important to note that not all heterochronic mechanisms will produce heterochronic patterns at the morphological level and heterochronic patterns of development are not necessarily derived from a heterochronic mechanism (Hall 1999).

Developmental patterns are often plotted as linear trajectories even though morphogenesis may exhibit abrupt developmental changes and asymptotic behaviors. Comprehensive comparisons of complete ontogenetic trajectories can reveal more subtle vagaries in ontogenetic divergence and provide important insights into potential developmental mechanisms (Rice 1997). If a truncated pattern of somatic development is eventually manifested late in ontogeny (e.g., late adulthood), then this indicates a mechanism that reduces the rate (or delays the onset) of differentiation or morphogenesis but does not necessarily eliminate it entirely. For example, some salamanders can exhibit "facultative paedomorphosis," whereby an individual can reproduce in the larval form, but then will metamorphose later in ontogeny if subject to environmental stress or treated with thyroid hormone (Bonett 2016). By comparison, the larval traits of some "obligately paedomorphic" salamanders

may never transform no matter how long the salamander lives or even when treated with a substantial doses of metamorphosis-inducing agents (e.g., thyroid hormone). This suggests that the heterochronic mechanisms that govern facultative paedomorphosis alter the rate (or timing) of reproduction or somatic morphogenesis, whereas metamorphosis of "obligate paedomorphs" is permanently displaced. Indeed, even though thyroid hormone can regulate gene expression in the larval tissues of obligate paedomorphs, the thyroid hormone system has become decoupled from transforming these tissues (Safi et al. 2006). This illustrates that examining heterochronic patterns can inform hypotheses about how processes are regulated.

The correlation of different traits among individuals of a population or across a phylogeny indicates integration, which could be a product of selective, genetic, functional, or developmental constraints. Strong developmental correlations among many traits suggest that they are influenced by a systemic (*global*) mechanism (Hall 1999). There are indeed a variety of mechanisms that can produce such patterns including *ontogenetic repatterning* (Wake 1989) from alterations to early development that reverberate across ontogeny. Also, changes to developmental signals such as hormones that have multiple genetic targets could also produce global heterochronic shifts.

Other developmental mechanisms may be more restricted (*local*) in their control. Hall (1999) recognizes *primary local* heterochronic mechanisms that directly influence the trait of interest and *secondary regional* heterochronic mechanisms that are an indirect by-product of alterations to other aspects of development. Generally, a lack of correlation between traits suggests that they are not coupled and can potentially evolve independently. For example, the "independence" of reproductive and somatic development allows salamanders to achieve reproductive maturity while still in their larval form (paedomorphosis). In salamanders these systems are relatively independent compared to frogs, which are altogether unable to reproduce as tadpoles. However, it is important to recognize that independence is relative when considering any traits within an individual. Even developmental components that are considered to be mostly independent may still exert considerable influence on one another. This is the case for vertebrate sex steroids, which are produced in the gonads and can broadly impact somatic development. These hormones can accelerate or inhibit amphibian metamorphosis (reviewed in Bonett 2016), and therefore maturation can fundamentally alter the developmental processes that regulate the somatic developmental trajectory.

Integrating Heterochrony

Patterns of punctuated phenotypic evolution are common in the fossil record and strike a contrast to the gradual changes originally suggested by Darwin. Such patterns can be explained by local phenotypic evolution followed by shifts in geographic distribution. It has also been suggested that punctuated changes arise from the evolution of global heterochronic mechanisms that simultaneously induce multiple alterations in descendants. This can give the appearance of an abrupt shift

in phenotype and is consistent with heterochronic variation observed from parents to offspring or among populations of extant species.

Heterochronies are evident from evolutionary analyses of developmental patterns and the fossil record. Developmental biology can inform us about the endogenous mechanisms that produce ontogenetic differences, but ecological analyses are necessary to understand the distributions of these phenotypes in nature. Comparing shifts in the timing and rate of development serves as a powerful framework to test the ecological scenarios that drive developmental patterns. This could include studying how niche divergence, competition, trophic interactions, sexual selection, or fecundity influence heterochronic evolution (reviewed in McKinney and McNamara 1991). Timing of reproduction and fecundity are fundamental parameters of population ecology. Understanding whether a heterochronic pattern results from shifting reproduction or shifting somatic development may shed light on whether the pattern is driven by habitat versus fecundity. Ryan and Semlitsch (1998) showed that paedomorphosis in *Ambystoma talpoideum* results from "reproductive predisplacement." Early maturation allows for early breeding, prior to the arrival of metamorphosed adults. This advance on the timing of reproduction provides the larvae of paedomorphs with an age (size) advantage over later breeders. Ryan and Semlitsch (1998) argue that selection is likely acting on age at first reproduction, which would drive paedomorphosis in this species. The maintenance of a larval morphology for remaining in the aquatic larval environment was considered to be secondary.

Across species or populations, phylogenetic comparative methods can be applied to test for the ecological causes that drive the evolution of heterochronic patterns. In the plethodontid tribe Spelerpini, larval form paedomorphic species are associated with stable aquatic environments such as caves, while biphasic species occur in regions with increased precipitation that provide stable terrestrial habitats (Bonett et al. 2014b). This demonstrates how environmental parameters can drive patterns of heterochrony across clades. There are indeed major consequences of heterochronic evolution. Paedomorphic plethodontids have relatively small geographic range sizes and show limited dispersal among physiographic regions in eastern North America. This is a strong negative trade-off of paedomorphosis, which has implications for patterns of lineage diversification. Across salamanders the consequences of heterochronic evolution can also be discerned in the patterns of body form diversification (Bonett and Blair 2017). Biphasic salamanders with aquatic-to-terrestrial life cycles exhibit relatively constrained body forms, compared to completely terrestrial (direct-developing) and completely aquatic (obligately paedomorphic) lineages. These studies highlight how analyses of heterochronic patterns can be used to understand the links between development, ecology, and evolution. Heterochrony has largely been tested in morphological traits, but behavioral, ecological, and physiological traits can also vary in developmental timing and may in fact be directly linked to the evolution of developmental processes (McKinney and McNamara 1991; Spicer and Rundle 2006). The study of ontogenetic evolution across different levels of organization provides a means for integrating diverse disciplines of biology.

Cross-References

- ▶ A Process-Based Approach to the Study of Flower Morphological Variation
- ▶ Convergence
- ▶ Developmental System Drift
- ▶ Morphometrics in Evolutionary Developmental Biology
- ▶ Pere Alberch (1954–1998)
- ▶ Stephen Jay Gould (1941–2002)

References

Alberch P, Alberch J (1981) Heterochronic mechanisms of morphological diversification and evolutionary change in the neotropical salamander, *Bolitoglossa occidentalis* (Amphibia: Plethodontidae). J Morphol 167:249–264

Alberch P, Gould SJ, Oster GF, Wake DB (1979) Size and shape in ontogeny and phylogeny. Paleobiology 5:296–317

Bonett RM (2016) An integrative endocrine model for the evolution of developmental timing and life history of plethodontids and other salamanders. Copeia 104:209–221

Bonett RM, Blair AL (2017) Evidence for complex life cycle constraints on salamander body form diversification. Proc Natl Acad Sci USA 114:9936–9941

Bonett RM, Steffen MA, Robison GA (2014a) Heterochrony repolarized: a phylogenetic analysis of developmental timing in plethodontid salamanders. EvoDevo 5:27

Bonett RM, Steffen MA, Lambert SM, Wiens JJ, Chippindale PT (2014b) Evolution of paedomorphosis in plethodontid salamanders: ecological correlates and re-evolution of metamorphosis. Evolution 68:466–482

Denoël M, Joly P (2000) Neoteny and progenesis as two heterochronic processes involved in paedomorphosis in *Triturus alpestris* (Amphibia: Caudata). Proc R Soc Lond B 267:1481–1485

Fink WL (1982) The conceptual relationship between ontogeny and phylogeny. Paleobiology 8:254–264

Gould SJ (1977) Ontogeny and phylogeny. Harvard University Press, Cambridge

Hall BK (1999) Evolutionary developmental biology, 2nd edn. Kluwer Academic Publishers, Dordrecht

Hanken J (1984) Miniaturization and its effects on cranial morphology in plethodontid salamanders, genus *Thorius* (Amphibia, Plethodontidae). I. Osteological variation. Biol J Linn Soc 23:55–75

Hanken J (2015) Is heterochrony still an effective paradigm for contemporary studies of evo-devo? In: Love A (ed) Conceptual change in biology: scientific and philosophical perspectives on evolution and development. Springer, New York

Kerney RR, Blackburn DC, Müller H, Hanken J (2012) Do larval traits re-evolve? Evidence from the embryogenesis of a direct- developing salamander, *Plethodon cinereus*. Evolution 66:252–262

Klingenberg CP, Spence JR (1993) Heterochrony and allometry: lessons from the water strider genus *Limnoporus*. Evolution 47:1834–1853

Maxwell E, Harrison LB (2009) Methods for the analysis of developmental sequence data. Evol Dev 11:109–119

McKinney ML, McNamara KJ (1991) Heterochrony, the evolution of ontogeny. Plenum Press, New York

Reilly SM, Wiley EO, Meinhardt DJ (1997) An integrative approach to heterochrony: the distinction between interspecific and intraspecific phenomena. Biol J Linn Soc 60:119–143

Rice SH (1997) The analysis of ontogenetic trajectories: when a change in size or shape is not heterochrony. Proc Natl Acad Sci USA 94:907–912

Ryan TJ, Semlitsch RD (1998) Intraspecific heterochrony and life history evolution: decoupling somatic and sexual development in a facultatively paedomorphic salamander. Proc Natl Acad Sci USA 95:5643–5648

Safi R, Vlaeminck-Guillem V, Duffraisse M, Seugnet I, Plateroti M, Margotat A, Duterque-Coquillaud M, Crespi EJ, Denver RJ, Demeneix B, Laudet V (2006) Pedomorphosis revisited: thyroid hormone receptors are functional in *Necturus maculosus*. Evol Dev 8:284–292

Spicer JI, Rundle SD (2006) Out of place and out of time – towards a more integrated approach to heterochrony. Anim Biol 56:487–502

Wake DB (1966) Comparative osteology and evolution of the lungless salamanders, family Plethodontidae. Mem South Calif Acad Sci 4:1–111

Wake DB (1989) Phylogenetic implications of ontogenetic data. In: David B, Dommergues JL, Chaline J, Laurin B (eds) Ontogenèse et Évolution, Geobios, Mémoire Spécial, vol 12. Département des Sciences de la terre de l'Université Claude Bernard, Lyon, pp 369–378

Wake DB, Hanken J (1996) Direct development in the lungless salamanders: what are the consequences for developmental biology, evolution and phylogenesis? Int J Dev Biol 40:859–869

Webster M, Zelditch ML (2005) Evolutionary modifications of ontogeny: heterochrony and beyond. Paleobiology 31:345–372

Developmental Exaptation

Ariel D. Chipman

Contents

Abstract

Developmental exaptation is the process through which pre-existing characteristics of the developmental program facilitate evolutionary changes in development, by providing an internal environment that is conducive to a certain change. Developmental exaptation can be manifested at all levels of the developmental process, including gene regulation, cellular behavior, tissue differentiation, morphogenetic movement, relative timing of developmental events, and more. Developmental exaptation can facilitate changes in highly conserved, slowly evolving genes. It is the inherent prerequisite for cases of gene or gene network co-option in the evolution of development and is probably involved in many cases of evolutionary novelties.

A. D. Chipman (✉)
The Department of Ecology, Evolution and Behavior, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel
e-mail: ariel.chipman@mail.huji.ac.il

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_73

Keywords

Constraint · Gene Regulatory Networks Morphogenesis · Novelty · Cooption

Introduction

Evolutionary theory has debated the question of internal factors vs. external factors since the days of the Evolutionary Synthesis (Whyte 1965). Conceptually, there are thought to be differences in the way an organism responds to selection, based on whether the selective forces are imposed by the environment: external selection; or by the need for integration between the different parts of the organism: internal selection (but see Fusco 2001 for an alternative view). This distinction can be expanded in several directions. Internal selection during embryonic development is one of the sources of the phenomenon known as developmental constraint; a limitation in the range of available phenotypes that is due to the limitation and requirements of the developmental system (Gould 1980; Alberch 1982; Resnik 1995; Schwenk 1995; Richardson and Chipman 2003; Arthur 2004; see chapter "Developmental Constraints"). Conversely, internal selection shapes the developmental program and leads to an optimal integration of all the myriad functions involved therein. Any change in development must be consistent with the rest of the process. In other words, the developmental program is under constant selection by the developmental program itself (Cheverud 1996; Beldade and Brakefield 2003; Richardson and Chipman 2003; see chapter "Internal selection"). If the developmental system is adapted to itself, there must also be instances of preadaptation, which allows exaptation within development. Gould and Vrba (1982) define exaptations as "...characters, evolved for other usages (or no function at all), and later coopted for their current role..." Note that preadaptation is normally understood to be the condition of having an existing adaptation that can facilitate the future change, whereas exaptation is used to refer to the evolutionary mechanism through which this happens. The concept of exaptation can be extended to include development and cases when pre-existing aspects of the developmental system facilitate its evolution in certain directions.

The concept of Developmental Exaptation was first raised by Chipman (2001) in a short opinion piece. This was in the early days of the evo-devo revolution, and the field was struggling to define its research agenda and conceptual framework (Arthur 2002). At the time, very little was known about how selective forces shape the developmental program, or indeed about how gene regulation evolves. The main idea of the 2001 paper was that changes in the development program are analogous to changes in the external environment in the effect they have on an organism's selective regime and evolution. Developmental exaptations are thus pre-existing characteristics of the developmental program that facilitate adaptation to the novel selective regime. The core examples given had to do with changes in expression patterns of transcription factors that are facilitated by the presence of downstream factors, which are compatible with the new expression pattern. These preliminary

ideas can be reframed today within a context of a better understanding of gene regulation and how it evolves and within a broader context of not only transcription factors, but entire networks.

Levels of Adaptation in Developmental Programs

Before discussing examples of exaptation, one must define the sources of selection, and hence adaptation, within the developmental program. Development includes processes that take place anywhere from the transcriptional and translational control of individual genes, through the control of cellular behavior and tissue differentiation, and up to the movement of entire tissue layers. All of these levels must be under constant selection (stabilizing selection during periods of stasis and directional selection following changes in development) for the developmental program to proceed successfully. In this section, the different levels of developmental selection are defined, followed by conceptual examples of how exaptation can work at these levels of selection.

Selection on Gene Regulation

The regulation of gene expression occurs at multiple levels. The spatial and temporal control of expression levels of developmental genes is mostly through cis-regulatory elements, which are composed of binding sites for specific transcription factors and for other components of the cellular transcriptional machinery. Control of levels and rate of translation is mostly through untranslated regions in the transcripts and microRNAs that bind to them. Additional levels of regulation can be through peptide signals in the translated gene product on the one hand or through changes in chromatin structure, which affects the accessibility of the gene to the transcriptional machinery, on the other. All of these levels of control can vary throughout evolution in response to selection for a modification in the timing or location of the gene's activity. Conversely, all of these levels of regulation will normally be under strong stabilizing selection.

The nucleotide sequence in upstream control elements fluctuates through random mutations. Certain stretches of sequence will be under strong selection if they contain binding sites for transcription factors that are necessary for the activity of the gene under control, and their sequence will be more stable over time. Other stretches will be under weaker selection and their sequence will vary more rapidly. These random fluctuations could lead to the appearance of a binding site for a transcription factor that is not expressed at a relevant time or place for the gene under control of the element in question. However, a change in the expression domain of the transcription factor represents a change in the internal selection regime. Genes that have pre-existing binding sites for this factor can be said to be exapted to being controlled by it. If the binding sites had not appeared at random,

the change in the expression pattern of the transcription factor would have had no effect.

Similarly, random changes in untranslated regions of a gene transcript could bring that transcript under the control of a specific miRNA. This can happen even when the miRNA has no existing regulatory control of the transcript, and no role in the developmental process in which the transcript is expressed. A random sequence change exapts the transcript to being regulated by a novel regulatory agent.

The example given by Chipman (2001) in the paper introducing the concept of developmental exaptation is a possible (if imprecise) example. We know that the expression domains of Hox genes control morphological identity along the anterior-posterior axis, at least in vertebrates (Burke et al. 1995) and in arthropods (Hughes and Kaufman 2002) (see chapter "Hox genes"). Axial identities are thus believed to evolve through changes in the expression domains of specific Hox genes. However, changes in the expression domains of Hox genes will have a deleterious effect, unless there are existing control regions in downstream genes that are normally expressed neither in the old Hox expression domain nor in the new Hox domain, but would fall under the control of the Hox gene following its expansion. Thus, the existing control regions would be nonfunctional and selectively neutral before the change in Hox gene expression, but could facilitate the change and allow it to have a nondeleterious effect.

Selection on Cellular Behavior

In the simplest terms, development is a process of cells proliferating and moving to their correct locations. Cells divide following specific signals from neighboring cells or following intracellular cyclic processes. The rate and timing of cell division is closely linked to absolute and relative size of organs and must be tightly controlled during development. It is thus under strict stabilizing selection. Selection for size is often selection on the rate or duration of cell proliferation, thus cell division can also be under directional selection in certain cases. Once cells have proliferated, they must migrate while following positional cues. These cues must be adapted to changes in scale. Thus, a change in the timing or rate of cell proliferation exerts a selective pressure on the positional cues to adapt to the new scale so that cells continue to migrate to their correct location.

A change in size – heterometry – through modifying the rate of cell proliferation or through modifying the duration of cell proliferation is one of the simplest evolutionary transformations. However, such a change is likely to be maladaptive in a tightly controlled embryo. Nonetheless, pre-existing variability in positional cues that guide proliferating cells to their correct location could facilitate a heterometric change. Certain combinations of positional cues within this neutral variation could act as exaptations for change.

There are dramatic cases of rapid changes in organ size in what are known as exaggerated traits (Lavine et al. 2015). These exaggerated traits could either be under

sexual selection, or as part of an ecological switch in the evolution of an organism that requires significant modification in the size of a single organ. Examples of the former are deer antlers or rhinoceros beetle horns, whereas examples of the latter are water strider legs or human brains. In these cases, external selection dictates an increase in cell proliferation, which in turn exerts internal selection on all signaling and patterning mechanisms within the developing exaggerated organ. While there has been a significant amount of work on the factors that drive the change in size, there has been almost no consideration of the necessary changes to developmental integration following it. It is likely that at least in some of the cases of exaggerated traits, a pre-existing neutral variability in the patterning of the modified organ allowed the increase in size to be more rapid, and prevented maladaptive patterning errors.

Selection on Tissue Differentiation

Every cell must also adopt the correct cellular identity through a series of differentiation events. These are controlled by positional cues and/or by a cascade of interacting transcription factors, with the different levels of control discussed above. The wrong type of cell in the wrong place can disrupt downstream developmental events, so this process is equally tightly regulated and under stabilizing selection. When there are changes in scale and positional parameters, cells might need to adopt a different fate, thus there is internal directional selection for specific cells to change their differentiation cascade, leading to selection on all upstream levels of control.

As development proceeds, cells change their identity and differentiate. Modification of this process throughout evolution can lead to a different tissue or structure – heterotypy. Selection for heterotypic modification can be facilitated by the existence of a pool of cells of the correct type, which may have originally been destined for a different tissue, but can be recruited to the modified tissue. These pre-existing cells are an exaptation for the evolution of the modified tissue.

A possible example of exaptation at the tissue level is the evolution of novel dermal bone structures in several vertebrate lineages (e.g., boxfish, crocodilians, caecilians, armadillos). Dermal bones are distinguished from endochondral bone mostly based on their developmental origin, with dermal bone arising from mesenchyme in the dermal layer (Hirasawa and Kuratani 2015). For dermal bone to evolve in a novel position, a pre-existing pool of correctly located and differentiated mesenchymal cells is needed as an exaptation. Without the necessary cells in the dermal tissue, the dermal bone could not evolve.

Selection on Morphogenetic Movements

Morphogenetic movements lead to the final organization of all cells and tissues in space (see chapter ▶ "Mechanisms of Pattern Formation, Morphogenesis, and

Evolution"). These movements are a direct result of all the processes described above and are usually mediated by differential control of cell-cell adhesion and changes in cell-shape or cell-type. These movements are under selection to end up with a correctly structured embryo. Any change in cell identity carries with it a selection to change some aspects of the morphogenetic movements.

Evolution can proceed through changes in the relative positions of structures – heterotopy. A structure that develops in a novel location, following a modification of positional cues or of cell shape is under selection to integrate into its novel embryonic environment. The exact identity of surrounding cells, the factors they secrete, and even their morphological arrangement can influence the likelihood of the heterotopic change being selectively advantageous. Specific conditions can act as exaptations for this change.

The evolution of front-fanged snakes involved the shift of a posterior dental lamina to the anterior of the upper jaw through allometric growth (Vonk et al. 2008). For the fang to differentiate and develop in its anterior position, the new surroundings had to have been exapted to provide the necessary cues and support tissues. As with the previous examples, we cannot reliable know what the intermediate stages were in the evolution of the novel anterior position of fangs. However, in this case we do have developmental data from two convergent events in two snake lineages of fang heterotopy, and these support the idea of an unknown developmental exaptation that allowed the fangs to develop anteriorly.

Selection on Relative Timing of Events

All of the above processes must be carefully coordinated in time, since there is a great deal of signaling and cross-regulation between different parts of the embryo and different events that are dependent on each other. It is this temporal integration which exerts the strongest internal selection on development. Any change in any process during development must remain adapted to its dependence on or requirement for other processes, or lead to a change in dependent or resultant process.

One of the most frequently discussed modes of developmental modification is a shift in relative timing of developmental events – heterochrony (Gould 1977; McKinney and McNamara 1991; see chapter ▶ "Heterochrony"). As in the examples discussed above, a change in timing will only be possible if the temporally shifting processes are exapted for the new relative timing. This can take the form of any of the aforementioned exaptations at any level.

There are numerous examples of heterochrony in the evo-devo literature, at all levels of regulation. The shift between anamorphic and epimorphic segmentation in several arthropod lineages is a well-studied case of a significant organism-level heterochronic shift and has presumably occurred in both directions. In epimorphic species, all segments are patterned before the embryo hatches, while in contrast, in anamorphic species the patterning of some of the segments is shifted to postembryonic ontogeny. Such a major restructuring of ontogeny requires a significant number of changes in integration and internal coordination. It is reasonable to assume that at

least some changes in timing occurred and were integrated into the program before the shift in timing of segmentation relative to hatching, and that these previous changes provided an exaptation to the organism-level heterochronic shift.

Note that this list of examples of exaptation is divided conceptually into levels of complexity, but in practice it can be difficult to differentiate between exaptations at different levels. Furthermore, almost all developmental modifications are ultimately down to a change in gene regulation, which is what drives development.

Rare Changes as Indicators of Exaptation

The fact that different genes evolve at highly different rates is well-documented (Bromham et al. 2002; Lanfear et al. 2010). This difference in rate is used as a tool for constructing and dating phylogenetic trees. Some genes are highly conserved and change at a very slow rate (e.g., transcription factors that are involved in crucial developmental events). Implicitly, mutations leading to sequence variation in these genes are very rare. In fact, there is no reason to assume that slowly evolving genes actually have a lower mutation rate. The common explanation for highly conserved – or slowly evolving – genes is that they are under very strong stabilizing selection. This raises the question how they change after all, even when there is selection for the sequence to remain unchanged. Change in a conserved gene can be explained by the presence of an unrelated modification of factors they interact with. These modifications can be in response to a novel selective pressure or as a result of random neutral drift. Either way, they form an exaptation which allows a sequence change in a conserved gene, which was previously impossible due to strong selection for maintaining a functional interaction with the factor, to take place while maintaining the interaction. Absence of change is evidence for strong stabilizing selection. The presence of rare changes, despite stabilizing selection, is evidence of exaptation.

Developmental Exaptation and Co-Option

The evo-devo literature discusses numerous examples for the co-option of existing genes or gene regulatory networks to novel functions, e.g., the recruitment of segment polarity genes to patterning butterfly eyespots (Monteiro and Podlaha 2009), or the recruitment of Hox genes to axial patterning of the vertebrate limb (Shubin et al. 1997; Petit et al. 2017; see chapter “Co-option”). In essence, co-option is a specific case of developmental exaptation, although it is not normally thought of in these terms. Genes and simple gene networks are not normally responsible for a morphological character, but for a specific cellular or developmental function. For example, a network that includes strong mutual repression among its components can form sharp boundaries, while a network that includes a negative feedback loop can generate a repeated pattern. These functions are exaptations for generating novel

structures or modifying existing ones. Thus, if there already exists a network for generating repeating structures through a reiterative process (such as repeated neural ganglia along the anterior-posterior axis), it can be used as the basis for the evolution of more complex repeating structures such as segments with units from different organ systems.

Developmental Exaptation and Novelties

A common idea about the evolution of novelties is that the novel structure does not appear out of nowhere, but represents a rapid diversification of a pre-existing feature or network, following a dramatic shift in selective pressure (Moczek 2008; Shubin et al. 2009; see chapter "Novelty and Innovation"). Novelty can also be placed in the framework of developmental exaptation, by considering the pre-existing features as exaptations. The best-studied example is that of the horns of beetles from the genus *Onthophagus.* These horns are understood to have evolved from the exaggeration of a pre-existing structure with a role in ontogeny: the pronotal horns necessary for eclosion of the final larval molt. These pronotal horns represent a suite of organized cells, which are present at the right place and time in development to be elaborated by natural selection in the generation of a novel structure (Moczek 2005, 2008). As in the beetle example, in almost every case of a novel structure there is a pre-existing developmental structure or network, which forms the exapted basis for the novelty.

Conclusions

Developmental exaptation was originally defined as "characters of the developmental program that were initially created through random events, or in response to selection for a specific function, and subsequently confer an internal selective advantage in a new role, following a change in the embryonic environment" (Chipman 2001 p. 299). These "characters" can include anything from gene expression domains, through cellular identity to relative position and timing of structures. Nonetheless, the actual modifications that these characters are exapted for are almost invariably changes in gene regulation and interactions. Indeed, the characters themselves are the outcome of gene regulatory networks, or to use the terminology from Wagner (2007), Character Identity Networks (ChIN's). A problem with studying developmental exaptations is that they are virtually impossible to identify, since we do not know the sequence of events leading to the fixation of a modified developmental process, nor can we predict a priori which current characters are likely to have a future adaptive value. Nevertheless, an updated view of developmental exaptation must include thinking about genes, their regulation, and the networks they are part of, both in characters modified through evolution and in characters that are exapted to facilitate their modification.

Cross-References

- ▶ A Macroevolutionary Perspective on Developmental Constraints in Animals
- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Evolvability
- ▶ Heterochrony
- ▶ Mechanisms of Pattern Formation, Morphogenesis, and Evolution
- ▶ The Evolution and Development of Segmented Body Plans

References

Alberch P (1982) Developmental constraints in evolutionary processes. In: Bonner JT (ed) Evolution and development, vol 22. Life sciences research report. Springer, Berlin, p 313–332

Arthur W (2002) The emerging conceptual framework of evolutionary developmental biology. Nature 415:757–764

Arthur W (2004) Biased embryos and evolution. Cambridge University Press, Cambridge

Beldade P, Brakefield PM (2003) Concerted evolution and developmental integration in modular butterfly wing patterns. Evol Dev 5:169–179

Bromham L, Woolfit M, Lee MSY, Rambaut A (2002) Testing the relationship between morphological and molecular rates of change along phylogenies. Evolution 56:1921–1930

Burke AC, Nelson CE, Morgan BA, Tabin CJ (1995) *Hox* genes and the evolution of vertebrate axial morphology. Development 121:333–346

Cheverud JM (1996) Developmental integration and the evolution of pleiotropy. Am Zool 36:44–50

Chipman AD (2001) Developmental exaptation and evolutionary change. Evol Dev 3:299–301

Fusco G (2001) How many processes are responsible for phenotypic evolution? Evol Dev 3:279–286

Gould SJ (1977) Ontogeny and phylogeny. Harvard University Press, Cambridge

Gould SJ (1980) The evolutionary biology of constraint. Daedalus 109:39–52

Gould SJ, Vrba ES (1982) Exaptation – a missing term in the science of form. Paleobiology 8:4–15

Hirasawa T, Kuratani S (2015) Evolution of the vertebrate skeleton: morphology, embryology, and development. Zoological Lett 1:2

Hughes CL, Kaufman TC (2002) Hox genes and the evolution of the arthropod body plan. Evol Dev 4:459–499

Lanfear R, Welch JJ, Bromham L (2010) Watching the clock: studying variation in rates of molecular evolution between species. Trends Ecol Evol 25:495–503

Lavine L, Gotoh H, Brent CS, Dworkin I, Emlen DJ (2015) Exaggerated trait growth in insects. Annu Rev Entomol 60:453–472

McKinney ML, McNamara KJ (1991) Heterochrony: the evolution of ontogeny. Plenum Press, New York

Moczek AP (2005) The evolution and development of novel traits, or how beetles got their horns. Bioscience 55:937–951

Moczek AP (2008) On the origins of novelty in development and evolution. BioEssays 30:432–447

Monteiro A, Podlaha O (2009) Wings, horns, and butterfly eyespots: how do complex traits evolve? PLoS Biol 7:209–216

Petit F, Sears KE, Ahituv N (2017) Limb development: a paradigm of gene regulation. Nat Rev Genet 18:245–258

Resnik D (1995) Developmental constraints and patterns: some pertinent distinctions. J Theor Biol 173:231–240

Richardson MK, Chipman AD (2003) Developmental constraints in a comparative framework: a test case using variations in phalanx number during amniote evolution. J Exp Zool 296B:8–22
Schwenk K (1995) A utilitarian approach to evolutionary constraint. Zoology 98:251–262
Shubin N, Tabin C, Carroll S (1997) Fossils, genes and the evolution of animal limbs. Nature 388:639–648
Shubin N, Tabin C, Carroll S (2009) Deep homology and the origins of evolutionary novelty. Nature 457:818–823
Vonk FJ, Admiraal JF, Jackson K, Reshef R, de Bakker MAG, Vanderschoot K, van den Berge I, van Atten M, Burgerhout E, Beck A, Mirtschin PJ, Kochva E, Witte F, Fry BG, Woods AE, Richardson MK (2008) Evolutionary origin and development of snake fangs. Nature 454:630–633
Wagner GP (2007) The developmental genetics of homology. Nat Rev Genet 8:473–479
Whyte LL (1965) Internal factors in evolution. Tavistock, London

Concept of Burden in Evo-Devo

Diego Rasskin-Gutman and Borja Esteve-Altava

Contents

Abstract

The concept of burden was developed around the 1970s by Austrian zoologist Rupert Riedl, based on morphological insights rooted in Karl Ernst von Baer's embryological tradition. Burden's main tenet is that as a morphological character evolves, it develops more relationships with other characters, becoming more and more interconnected. Through this process, the morphological character acquires more biological "responsibilities" within the organism. Two main consequences of the burden hypothesis are that (1) a character's evolvability will be limited by these responsibilities and (2) a set of heavily burdened characters could be considered as part of the body plan of a taxonomic group. The concept of burden

D. Rasskin-Gutman (✉)
Theoretical Biology Research Group, Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Valencia, Spain
e-mail: diego.rasskin@uv.es

B. Esteve-Altava
Institut de Biologia Evolutiva (UPF-CSIC), Universitat Pompeu Fabra, Barcelona, Spain
e-mail: boresal@gmail.com

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_48

is intimately related to that of developmental constraint, and as such, it is central to evo-devo.

Keywords

Morphology · Philosophy of biology · Rupert Riedl · Developmental constraints

Introduction

The interplay between development and evolution forms the central issue of evo-devo. This apparently trivial statement harbors a very complex suite of concepts and associated problems, especially with regard to causality. As a fundamental part of the phenotype, developmental processes can be viewed as a series of intermingled characters shaped by evolutionary forces, including, but not limited to, the action of natural selection. Thus, development, as any other character, is a complex product of evolution. The converse statement – that evolution is shaped by development – has been less apparent throughout the history of biology and, to say the least, is more controversial. The problem at hand is, then, how the dynamics of development can shape (cause) evolution. This relationship is captured by a family of concepts that more or less have settled under the encompassing umbrella of "constraints," to which the concept of "burden" owes its existence. Several authors have contributed to putting forward these concepts and although it is tempting to draw a linear conceptual genealogy starting with Ernest Haeckel's biogenetic principle, Gavin de Beer's heterochrony, and Stephen J. Gould's and allied ideas on developmental constraints (including the likes of Pere Alberch, John Maynard-Smith, and several architects of the famous 1981 Dahlem Conference), the story is a bit more convoluted. Indeed, other important authors have contributed with additional concepts, such as von Baer's embryological laws; Thompsons' emphasis on the physical underpinning of growth and form; Waddington's genetic assimilation, canalization, and epigenetic landscape; Goldschmidt's "hopeful monsters"; and even Jacob's evolutionary tinkering (von Baer 1828; Haeckel 1874; Thompson 1917; de Beer 1940; Goldschmidt 1940; Waddington 1956; Gould 1977; Jacob 1977; Bonner 1982; Maynard Smith et al. 1985).

Thus, the concept of burden belongs within this solid but disparate family of related ideas which, in essence, form the nucleus of modern evo-devo. Burden appeared originally in Rupert Riedl's book, *Order in Living Organisms* (Riedl 1978), which was first published in German as *Die Ordnung des Lebendingen* in 1975. Riedl, an Austrian morphologist of the second half of the past century, devised the concept of burden with the clear goal of linking the morphological organization (Bauplan) of species with their evolvability in the broad framework of a particular idea of selection, namely that of "internal selection" (Schoch 2010). His good morphological intuition, rooted in German Idealist Morphology school, made him lean naturally on several classical nineteenth century ideas: von Baer's embryological laws, Geoffroy's principle of connections, and Cuvier's conditions of existence.

It is also worth mentioning that the concept of burden shows many parallelisms to Wimsatt's concept of entrenchment, developed independently almost at the same time (Wimsatt 1986) but which will not be discussed here (Wimsatt's own work on the issue, Wimsatt 2007). Wallace Arthur, whose idea of developmental bias is also close to the concept of constraint, is somewhat more neo-Darwinian in his account of internal factors in evolution, taking them as cases of selection: "in addition to directional selection for adaptation to particular environments, there would always be stabilizing selection for internal integration." Thus, burden, just like entrenchment, constraint, and developmental bias, would all be consequences of internal selection, whose weight would "fall disproportionally on early developmental stages" (Arthur 2015).

What Is Burden?

Since pre-evolutionary days, comparative anatomists have been noting that large groups of animals and plants share similar features; the suite of all these most characteristic features is what forms the Bauplan or "body plan". For example, the vertebral column is one of the most salient features of the vertebrate body plan, consisting of a repetitive series of bony elements, the vertebrae, that not only protect the spinal cord, but also anchor other bones and muscles, giving shape to the main anatomical arrangement of the body axis. During embryonic development, many transient features are also shared, such as the formation of a morula or a gastrula, or more specifically for vertebrates, the formation of the notochord. Thus, within the body plan, features are at some level constrained and invariable for large taxonomic groups: they are always present. The study of developmental constraints attempts to answer how these features come about and how they stay constant in diverse groups for hundreds of millions of years.

More specifically the question asked by the concept of burden is: do characters vary in their possibilities to transform during evolution? And, if so, are there any that can change more likely than others? In essence, Riedl's concept of burden represents a way of trying to explain the origination and maintenance of the body plan (Wagner and Laubichler 2004). In other words, how do groups of organisms acquire a set of characters and how do they retain them in the face of strong tendencies for change through natural selection. The question of origination cannot be answered directly, but the question of maintenance can. To answer this question, Riedl proposed that some characters are strongly constrained (so much so that they might become invariable during evolution, and hence become a part of the body plan), while others can change more freely (i.e., they are more evolvable).

According to Riedl, the source of these constraints or biological burden takes two forms: one is based on their embryological generality and another on their functional and developmental interdependencies. Raff (1996) refers to these two aspects as vertical and horizontal constraints. To him, while the former has been dispensed with by the embryological evidence (although not completely, see below), it is the latter

that gives the concept of burden such a central place in modern evo-devo. In the next section, we will examine the value of embryological generality. The subsequent section looks at the merits of biological dependencies. In the final section, we will present the notion of "burden rank" along with a metric based on anatomical networks, which allows a precise quantification of burden that can be tested in a phylogenetic context.

The Question of Embryological Generality

When embryos develop, processes follow one after another in a rather rigorous and precise way. Every morphogenetic process is followed by subsequent ones, beginning with general features such as the formation of a morula or the process of gastrulation followed by neurulation that will form typical gastrulas and neurulas. These embryological features are very general, insofar as they can be found in most triplobastic animals: any perturbation to their formation would cause severe consequences for the future organism. This was noted by Karl Ernst von Baer at the beginning of the nineteenth century, in pre-evolutionary times. His careful observations were encapsulated in several generalizations that came to be known as von Baer's laws (Gilbert 2013):

1. The more general characters of a large group of animals appear earlier in their embryos than the more special characters.
2. From the most general forms, the less general are developed, and so on, until finally the most special arise.
3. Every embryo of a given animal form, instead of passing through the other forms, becomes separate from them.
4. Fundamentally, therefore, the embryo of a higher form never resembles any other form but only its embryo.

Von Baer's laws have been revisited in the concept of phylotypic stage, which is most relevant to the discussion of burden. Indeed, embryologists have realized that, in contrast to Von Baer's laws, embryos can present a great variety of ways to go through their first stages, especially those concerning early cleavage and gastrulation. However, they all have to pass through some sort of middle stage, called the phylotypic stage, which is constrained by the multiple interactions of the early developmental processes (Slack et al. 1993). In vertebrates, the phylotypic stage corresponds to the late neurula. Following the phylotypic stage, embryos unleash variation in their morphogenetic processes that are species specific. This has been captured by the metaphor "phylogenetic hourglass" starting with early stages at which many evolutionary changes are possible and ending up with late stages where, again, many changes are possible. In the middle part, the phylotypic stages that characterize each phylum, there exist periods of highly constrained, less alterable interactions.

In light of this hourglass pattern of variation, as discussed in Raff (1996), the concept of burden shows its deficiencies. The question of the generality of characters according to their temporal embryological position is the weakest part of the concept of burden. Indeed, Raff presents counter examples showing that early entrenchment does not necessarily mean less variation. For example, the varied ways in which different early embryos start development show that variation can occur very early. This is more in line with the hourglass model, which dictates that variation will be greater both before and after the phylotypic stage. From the phylotypic stage onwards, every single downstream process becomes more and more specific for each species, both in morphogenetic process and in gene expression. Thus, the first aspect of burden can only be vindicated by the variation that occurs after the embryo has reached the phylotypic stage.

The Question of Dependencies

As we have just seen, of the two major tenets of the concept of burden, the question of dependencies is the stronger one, making burden a major research agenda for evo-devo. Indeed, biological dependencies resonate with other associated fields and concepts such as morphological integration, which was originally initiated by Olson and Miller (1958) in the context of morphometric analysis, as well as with modern ideas about the hierarchical nature of gene regulation during development (Gene Regulatory Networks or GRNs) as posited by Davidson and others (see, for example, Erwin and Davidson 2009). Both areas, morphological integration and GRNs, are very active on their own and often intermingled in evo-devo research.

Dependencies are understood as relations among parts of anatomical or functional systems that are in place during embryological development. As such, burden can be understood as "a direct measure of the organismal integration of a trait" (Schoch 2010). These dependences operate at all scales, from GNRs to articulations among bones (as we will see in the next section) and from cell-to-cell transport to hormone-regulated concerted growth of organs. In the context of burden, the more relations a part has with others, the more burden it will hold. This "burdened" part will then be constrained by the summary load of these dependences. In turn, the heavier the constraint, the less evolvable the part will be (see chapter ▶ "Evolvability"). But evolvable in which sense?

Evolvability has been defined in population genetics as the ability to generate adaptive genetic diversity that is susceptible to natural selection. This definition, as is often the case with evolutionary definitions that are suitable for population dynamics, asks a different question from the one evo-devo is interested in. In evo-devo, unlike the population genetic definition just given, the question of evolvability has to do with the ability of an anatomical element to change under any kind of evolutionary influence, most importantly, during embryogenesis. And this is where the concept of burden has a lot to say: the more dependencies to other elements, the more constrained a trait will be. In other words, the concept of burden speaks directly

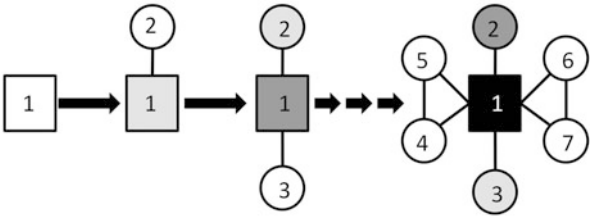


Fig. 1 Schema of the origination of a body plan through increasing burden. As evolution proceeds, character **1** develops more and more dependencies to new characters, eventually becoming so "burdened" that any changes will have big consequences. Eventually, **1** becomes part of the body plan and because of this high rank burden, it loses evolvability or capacity to change (Modified from Wagner and Laubichler 2004)

to the problem of phenotypic evolvability, Riedl's cornerstone (Fig. 1). Furthermore, there is an explicit connection between evolvability, burden, and genotype-phenotype mapping in the ways in which it constrains the possibility for the appearance of novelties since, "newly arising variation is structured by development and presented to selection in a nonrandom way." Thus, "(b)y conceptualizing the organisms in terms of patterns of variation, Riedl also created the much needed connection between organismal comparative biology and the variation based Neo-Darwinian theory of evolution" (Pavlicev and Wagner 2012).

Quantifying Burden Using Anatomical Network Analysis

The use of network theory to study anatomy has been implemented in the past decade (see Rasskin-Gutman and Esteve-Altava 2014, and the chapter on ▶ "Anatomical Network Analysis in Evo-Devo" for an overview). This new methodology, called anatomical network analysis (AnNA), explores the connectivity relations among anatomical elements. Anatomical parts, such as the skull, are analyzed by looking at all the patterns of articulations among bones. What is important in this method is the neighborhood of each bone as it connects to others by sutures and joints, thus providing a natural way to quantify burden. When bones are taken as units of developmental or evolutionary change, a direct measure of burden is the degree of connectedness of a bone, that is, the number of its connections. Other measures, such as the betweenness centrality or the clustering coefficient, could also be used as proxies for burden. For example, a typical human skull has 21 bones. Each bone has a specific pattern of connections that can be quantified using AnNA. Some bones have many connections, such as the sphenoid, the ethmoid, and the frontal, whereas most of them have few connections, such as the nasals and the lacrimals. In addition, these connectivity patterns organize into two modules, the facial and the cranial. To explain the observed relationship between the number of contacts that a bone has and the importance of this bone within the organization of

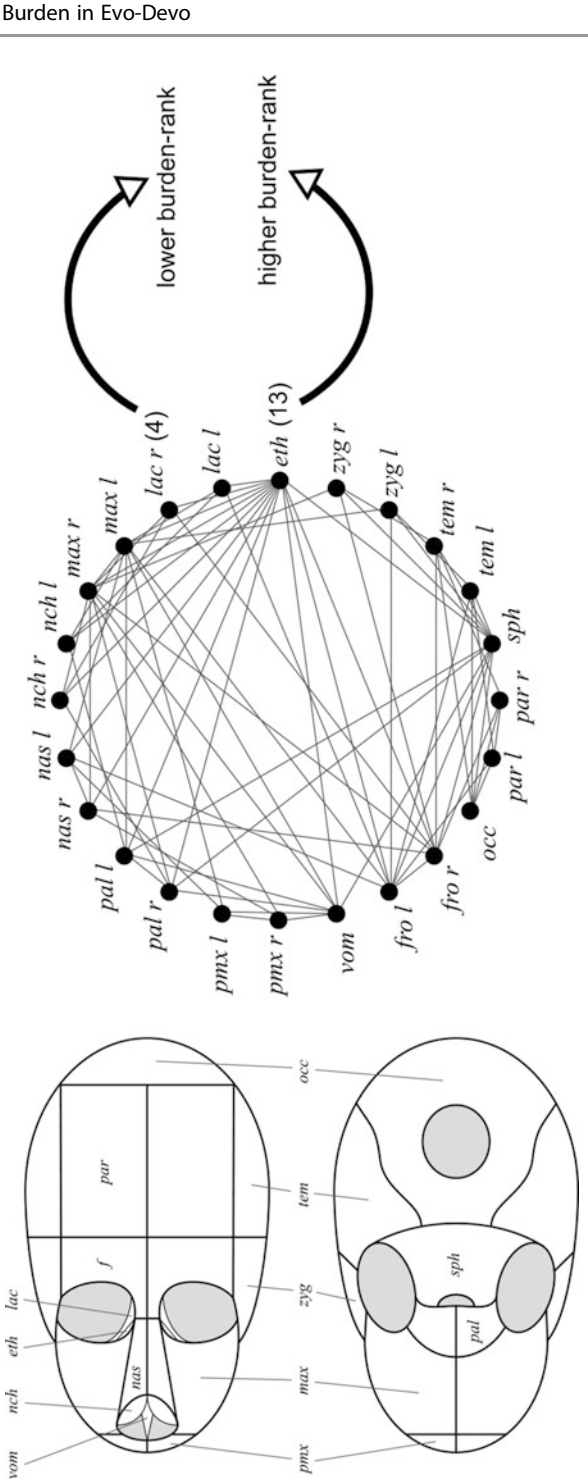


Fig. 2 Schema of the human skull and its corresponding network model highlighting the different number of connections or burden rank between the ethmoid and the lacrimal bones

the entire skull, Esteve-Altava and co-workers (2013a) proposed "the burden-rank hypothesis," (Fig. 2) based on Schoch's analysis of the concept of burden (Schoch 2010).

In light of the concept of burden, this hypothesis states that the number of contacts that a bone has increases the number of developmental and functional co-dependences constraining its variation, which in turn would favor the conservation of this bone during evolution (Rasskin-Gutman and Esteve-Altava 2014). Thus, the more interconnected a bone is, the less likely it is to change over evolutionary time. Moreover, many bones have fused during the evolutionary history of mammals; thus, fusion can be seen as the ultimate fate of heavily burdened bones. In fact, fused bones could lead to an entirely new skull architecture, which could be said to have emerged from the "forces" exerted by the burden of the individual bones of the ancestor.

The burden-rank hypothesis predicts that modules with fewer interactions (i.e., contacts) will be more evolvable than modules with a greater number of interactions. In the context of AnNA, the fewer the number of contacts in a module, the greater the module's capacity to exhibit phenotypic variation and, therefore, to evolve. A prediction from this study of the human skull is that the facial module should show more morphological evolvability than the cranial one, because the facial skull has fewer contacts among its bones than the neurocranium. Although classical morphometrics studies demonstrated that adjacency is a key factor in shape correlations, and more recent approaches vindicate the inclusion of topological considerations in shape analyses, translating these predictions of evolvability at the connectivity level to predictions of variability in the shape and size of the skull is a major challenge.

The burden-rank hypothesis explains the difference in evolutionary conservation between the facial and the cranial phenotypic modules as a consequence of differences in their complexity. By virtue of the greater richness of bone-to-bone interactions, more complex modules entail more developmental and functional codependences, which constrain module variation. In this context, the lower complexity and greater anisomerism of the facial module suggests specialization due to anatomical differentiation of bones in terms of number of contacts. Morphometrics studies in primates support the finding that regions with lower disparity of the cranial module are less evolutionarily plastic than parts of the facial region (Goswami and Polly 2010).

Criticisms

Riedl's theory of burden is far from being a perfect account of the behavior of anatomical traits in a neo-Darwinian framework. However, it provides a solid departure point to bring back the importance of development to understanding how evolution shapes multicellular organisms. Thus, as so often happens when new theories are put forward, several criticisms have been raised against the concept

of burden by different authors. We will just mention three of them, by Raff (1996), Schoch (2010), and Budd (2006):

Raff has questioned the validity of the theory of burden regarding the hierarchy of developmental processes (Raff 1996), a question that has been solved by the hourglass model of development. Only if we examine development from the phylotypic stage onwards, rather than from the beginning of embryo development, the process seems to conform to the notion that early morphogenetic events are less variable than later ones.

Budd has pointed out that, as it has been formulated, the concept of burden would entail that characters get more and more burdened to the point of irreversibility, which might lead to dead ends (Budd 2006). However, as we have noted in the precedent section, intimately connected elements that might end up fusing into one new element, as has happened repeatedly in the evolution of the skull, can overcome such irreversibility by changing the whole dynamics of the system (Esteve-Altava et al. 2013b and references therein).

Schoch has noted that developmental processes are full of pleiotropic effects, as well as nestedness among them, making the gradual and linear acquisition of burden for single characters unlikely (see chapter ▶ "Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics"). Also, Schoch has criticized the use of burden ranking on the grounds of characters not being clearly linked causally, because the dynamics of morphogenesis is not linear but consists of a complex network of cause-effect relationships that involve genetic-epigenetic regulation of morphogenetic mechanisms (Schoch 2010). These two criticisms, the pleiotropy effect and the causal linkage, are the most compelling ones against the concept of burden as it was originally formulated.

Conclusions

The most interesting evolutionary aspect of burden is its dynamic properties: as characters change their dependencies to other characters (sometimes new ones), they increase their resilience towards change since more is at stake or, in Riedl's words, their "responsibility" towards the developing embryo is greater. Thus, characters are not fixed entities subject to change by natural selection. Rather, they are constrained by their burden, so that their evolvability would also be compromised. We have seen that the concept of burden relies on two types of mechanisms: the hierarchical importance of the time of appearance of a character and the biological dependencies. Of these two, the latter seems to resonate more strongly in modern evo-devo.

Beyond the mentioned criticisms, the concept of burden is central to evo-devo because it is a statement about organization and dynamics explained as the morphogenesis constraining the organization of the body plan. Conversely, we might also say that the concept of burden explains how the organization of the body plan

constrains the dynamics of morphogenesis, and thus the possible innovations that might arise.

Cross-References

- ▶ Anatomical Network Analysis in Evo-Devo
- ▶ Evolvability
- ▶ Mechanisms of Pattern Formation, Morphogenesis, and Evolution
- ▶ The Developmental Hourglass in the Evolution of Embryogenesis
- ▶ Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics

Acknowledgments DRG funded by MINECO-FEDER BFU2015-70927-R. BE-A is funded by a European Union's Horizon 2020 Marie Skłodowska-Curie grant No. 654155.

References

Arthur W (2015) Internal factors in evolution: the morphogenetic tree, developmental bias, and some thoughts on the conceptual structure of evo-devo. In: Love A (ed) Conceptual change in biology, Boston studies in the philosophy and history of science, vol 307. Springer, Dordrecht

Bonner JT (ed) (1982) Evolution and development. Report of the Dahlem workshop on evolution and development Berlin 1981, May 10–15. Springer, Berlin

Budd GE (2006) On the origin and evolution of major morphological characters. Biol Rev 81:609–628

de Beer GR (1940) Embryos and ancestors. Clarendon Press, Oxford

Erwin DH, Davidson EH (2009) The evolution of hierarchical gene regulatory networks. Nat Rev Genet 10:141–148

Esteve-Altava B, Botella H, Marugán-Lobón J, Rasskin-Gutman D (2013a) Structural constraints in the evolution of the skull: Williston's law revisited. Evol Biol 40:209–219

Esteve-Altava B, Marugán-Lobón J, Botella H, Bastir M, Rasskin-Gutman D (2013b) Grist for Riedl's mill: a network model perspective on the integration and modularity of the human skull. JEZ Part B Mol Dev Evol 320(8):489–500

Gilbert S (2013) Developmental biology, 10th edn. Sinauer Associates, Inc., Sunderland

Goldschmidt R (1940) The material basis of evolution. Yale University Press, New Haven

Goswami A, Polly PD (2010) The influence of modularity on cranial morphological disparity in Carnivora and Primates (Mammalia). PLoS One 5:e9517

Gould SJ (1977) Ontogeny and phylogeny. Harvard University Press, Cambridge, MA

Haeckel E (1874) Anthropogenie oder Entwickelungsgeschichte des Menschen. Englemann, Leipzig

Jacob E (1977) Evolution and tinkering. Science 196(4295):1161–1166

Maynard Smith J, Burian R, Kauffman S, Alberch P, Campbell J, Goodwin B, Lande R, Raup D, Wolpert L (1985) Developmental constraints and evolution: a perspective from the Mountain Lake conference on development and evolution. Q Rev Biol 60(3):265–287

Olson EC, Miller RL (1958) Morphological integration. The University of Chicago Press, Chicago

Pavlicev M, Wagner GP (2012) Coming to grips with evolvability. Evo Edu Outreach 5:231–244

Raff RA (1996) The shape of life: genes, development, and the evolution of animal form. University of Chicago Press, Chicago

Rasskin-Gutman D, Esteve-Altava B (2014) Connecting the dots: anatomical network analysis in morphological EvoDevo. Biol Theory 9:178–193

Riedl R (1978) Order in living organisms: a systems analysis of evolution. Wiley, New York
Schoch RR (2010) Riedl's burden and the body plan: selection, constraint, and deep time. J Exp Zool Mol Dev Evol 314B:1–10
Slack JMW, Holland PW, Graham CF (1993) The zootype and the phylotypic stage. Nature 361:490–492
Thompson DW (1917) On growth and form. Cambridge University Press, Cambridge, UK
von Baer KE (1828) Entwicklungsgeschichte der Thiere: Beobachtung und Reflexion. Königsberg, Bornträger
Waddington CH (1956) Genetic assimilation. Adv Genet 10:257–290
Wagner GP, Laubichler MD (2004) Rupert Riedl and the re-synthesis of evolutionary and developmental biology: body plans and evolvability. J Exp Zool Mol Dev Evol 302B:92–102
Wimsatt WC (1986) Developmental constraints, generative entrenchment and the innate acquired distinction. In: Bechtel W (ed) Integrating scientific disciplines. Martinus Nijhoff, Dordrecht, pp 185–208
Wimsatt WC (2007) Echoes of Haeckel? Reentrenching development in evolution. In: Laubichler MD, Maienschein J (eds) From embryology to evo-devo: a history of developmental evolution. MIT Press, Cambridge, pp 309–355

A Macroevolutionary Perspective on Developmental Constraints in Animals

Frietson Galis and Johan A. J. Metz

Contents

F. Galis (✉)
Naturalis Biodiversity Center, Leiden, The Netherlands
e-mail: frietson.galis@naturalis.nl

J. A. J. Metz
Naturalis Biodiversity Center, Leiden, The Netherlands

International Institute for Applied Systems Analysis, Laxenburg, Austria

Mathematical Institute, University of Leiden, Leiden, The Netherlands
e-mail: j.a.j.metz@biology.leidenuniv.nl

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_69

Abstract

We review the importance of developmental mechanisms in animals in constraining evolutionary changes. We first discuss the importance of time scales at which such constraints are relevant and after that focus on near absolute constraints that act on macroevolutionary scales. We could find only a few well-underpinned examples of such near absolute constraints. We discuss three outstanding cases, the ancient metazoan constraint that differentiated cells cannot divide, constraints against changes of phylotypic stages in vertebrates and other higher taxa, and constraints against the evolution of parthenogenesis. These constraints all have major consequences, including many secondary constraints, and they have in common that they are caused by high levels of global developmental interactivity.

The global developmental interactivity almost inevitably causes mutations to have many harmful pleiotropic effects, and thus will be strongly selected against, leading to long-term evolutionary conservation. The discussed developmental constraints have major consequences for evolution and critically restrict regeneration capacity, life-history evolution, and body plan evolution.

Keywords

Body plan evolution · Evolutionary constraint · Parthenogenesis · Phylotypic stages · Early organogenesis · Regeneration capacity · Cilia · Centrosome · Mitosis · Meiosis

Introduction

We speak of developmental constraints when there is a bias on the production of variant phenotypes or a limitation on phenotypic variability caused by developmental mechanisms (Maynard Smith et al. 1985). Earlier more intuitive mechanistically oriented arguments were put forward by among others Gould and Lewontin (1979), who argued that developmental constraints must be important, based on the apparent conservation of early developmental stages, the required integration of these stages, and the accumulating effects of early errors. Even earlier, Whyte (1964) argued that these constraints relate to internal selection, i.e., the necessity for the machinery of the body, in particular development, to be well-concerted.

The importance of developmental constraints in evolution is still subject to controversy: In which ways and to what extent do developmental mechanisms restrict the range of possible phenotypes? The answer depends largely on the time scale at which the constraints are supposed to act. In evolutionary biology it pays to make at least the following gross distinctions: microevolution (changes in gene frequencies on a population dynamical time scale), meso-evolution (the evolution of quantitative traits through repeated mutation substitutions), and macroevolution (large-scale changes, such as innovations). Quantitative genetics and adaptive dynamics provide the main frameworks for dealing with trait evolution on the

micro- and meso-evolutionary scales, respectively, while macroevolutionary discourse is dominated by arguments from functional morphology and evo-devo. We will first discuss why genetic constraints are expected not to lead to constraints acting at macroevolutionary scales and follow-up with a discussion of three well-documented examples of near absolute macroevolutionary developmental constraints, their causes, and their vast evolutionary impact.

Genetic Constraints Do Not Constrain Evolution on Macroevolutionary Scales

In the past, discussions on developmental constraints, in particular among geneticists, have often focused on so-called genetic constraints, especially the potential of genetic covariation to steer evolution (e.g., Conner 2012). Note that genetic covariation is to a large extent caused by the genotype-phenotype map, i.e., by developmental mechanisms. In quantitative genetics, the term genetic constraint is used for the differential responsiveness to selection in the directions of the principal components of the genetic covariance matrix in proportion to their size. Principle components correspond to the direction in trait space supporting the largest variation, the direction orthogonal to that first direction supporting the largest variation, etc. In particular, any zero principal component corresponds to an absolute, i.e., dictionary style, constraint. Although little is known yet about the prevalence of such zero principal components, the general tendency in high-dimensional biological data is that principal components peter out roughly exponentially, suggesting that absolute genetic constraints will be extremely rare, except when directly caused by a physical conservation law. This unlikeliness becomes even greater since the effects of new mutations on phenotypes, as captured by their mutational covariance matrices, and thus their principal components, generally change with progressive evolution. Note that both types of covariances are but phenomenological representations of the phenotypic effects of mutational possibilities combined with development and in the case of the genetic covariances also linkage disequilibrium.

For smooth genotype to phenotype maps the effect of small changes in gene expression is bound to be locally additive. In that case, we can treat the microevolutionary process as governed by additive genetics, leading to a seamless transition from the arguments about microevolution, in terms of shifts in standing variation to those about meso-evolution based on mutant substitutions. However, the genotype-to-phenotype map is invariably nonlinear in the large. Moreover, phenotypic change on that scale necessarily influences the fitness landscape through its effect on the ecology. Adaptive dynamics (e.g., Metz (2012)) focuses on the effects of the latter changes.

Meso-evolution presumably is largely driven by mutations of small effect. This expectation has both a mechanistic and a Darwinian reason. Most trait evolution appears to be regulatory. Most mutational changes in the regulatory mechanisms may be expected to result in small changes in the quantities of the relevant proteins at different points and times in the body and thus to small changes in development, physiology, and behavior. In addition, mutations with large effect tend to bring

an otherwise harmoniously operating system into disarray and will therefore contribute little to meso-evolutionary change. The ecology-mediated changes in invasion fitnesses that drive meso-evolution tend to be minor relative to the fitness effects deriving from the need for a well-concerted organismal development and functioning. The latter effects presumably also underlie most macroevolutionary regularities. However, on macroevolutionary scales, trait spaces themselves change through the breaking of hard meso-evolutionary constraints, permitting innovations (e.g., Peterson and Müller 2016). Together the above considerations support the metaphor of meso-evolution as a smooth uphill movement along the crests of a high-dimensional fitness landscape. Selection pushes in the steepest direction with the realized uphill movement determined by the interplay between this push and the current mutational covariances. The relatively featureless landscape on top of the crests continually changes thanks to the ecologically mediated feedback from traits to fitnesses. The gross landscape structure, on the other hand, stays roughly constant as it is dominated by internal selection, i.e., the need for organisms to stay well-concerted. In contrast, macroevolution is guided by large-scale landscape features with key innovations providing wormholes to higher dimensions. This allows little chance for the directional effects of the mutational covariances to leave a visible trace. Thus, genetic covariation undoubtedly steers evolution on micro- and meso-evolutionary scales yet is unlikely to constrain on macroevolutionary scales, except for the effects of rampant pleiotropy discussed below.

Macroevolutionary Constraints

Absolute developmental constraints on the evolution of specific adaptive phenotypes are extremely rare, and Vermeij (2015) even argues that they are absent, given sufficient time. Yet, we argue that there exists one exceptional category of near absolute developmental constraints: when development is highly interactive, the many cascading pleiotropic effects caused by development (relational pleiotropy, sensu Hadorn (1961)) result in high-dimensional variation that, combined with stabilizing selection in most directions, will strongly constrain evolution (Galis et al. 2018). As a result, developmental changes that are initiated in highly interactive developmental stages tend to be constrained even on macroevolutionary scales. We shall discuss the three best established examples of such near absolute developmental constraints in animals: the metazoan constraint that differentiated cells cannot divide, the constraints against changes of phylotypic stages in vertebrates and other higher taxa, and constraints against the evolution of parthenogenesis.

Metazoan Cells Cannot Divide While Differentiated

In 1898, Henneguy and Lenhossek independently proposed a universal developmental constraint for metazoans: ciliated cells cannot divide (Henneguy 1898; Lenhossék 1898). They had observed that the basal bodies of cilia were transformed

centrosomes and had never observed ciliated cells divide. They proposed that when a centrosome becomes involved in cilium formation, it cannot form a spindle for cell division. The centrosome, in its entirety with two centrioles, forms a spindle pole. It is duplicated shortly before cell division, and each centrosome forms one of the two poles of the spindle that segregate the duplicated chromosomes. When a cilium is formed, one of the two centrioles of the single centrosome converts to a basal body and migrates to the cell surface, anchors to the cell membrane, and organizes the assembly of the cilium that protrudes from the cell membrane. Hence, the proposed incompatibility of functioning of the centrosome in cilium formation and mitosis, a hypothesis that for metazoans thus far remains uncontested.

Buss (1987) extended the hypothesis from ciliated cells to all differentiated cells of metazoans. In his thought-provoking book, *The Evolution of Individuality*, he proposed that cell division by mitosis and differentiation are mutually exclusive. He assumed that the cilium is usually involved in the differentiation process of a cell and, additionally, that the centrosome is the only microtubule-organizing center (MTOC) in a cell and that any commitment of the single MTOC to a cilium or to another microtubule-based structure would preclude commitment to the poles of the mitotic spindle, thus inhibiting mitosis. Buss further assumed that there cannot be more than one centrosome in a cell and that this is a phylogenetic constraint inherited from unicellular protist ancestors, whereas other unicellular taxa, such as Euglenophytes, Cryptophytes, and Chlorophytes possess multiple MTOCs, and therefore can simultaneously achieve cell movement with cilia or flagella and cell division. Bell (1989) challenged the phylogenetic constraint hypothesis, arguing that the duplication of centrosomes in cells shortly before mitosis suggested that there cannot exist a constraint preventing the production of more than one centrosome. Indeed, it is now known that, exceptionally, extra centrosomes are formed in cells (e.g., Gönczy 2015). The explosively expanding knowledge of cellular processes has revealed more challenges to Buss's hypothesis, e.g., experimental removal of centrosomes has indicated that they are not essential for the formation of radial spindles and mitotic cell division (Wu and Akhmanova 2017). However, as we shall discuss, new knowledge indicates that in metazoans it is important that normally there is precisely one centrosome in a cell and primary cilia perform crucial functions in virtually all cells, which again explains that ciliated and differentiated cells cannot divide in metazoans.

No or More than One Centrosome in a Cell

Centrosomes are not absolutely required for mitotic spindle formation and division in many cells, but mitosis in the absence of centrosomes is slower, which increases the risk of chromosomes lagging during the separation and thus causing aneuploidy. Centrosomes thus are necessary for rapid, robust, and error-free separation of the chromosomes during mitosis (Gönczy 2015; Wu and Akhmanova 2017). Very rarely in a cell extra centrosomes are generated de novo. The presence of multiple centrosomes poses grave risks as it can lead to the formation of multiple spindle poles,

aneuploidy, genomic instability, abnormal cell migration, cell cycle arrest, and cancer (e.g., Gönczy 2015). This is not surprising given the many key functions of centrosomes in cell cycle control and cell differentiation. They play an important role, among others, in establishing cell fate determination, cell polarity, transmission of polarity to daughter cells, the positioning of cell organelles, de-epithelialization, DNA damage repair, adhesion, migration of cells, and functioning as signaling hubs (Wu and Akhmanova 2017).

More than One MTOC in a Cell

In addition to the centrosome, there are other cell organelles that organize microtubule, like the Golgi apparatus, the nuclear envelope, the cell cortex, and pre-existing microtubules. During differentiation, the microtubule-organizing capacity of the centrosome is partially or fully transferred to such non-centrosomal MTOCs (e.g., Wu and Akhmanova 2017). The division of tasks between the centrosomal and non-centrosomal MTOCs appears to be tightly regulated during cell cycle progression and differentiation, probably in a competitive way, which presumably limits the possibility of centrosomes in differentiated cells to function as spindle poles.

Importance of Primary Cilia

Primary cilia were long thought to be vestigial organelles, which if true, would complicate Buss' argument that the presence of primary cilia constrains the functioning of centrosomes as mitotic spindles. However, in the last few decades, it was first shown that they function as antennae on almost all metazoan cells and as such play a crucial role in intercellular signaling (e.g., *Shh* signaling in vertebrates, Walz 2017). Cilium signaling is involved in the organization of most, if not all, developmental processes, including left-right patterning, cell migration, proliferation, cell size and shape, apoptosis, and cell fate decisions. The many diseases caused by malfunctioning cilia, so-called ciliopathies, emphasize the primordial role of cilia in development and tissue homeostasis (among many others, diabetes, polycystic kidney disease, and retinal degeneration (Walz 2017)). As virtually all differentiated cells appear to have a primary cilium, this essentially equates the hypothesis of Henneguy and Lenhossek to the one of Buss that there is a constraint on mitotic divisions of differentiated cells.

Cilia and Centrosomes Regulate Cell Cycle Progression

At the exit of mitosis, cells typically form a primary cilium, unless they continue proliferating, when ciliogenesis appears to be actively suppressed (Walz 2017). In all other cases, also in quiescent stem cells, the mother centriole converts to a basal body and assembles the primary cilium. Upon cell cycle reentry, ciliary resorption

begins, the basal body is detached from the cell surface, and the centrosome migrates to near the nucleus. Recent studies have shown that the cell cycle is not so much regulating centrosome and cilium dynamics, but instead, the dynamics of the centrosome and primary cilium actively regulate cell cycle progression and arrest or exit followed by differentiation (Walz 2017). For instance, the physical presence of the primary cilium appears to block cell division, while primary ciliary resorption is thought to unblock it and the length of the cilium influences cell cycle duration, which in turn influences cell fate decisions.

Pleiotropic and Developmental Constraint

The centrosome and primary cilium play a key role in the complex organization of almost all cellular processes in multicellular metazoans. Abnormal numbers of centrosomes and primary cilia disrupt the highly controlled interactivity during mitosis and cell cycle progression. A further contribution to the complexity comes from the competitive interactions between the centrosome and non-centrosomal sites that organize microtubuli. As a result, mutations affecting centrosomal and ciliary functions will have a multitude of deleterious pleiotropic effects that will be strongly selected against, such as apoptosis, genomic instability, aneuploidy, cell cycle progression, and cancer (e.g., Gönczy 2015; Walz 2017). Hence, there is a strong constraint against changes in the number of centrosomes (and primary cilia). As the interactivity is part of the intracellular development, the constraint should be considered developmental, as opposed to genetic. The constraint causes several other fundamental developmental constraints, of which the one that differentiated cells cannot divide is the hardest, i.e., impacting the conservation of phylotypic stages and, thereby, the evolution of body plans.

Evolutionary and Developmental Consequences of the Constraint

Low Fidelity of Meiotic Divisions of Oocytes

Meiotic divisions of animal oocytes occur without centrioles. The centrioles degenerate beforehand, presumably to avoid the problematic presence of a second centrosome in the zygote. The centriole(s) in the zygote are contributed by the sperm. It is thought that the centrioles of the egg cells rather than of the sperm cells degenerate, because of the necessity for the motile sperm cell to have a centriole organizing its flagellum (Manandhar et al. 2005). The absence of a centrosome during the meiotic divisions of the oocyte is associated with a cost of lower fidelity of the divisions, with increased rates of aneuploidy and genetic instability, presumably playing a role in the high rates of miscarriages in humans (Manandhar et al. 2005).

In an unfertilized ovum, the paternal contribution of centrioles is missing, and this forms a constraint against parthenogenesis, as a centrosome is generally necessary to initiate mitotic divisions (e.g., Eisman and Kaufman 2007; see “Constraints against the evolution of parthenogenesis”).

Limited Capacity for Wound Healing and Regeneration

Wound healing and regeneration would presumably be much more effective if all differentiated cells could divide to replace damaged cells of the same type. Regeneration now typically proceeds from a blastema of undifferentiated cells that are either dedifferentiated cells or already locally present tissue-specific progenitor cells (Tanaka and Reddien 2011). Subsequently, complex interactions are required, often similar to those that occurred during development of the part to be regenerated. This requirement of developmental interactions after dedifferentiation seriously limits the possibility of regeneration in more complex metazoans. This is problematic in particular when interactions with transient organs of early embryogenesis are involved, such as the somites and neural tube in vertebrates (Galis et al. 2018).

Constraints on Development and the Evolution of Body Plans

The conflict between differentiation and mitosis has, without doubt, crucially shaped the development and evolution of metazoan body plans. The body plan is mostly defined during embryonic development, when there are still zones that produce pluripotent stem cell colonies that subsequently migrate to other places in the embryo, to initiate their paths of differentiation. The absence of pluripotent cells later in life restricts the building of organ primordia to an early embryonic stage, generally known as the phylotypic stage. This stage, which includes the production of organ primordia, is highly conserved (see the section "Conservation of Phylotypic Stages"). Adults generally lack pluripotent stem cells and only have multipotent, tissue-specific, stem cells that function in cell renewal, wound healing, and regeneration (Tanaka and Reddien 2011). In contrast, plants, that do not have this incompatibility of cell division and differentiation, can generate complete organs throughout their life (Heidstra and Sabatini 2014). Even cells outside the stem cell niches are able to return to a proliferative pluripotent state, whereas in animals the capacity for pluripotency is limited to embryonic stem cells. The largest post-embryonic flexibility in animals is provided by changes in the number of segments or the vegetative production of modules that are morphological repeats of the body plan (e.g., cnidarians and bryozoans).

Conservation of Phylotypic Stages

The abovementioned early specification of most organ primordia in animals leads to further constraints on the evolution of body plans due to the intense global interactivity in the embryo during the phylotypic stage. Embryologists have long noticed that the early organogenesis stage is less variable morphologically than both earlier and later stages. Recently, a large number of studies have shown that during that period there is also strong conservation of gene activity and of epigenetic mechanisms (e.g., Cridge et al. 2016; Hu et al. 2017).

Support for the Pleiotropy Hypothesis

Sander (1983) was the first to propose the interactivity of early organogenesis in the embryo as the root cause of the conservation of the phylotypic stage. His implicit hypothesis is that strong global interactivity causes disturbances to

cascade into deleterious pleiotropic effects in other parts of the embryo that become amplified as development proceeds. As a result, mutants with a change during such a highly interactive stage will be strongly selected against (Galis and Metz 2001; Galis et al. 2018). We have called this suite of ideas the pleiotropy hypothesis.

Teratological data on rodents strongly support the pleiotropy hypothesis: disturbances of early organogenesis lead to many deleterious pleiotropic effects, and mortality is considerably higher than during earlier or later stages (Galis and Metz 2001). The interdependent pattern of abnormalities shows that the vulnerability of the stage is not due to one specifically vulnerable process (e.g., neural tube closure) but to the high interactivity of the stage. This implies that a particular, potentially useful, change of this stage, e.g., the induction of a change in the number of cervical vertebrae, kidneys, digits, or even arms, almost always will induce other abnormalities and lethality even before the organism is exposed to ecological selection. Indeed, in humans ca. 90% of individuals with polydactyly or a changed number of cervical vertebrae are dead at birth, while these changes are generally associated with a multitude of deleterious pleiotropic effects (Galis et al. 2018). As organ primordia typically originate during the phylotypic stage, this implies that the conservation of the stage leads to conservation of the number *and* earliest development of most organs (e.g., lungs, kidneys, limbs, long bones, eyes, ears), which thus can be viewed as due to secondary constraints. Further support for the pleiotropy hypothesis comes from transcriptomics studies on vertebrates and insects that show that during phylotypic stages, there is not only stronger conservation of gene expression than during earlier and later stages but also that in particular regulatory genes and genes with pleiotropic activity in other parts of the embryo are involved (e.g., Hu et al. 2017).

Why So Much Pleiotropy?

During the phylotypic stage, the trunk can be considered to be one large developmental field. The global interactivity and consequent low effective modularity are probably to an important extent due to the interactivity of the patterning of the three body axes and the interactivity of axial patterning with the other simultaneously occurring morphogenetic processes that are simultaneously occurring in the trunk (e.g., Diez del Corral et al. 2003; Galis et al. 2018). In vertebrates, for instance, the opposing and antagonistic gradients of Fgf/Wnt and retinoic acid (RA) in interaction with the segmentation clock play a major role in their coordinated organization. Not only genetic interactions are important: often the crucial importance of self-organizing chemical and physical interactions steering these highly dynamic processes is overlooked, including extensive migration, epithelialization, de-epithelialization, cell division, and cell shape changes (see chapters ► "Mechanisms of Pattern Formation, Morphogenesis, and Evolution" and ► "Inherency"). A large study on deceased human fetuses and infants provides strong support for the coupling of axial patterning and morphogenetic processes as a cause of the vulnerability of the stage, indicating among others a particularly strong coupling between segmentation (somitogenesis) and A-P patterning of the vertebral column (Galis et al. 2018).

During the early phylotypic stage, there are only a few large developmental fields. Other than the trunk field, there is the large neural crest-related cardio-craniofacial field and the heart primordium, while at the end, the tail bud increases in importance, and more organ primordia appear. The scarcity and large size of the developmental fields and the intense signaling within, but also between them, can explain a major part of the pleiotropy and low effective modularity characterizing the stage. This holds in particular for the earliest, most strongly conserved part of the stage.

Modularity and Evolvability of Later Stages

As development proceeds, it becomes more compartmentalized, with the appearance of progressively more and more signaling centers, organizing more and more localized developmental fields. Concurrently, the expression patterns of key signaling molecules become more restricted. For example, the signaling in the neural crest-related cardio-craniofacial field and in somites becomes increasingly compartmentalized (Galis et al. 2018). Within these smaller developmental fields, intense signaling between tissues continues to occur, but the interactivity within modules is more intense than the signaling between modules. The higher effective modularity probably underlies the reduced pleiotropy and increased developmental stability of the later developmental stages. This decreased pleiotropy allows for greater evolvability and more evolutionary divergence.

Challenges to the Pleiotropy Hypothesis

A challenge to the pleiotropy hypothesis is that, notwithstanding the vulnerability of early cleavage processes to radiation and toxicants (Jacquet 2004), evolutionary changes in the earliest developmental stages are not uncommon. However, in contrast to early organogenesis, the vulnerability is that of a single process, cell division. As the dividing cells are highly similar and capable of self-renewal, either too many cells are killed and the embryo dies or the damage is reversible and development resumes. As a result, nonlethal mutants with a changed cleavage have a chance to get established. In addition, mutations have a larger chance to be successful, since simple patterns have a lesser chance to be fatally disrupted than complicated ones (Galis et al. 2018). The greater simplicity and associated robustness of early forms may thus be expected to allow greater diversification.

Another challenge to the pleiotropy hypothesis is that cleavage and gastrulation are sometimes remarkably similar, even more so than the phylotypic stages within metazoan phyla and classes. However, this similarity is largely inevitable, given the complete reset of development at the initial single-celled stage (Galis and Sinervo 2002). Only a limited number of permutations is possible when there are only a few undifferentiated cells present, due to the conflict between cell division and differentiation limiting possible developmental pathways. Convergent nutritional and locomotory adaptations cause further similarity, as well as maternal efforts to influence early development (Buss 1987). Gastrulation processes are more diverse than cleavage and, importantly, are far more diverse than their end product, phylotypic stages. Gastrulation almost always results in three germ layers, and the organ systems originating from these germ layers are highly conserved. Furthermore, a fundamental outcome of gastrulation is that sheets

of cells come into contact in a precise way, allowing the conserved embryonic inductions that are required for the organization of the body plan during the phylotypic stage. These inductions between adjacent cell populations appear to form a strict spatiotemporal constraint on the outcome of gastrulation, the starting point of the conserved phylotypic stage (Galis and Sinervo 2002).

Consequent Constraints on Body Plan Evolution

Most organ primordia originate during the phylotypic stage, and, as mentioned above, the strong conservation of this stage implies strong conservation of the number and early development of organs. Mutations for duplications of organs occur, but they co-occur with many often fatal pleiotropic effects (Galis et al. 2018). Similarly, the evolutionary loss of organs is constrained, as early developmental interactions cannot easily be done away with. For this reason, loss of organs typically occurs via the slow evolution of earlier and earlier developmental arrest, followed by degeneration. A good example is the many times that cave fishes and salamanders have evolved blindness: the lens always develops and then starts to degenerate. As a result of the slow accumulation of mutations, re-evolution of lost complex organs is virtually impossible, in agreement with Dollo's law. In contrast, when organ primordia appear during more compartmentalized later developmental stages, organ numbers are considerably more evolvable. Good examples are the number of segments in insects, phalanges and carpal and tarsal elements in tetrapods, nipples in mammals, and teeth in vertebrates. The variability of the number of cervical vertebrae in long-necked nonmammalian amniotes is a case in point. The neck-trunk boundary is determined late in these cases, and, in agreement with this, the more vertebrae in a neck, the more variable the number is (swans have 22–25). In contrast, not only in mammals, but in all amniotes with necks of eight or fewer vertebrae, the number is conserved, e.g., crocodiles, turtles, geckos, and many other limbed lizards. A further arthropod example is the number of segments of centipedes, which is variable, but always odd (Arthur and Farrow 1999). This constraint on even numbers appears to be caused by the conserved oscillatory pattern which generates two segments per cycle; hence variation is generated by the number of cycles, with the oscillatory mechanism set up during the phylotypic stage (see chapter ▶ "The Evolution and Development of Segmented Body Plans").

Breaking of Constraints: Relaxed Stabilizing Selection

Relaxed stabilizing selection occasionally allows the breaking of constraints. Such a relaxation can result from environmental changes like the opening up of new food niches or the disappearance of competitors and predators. Such changes are often associated with the start of adaptive radiations and the emergence of key innovations. Arguably relaxed selection allows novelties to persist for some time, permitting selection against some of the most deleterious pleiotropic effects, such that when stabilizing selection subsequently increases, the chance for persistence of the novelties is increased. Domestic dogs show a useful parallel, as human care allows dogs with extra digits to persist and only selection against some of the more deleterious pleiotropic effects (congenital abnormalities) occurs.

Internal factors can also cause relaxed selection, with slow behavior and low metabolic rates as good examples. Sloths and manatees are exceptional mammals with a changed number of cervical vertebrae. The breaking of the strong constraint on the number of cervical vertebrae in mammals appears to be due to a large tolerance for associated pleiotropic effects, due to their extremely slow activity rates. Adult sloths and manatees frequently have skeletal abnormalities that in deceased human fetuses and infants are commonly associated with cervical ribs (e.g., fused cervical vertebrae and serious ossification defects), and this apparently poses no major problem. Furthermore, in manatees and sloths, another pleiotropic effect of cervical ribs (documented for humans), embryonal tumors, may be less problematic due to the extremely low metabolic rates and presumably low cancer rates (c.f. Galis et al. 2018). Whales and dolphins are also exceptional in frequently having ribs on the seventh vertebra. This is probably similarly due to relaxed selection against skeletal abnormalities, caused by the supporting effect of water. Whales and dolphins also have low cancer rates. Thus, the difficulty of breaking specific constraints varies among taxa, due to differences in the experienced selection regimes and to differences in the specific pleiotropic effects associated with traits.

Developmental Constraints Against the Evolution of Parthenogenesis

In parthenogenesis, development generally starts with an unfertilized ovum and, thus, without contribution from a father. The most important missing paternal contributions are the centrosome and chromosomes. As mentioned above, the centrosome is presumably missing in the ovum as a consequence of the universal metazoan constraint against having more than one centrosome in a cell. If both the ovum and the sperm cell would contribute a centrosome, the zygote would end up with two centrosomes. The paternal chromosomes normally restore the ploidy of the ovum after meiosis, and as the development of haploid ova almost always fails in diploid animals, this forms another strong developmental constraint against the evolution of parthenogenesis. Although obligatory parthenogenesis has the disadvantage of missing genetic recombination, facultative parthenogenesis should be the most advantageous reproductive type, as females can choose between the production of asexual and sexual offspring and, thus, can combine the advantages of both modes of reproduction.

Replacing the Missing Paternal Centrosome

As mentioned earlier, centrosomes are required for rapid, error-free cell divisions. In sporadic parthenogenesis, centrosomes are assembled de novo. However, this occurs in an inefficient way, and the centrosomes are often malfunctioning, diminishing the success of ploidy restoration and mitosis (Eisman and Kaufman

2007). This is probably one of the reasons for the generally extremely low reproductive success of sporadic parthenogenesis and emphasizes the importance of the developmental constraint (Eisman and Kaufman 2007). In regularly parthenogenetic animals, the lack of the paternal centrosome is usually remedied by an efficient de novo production of a centrosome in the ovum, for which a variety of mechanisms has evolved in different taxa (Schön et al. 2009). In stick insects of the genus *Bacillus*, the centrosome is always assembled de novo in the fertilized ovum, thus removing the constraint (Schön et al. 2009, ch.16). An alternative remedy for the lack of a centrosome is sperm-dependent parthenogenesis, where a sperm cell of a related sexual species or a conspecific is used to initiate mitosis of the ovum, without the sperm-contributing genes (Schön et al. 2009, ch. 19). It is generally assumed that mitosis can only be initiated thanks to the sperm's centriole. In fishes and amphibians, but also in many other taxa, parthenogenesis is always sperm-dependent.

Replacing the Missing Paternal Chromosomes

In parthenogenetic organisms, the problem of the missing paternal chromosomes is usually solved by one of several mechanisms of ploidy restoration. In sporadic parthenogenesis ploidy is generally restored with no or minimal change of meiosis (Schön et al. 2009, ch. 4). In one of the two most common mechanisms, gamete duplication, the unfertilized ovum starts a cleavage division, and this is followed by fusion of the two daughter nuclei, without affecting meiosis. These nuclei have the same half of the genome of the mother, and hence all loci will be homozygous. In the other common mechanism, terminal fusion, also only half of the maternal genome is transmitted. Here ploidy is restored at the end of meiosis by the fusion of the generally large oocyte with the closest polar body (second, in the row of four meiotic products). This polar body shares the same half of the maternal genome. Heterozygosity is lost, except for some caused by crossing over. Sporadic parthenogenesis is, thus, generally characterized by ploidy restoration with no or minimal change of the meiosis, resulting in heterozygosity loss, which together with the abovementioned poor centrosome function in the ovum results in extremely low viability and fertility rates.

In contrast, in most regularly parthenogenetic animals, ploidy restoration occurs via a drastic change of the normally tightly controlled global interactivity in the cell during meiotic divisions. This is remarkable, as even minor disruptions usually lead to strongly deleterious effects, such as aneuploidy, embryonic death, and sterility. The most common mechanism in obligatory parthenogenesis is the almost complete suppression of meiosis, followed by a mitosis-like division (apomixis), resulting in the transmission of the entire genome of the mother (Schön et al. 2009, ch. 4). Another frequent mechanism involves the duplication of the genome at the onset of meiosis, such that meiosis restores ploidy and the complete genome of the mother is transmitted (so-called premeiotic doubling). Yet another common mechanism is so-called central fusion. Central fusion differs from terminal fusion in that the oocyte is not fusing with the second polar body, but instead there is a fusion of the two inner

polar bodies, that replace the oocyte and apparently receive sufficient protoplasm. The inner polar bodies each have a different half of the maternal genome. The entire genome of the mother is thus transmitted, provided crossing over is repressed, for instance, by large inversions, as found in the only obligate parthenogenetic *Drosophila* species, *D. mangabeirai*. *D. mangabeirai* studies show that central fusion involves drastic modifications of meiosis, leading to different relative positions of the polar bodies (Schön et al. 2009 ch).

In summary, in contrast with sporadic parthenogenesis, regular parthenogenesis is generally characterized by ploidy restoration that involves drastic disruptions of meiosis, usually resulting in the transmission of the entire genome of the mother and good centrosome function, and the outcome is good viability and fertility.

Evolutionary Mechanisms That Facilitate the Alteration of Meiosis: Hybridization and Endosymbionts

There is a close association of regular and obligatory parthenogenesis with hybridization or endosymbiont infections. It is probable that the radical alterations of meiosis usually observed in regular and obligatory parthenogenesis have their origin in sudden large cytological events, for instance, due to interspecific hybridization or paternal genome loss due to endosymbionts, such as *Wolbachia* (Schön et al. 2009). It is counterintuitive that gradual changes of meiosis would be selectively advantageous, given the strongly deleterious effects of even minor changes. All well-investigated unisexual vertebrates are interspecific hybrids, and this has been found for a large expanding pool of invertebrate asexuals as well (Schön et al. 2009). Both successful lab experiments and recent detailed and large-scale genomic analyses have shown that on (extremely) rare occasions, interspecific hybridization followed by backcrossing events can result in parthenogenetic reproduction with a restoration of ploidy that retains heterozygosity. Paternal genome loss due to infection with maternally inherited endosymbionts also radically disrupts meiosis and is found to be associated with the origin of parthenogenesis (Schön et al. 2009). For instance, the initial event in the evolutionary path to sperm-dependent parthenogenesis of males (so-called pseudo-arrhenotoky) is most likely infection with symbionts that inactivate the paternal genome, via cytoplasmic incompatibility or via male-killing (e.g., Engelstädter and Hurst 2006).

The two causes of developmental constraints against parthenogenesis, absence of paternal chromosomes and centrioles, probably combine to provide a near absolute constraint against the gradual evolution of regular parthenogenesis. The genetic constraint of enforced homozygosity associated with sporadic parthenogenesis will further impede this gradual evolution. This probably explains why the advantageous mode of facultative parthenogenesis is not more widespread and is even entirely absent in vertebrates. In contrast, stick insects of the genus *Bacillus* miss the developmental constraint caused by the missing centrosome, which perhaps has allowed the, otherwise exceptional, gradual evolution of parthenogenesis.

Conclusions

We found three well-underpinned examples of wide-ranging near absolute developmental constraints in animals on the macroevolutionary time scale, which in turn induce many secondary constraints. All three are caused by rampant pleiotropy, itself caused by the complex and highly controlled global interactivity associated with mitosis and meiosis in the cell and with organogenesis in the early embryo (Galis et al. 2018). A strong control adds to the interactivity in leading to a stronger developmental conservation. Such a strong control presumably evolved because of the complexity of the processes and the grave consequences of their disruption (aneuploidy, apoptosis, cancer, sterility, death). As a result, mutations that affect such highly interactive developmental processes almost unavoidably have many deleterious pleiotropic effects that drastically diminish their chance to be successful. Conservation is, thus, caused by consistently strong selection against mutational change thanks to developmentally caused pleiotropic effects. As the interactivity is an intrinsic property of the developmental processes, the constraints are developmental.

The strongest and most universal of the constraints is the incompatibility of ciliated and differentiated cells to undergo mitosis. This results from pleiotropic effects combined with selection against having more than one or no centrosome, since this dramatically affects the interactivity associated with mitosis. As a consequence, in almost all adult metazoans, there are no pluripotent stem cells, only tissue-specific ones, such in strong contrast to plants. For differentiated metazoan cells, the only way around the constraint is to first dedifferentiate and then divide, as happens in wound healing and regeneration (as well as cancer), severely restricting the potential for these processes and protecting us against cancer. Furthermore, the constraint strongly biases the order and timing of proliferation and differentiation during development, the more so the larger the developmental complexity. A further important consequence of the constraint is the early determination of almost all organ primordia, when development is still highly interactive (phylotypic stage). This, among others, leads to the hard constraint against changes of phylotypic stages in many higher taxa, impacting the evolution of body plans. Since most organ primordia originate during this stage, there is strong conservation of the number and earliest development of most organs, like cervical vertebrae, eyes, and digits. The universal constraint against having more than one centrosome in a cell further leads to a hard developmental constraint against parthenogenesis, as zygotes only receive a centrosome from the father and not from the mother. Two centrosomes can be expected to be strongly deleterious. Hence, the missing paternal contribution of a centrosome in an unfertilized ovum hinders reliable cleavage divisions. We also discussed another hard developmental constraint, this time against parthenogenesis, which involves the missing paternal chromosomes. Successful ploidy restoration also appears to be limited due to a constraint, caused by the strong interactivity of meiosis. The combination of the two developmental constraints against parthenogenesis arguably forms a near absolute constraint against the gradual evolution of parthenogenesis from sporadic to regular or obligatory.

Cross-References

- ▶ Concept of Burden in Evo-Devo
- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Heterochrony
- ▶ Inherency
- ▶ Mechanisms of Pattern Formation, Morphogenesis, and Evolution
- ▶ Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics
- ▶ The Developmental Hourglass in the Evolution of Embryogenesis
- ▶ The Evolution and Development of Segmented Body Plans

References

Arthur W, Farrow M (1999) The pattern of variation in centipede segment number as an example of developmental constraint in evolution. J Theor Biol 200:183–191

Bell G (1989) Darwin and biology. The evolution of individuality. Leo W. Buss, Princeton University Press Princeton, New Jersey 1988.197 pp. J Hered 80:417–421

Buss LW (1987) The evolution of individuality. Princeton University Press, Princeton

Conner JK (2012) Quantitative genetic approaches to evolutionary constraint: how useful? Evolution 66:3313–3320

Cridge AG, Dearden PK, Brownfield LR (2016) The mid-developmental transition and the evolution of animal body plans. Ann Bot 117:833–843

Diez del Corral R, Olivera-Martinez I, Goriely A, Gale E, Maden M, Storey K (2003) Opposing FGF and retinoid pathways control ventral neural pattern, neuronal differentiation, and segmentation during body axis extension. Neuron 40:65–79

Eisman RC, Kaufman TC (2007) Cytological investigation of the mechanism of parthenogenesis in Drosophila mercatorum. Fly 1:317–329

Engelstädter J, Hurst GDD (2006) Can maternally transmitted endosymbionts facilitate the evolution of haplodiploidy? J Evol Biol 19:194–202

Galis F, Metz JAJ (2001) Testing the vulnerability of the phylotypic stage: on modularity and evolutionary conservation. J Exp Zool B Mol Dev Evol 291:195–204

Galis F, Sinervo B (2002) Divergence and convergence in early embryonic stages of metazoans. Contr Zool 71:101–113

Galis F, Metz JAJ, van Alphen JJM (2018) Development and evolutionary constraints in animals. Ann Rev Ecol Evol Syst 49:499–522

Gönczy P (2015) Centrosomes and cancer: revisiting a long-standing relationship. Nature Rev 15:639–652

Gould SJ, Lewontin RC (1979) The spandrels of the San Marco and the Panglossian paradigm: a critique of the adaptationist program. Proc Royal Soc London B 205:581–598

Hadorn E (1961) Developmental genetics and lethal factors. Methuen, London

Heidstra R, Sabatini S (2014) Plant and animal stem cells: similar yet different. Nature Rev Mol Cell Biol 15:301–312

Henneguy LF (1898) Sur les rapports des cils vibratiles avec les centrosomes. Arch d'Anat Micr 1:481–496

Hu H, Uesaka M, Guo S, Shimai K, Lu S-M, Li F, Fujimoto S, Ishikawa M, Liu S, Sasagawa Y, Zhang G, Kuratani S, Yu J-K, Kusakabe TG, Khaitovich P, Irie N (2017) Constrained vertebrate evolution by pleiotropic genes. Nature Ecol Evol 1:1722–1730

Jacquet P (2004) Sensitivity of germ cells and embryos to ionizing radiation. J Biol Regul Homeost Agenets 18:106–114

Lenhossék M (1898) Ueber Flimmerzellen. Verhandl Der Anat Gesel Kiel 12:106–128

Manandhar G, Schatten H, Sutovsky P (2005) Centrosome reduction during gametogenesis and its significance. Biol Reprod 72:2–13
Maynard Smith J, Burian R, Kauffman S, Alberch P, Campbell J, Goodwin B, Lande R, Raup D, Wolpert L (1985) Developmental constraints and evolution. Q Rev Biol 60:265–287
Metz JAJ (2012) Adaptive dynamics. In: Hastings A, Gross LJ (eds) Encyclopedia of theoretical ecology. University of California Press, Berkeley, pp 7–17
Peterson T, Müller GB (2016) Phenotypic novelty in EvoDevo: the distinction between continuous and discontinuous variation and its importance in evolutionary theory. Evol Biol 43:314–335
Sander K (1983) The evolution of patterning mechanisms: Gleanings from insect embryogenesis and spermatogenesis. In: Goodwin BC, Holder N, Wylie CC (eds) Development and Evolution. Cambridge Univ. Press, Cambridge, UK, p 137
Schön I, Martens K, van Dijk P (eds) (2009) Lost sex. The evolutionary biology of parthenogenesis. Springer, Dordrecht
Tanaka EM, Reddien PW (2011) The cellular basis for animal regeneration. Dev Cell 21:172–185
Vermeij GJ (2015) Forbidden phenotypes and the limits of evolution. Interface Focus 5:20150028
Walz G (2017) Role of primary cilia in non-dividing post-mitotic cells. Cell Tissue Res 369:11–25
Whyte L (1964) Internal factors in evolution. Acta Biotheor 16:33–48
Wu J, Akhmanova A (2017) Microtubule-organizing centers. Ann Rev Cell Dev Biol 33:4.1–4.25

Developmental Innovation and Phenotypic Novelty

Gerd B. Müller

Contents

Abstract

The explanation of phenotypic novelties is a central goal of evo-devo. Building on earlier classifications, three types of novelties are distinguished, here renamed constituting novelty, discretizing novelty, and individualizing novelty. A discussion of developmental innovation processes and modeling approaches highlights the central roles of generic material properties and process dynamics in novelty formation. Whereas individualizing novelty is consistent with the standard modes of variation, constituting novelty and discretizing novelty give rise to evolutionary innovation, a class of phenotypic change regarded as distinct from adaptive variation. A developmental theory of innovation privileges emergent development over genetic variation in the explanation of phenotypic novelty.

G. B. Müller (✉)
Department of Theoretical Biology, University of Vienna, Vienna, Austria

Konrad Lorenz Institute for Evolution and Cognition Research, KLI Institute, Klosterneuburg, Austria
e-mail: gerhard.mueller@univie.ac.at

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_66

Keywords

Evolution · Development · Novelty · Innovation · Evo-devo · Inherency

Introduction

All science that deals with change over time is faced with an intertwined set of problems: how are existing entities modified, and how do new entities arise? In biology, evolutionary theory has traditionally concentrated on the former, the variation of the existing. Although Darwin had recognized that novelty may constitute an evolutionary problem distinct from adaptation, in which not selection but environmental induction would be the decisive agent (West Eberhard 2008), and other evolutionists occasionally reflected upon the conceptual challenges provided by the issue of novelty, later commentaries usually served the goal of bringing novelty into the fold of genetic and adaptive variation. It was only with the introduction of evo-devo that the topic received more differentiated attention. Indeed, novelty is frequently called a core issue of evo-devo, a claim supported by a host of publications that report empirical examples or provide theoretical contributions to this topic. Yet, these works use substantially different types of vocabularies, definitions, and interpretations of novelty, and there is no consensus as to whether and how these concepts can or should be integrated within the canonical evolutionary framework. Acknowledging that there are many approaches to novelty (see overviews in Fontana 2001; West-Eberhard 2003; Moczek 2008; Brigandt and Love 2012; Peterson and Müller 2016; Erwin 2019) and that the study of novelty is a highly interdisciplinary field, this treatise concentrates on the contributions of evo-devo to the novelty problem in light of their consequences for evolutionary theory.

One of the key issues in the debate is whether evo-devo explanations of novelty can be aligned with standard evolutionary theory. These discussions usually revolve around conventional themes, such as gradual vs. saltational change, the applicability of the proximate-ultimate distinction, or the never-ending micro- vs. macroevolution debate, as well as the potential roles of novelty in taxonomical contexts involving homology and apomorphy designation, and further issues like the ecological impact of novelties or their roles in speciation. Drawing the novelty problem into these established discourse structures usually leads to the complete exclusion of evo-devo-based reasoning. The micro-macroevolution narrative, for instance, has proven particularly effective in quelling earnest considerations of the role of development in evolutionary change, using the simple assertion that development is part of the macroevolutionary realm and hence does not affect the purportedly decisive micro-evolutionary genetic variation-inheritance-natural selection scheme. Casting the novelty problem in the phenotypic plasticity discourse often has a similar effect. Since plasticity and reaction norms are usually genetically defined, any novelty derived from the plasticity of developmental systems inevitably becomes a consequence of genetic variation and, hence, part of the evolutionary orthodoxy. The actual capacities of developmental systems play no role in these accounts.

It is before this background of established discourse patterns that the treatment of novelty gains its theoretical importance, and it seems necessary to adopt a fresh perspective that is not rooted in one of the habitual explanatory modes in order to include developmental mechanisms of novelty generation into the evolutionary framework. For a discussion of this topic in the last section (see also chapter on ▶ "Evo-Devo's Contributions to the Extended Evolutionary Synthesis"), it is first necessary to observe which kinds of results evo-devo has produced and how they elucidate the novelty problem. To this end, it will be useful to distinguish a strand of work focused on developmental mechanisms and a modeling strand. Before dealing with these domains in the sections below, several issues of terminology need to be addressed.

One is the usage of *innovation* and *novelty*. In the literature, these terms are frequently used interchangeably. But several authors have made distinctions, albeit in different ways. Whereas *novelty* is preferentially reserved for new structures or characters, *innovation* is often used to describe new functions. A related distinction is drawn by Erwin (2019) who also ascribes *novelty* to new structures, in the sense of newly individuated characters, but uses *innovation* for changes in a clade that have major ecological impact – defined by the effects of its removal from an ecological network. By contrast, Müller and Newman (2005) also apply *novelty* to new structures, traits, or characters but use *innovation* to distinguish their evolutionary origins from standard *variation*. Here, the term innovation points at the evolutionary mode of origination, whereas novelty refers to the outcome at the character level, i.e., novelty pairs with adaptation and innovation pairs with variation. Subsequent treatments (Müller 2010; Peterson and Müller 2016) have elaborated on the variation-innovation distinction, and I will continue this usage in the present text. At the same time, *developmental innovation* needs to be distinguished from *evolutionary innovation*. Developmental innovation refers to the mechanistic processes through which phenotypic novelty can be realized, which in turn may be classified as an evolutionary innovation in phylogeny.

In this context, it should be noted that *key innovation* refers to yet another usage of innovation. The term has usually been applied to outstanding evolutionary "inventions" that permit the invasion of a new ecological niche or adaptive zone by an organismal lineage and may serve as triggers of adaptive radiation and speciation (Galis 2001). Such innovations can occur at physiological, developmental, morphological, or behavioral levels and thus intersect with the novelty and innovation themes in evo-devo. But more often this kind of perspective is related to the earlier associations of novelty with the origin of higher taxa and speciation. The focus of this usage was on the dynamics of species diversification and not on the causal factors responsible for key innovations to be formed. Importantly, such key innovations don't necessarily have to be based on a novelty in the evo-devo sense but may also result from processes of standard variation at any one of the levels mentioned above. Whether a phenotypic novelty contributes to a key innovation or other kinds of evolutionary success will not be examined in the present chapter.

Finally, a word on causes. Traditional explanations of novelty, often not distinguished from a general notion of evolutionary change, have vacillated between the

mutation-first argument (irrespective of the kind of developmental process affected) and the environment-first argument (through either ecological opportunity or environmental stress). The former attributes causal primacy to changes in genetic control and the latter to the environment. Developmental systems themselves, in which these factors would play out, were not regarded as a cause or were designated as "proximate" mechanisms. In order to avoid the unfortunate separation of proximate from ultimate causes, a less universal distinction between initiating and realizing conditions has been introduced (Müller and Newman 2005; Müller 2010). It assumes that the locus for the specific realization of novelties (development) has causal primacy (when the explanandum is the phenotype), whereas mutational, selectional, or environmental triggers are taken to represent initiating conditions. Even if there is an Aristotelian ring to it, we might say that evo-devo is concerned with the efficient causes that define the phenotypic outcomes of innovation processes.

Definitions and Classifications

All treatments of novelty in evolution hinge on the question of what is meant by novelty. When is something novel? How do we recognize novelty? Are there different classes of novelty? As observed above, it only makes sense to speak of novelty if it represents an entity that is distinct from other forms of evolutionary change, such as variation or adaptation. Otherwise, if any kind of evolutionary modification that appears different from an ancestral state is synonymous with novelty, the term becomes meaningless. Usually, novelty is taken to refer to an entity that has not existed before. In biology, recognizable entities are called features, characters, or traits. Therefore, a definition of novelty is inextricably linked to the notion of organismal characters. We may say that a novelty is a character that has not existed in the ancestry of its bearer. This implies a notion of difference and sameness of characters, which takes us, whether we like it or not, to the notorious concept of homology. We may call a new homologue, i.e., a new character shared by the members of a derived clade, a novelty (Müller and Wagner 1991). Homologues are discrete, robust, heritable, and comparable entities of morphological structure and, therefore, suitable markers of novelty, even if homology assignments are not always easy or possible. In other definitions, the distinction between quantitative and qualitative change has been emphasized (Müller 1990; West-Eberhard 2003), and so was the requirement for a transition between adaptive peaks in a fitness landscape (Hallgrimsson et al. 2012). All definitions have their uses in particular theoretical settings. Since evo-devo deals with the way by which organisms produce morphological phenotypes, I will stay with the character definition and will restrict my further analysis to novel morphological characters.

Besides definitions, various classification schemes for morphological novelties have been proposed. I introduced a distinction of three types of novelty, based on the kinds of constructional change they represent (Müller 2010). In this classification, Type I refers to the primary morphological body plans that arose in conjunction with the origins of multicellularity. Type II are structural elements newly inserted into an

existing body plan, with no homologous counterpart in the ancestral species. Type III are major variations of an existing body plan element through progressive individualization, with a new quality or functional capacity. Using these three classes, a sufficiently reliable identification of morphological novelties is possible. A reformulation of these categories and example cases is provided below.

Wagner (2014) provided a partially overlapping classification, distinguishing two types of novelties based on his elaborate character concept. He defines Type I as a novelty that creates a new character identity, typically with no counterpart in the ancestral state, roughly corresponding to Type II in the classification above. Type II in Wagner's classification are characters already present in ancestral species, yet with new variational characteristics, roughly corresponding to Type III of the above classification. In his "typology," the explanation of Type I novelties is a question of the origin of gene regulatory networks that provide character identity, whereas Type II novelties require the differentiation of developmental modalities that underlie variational tendencies. Erwin (2019) adopts Wagner's classification and adds a third type of novelty, the combination of preexisting characters that leads to the formation of a different character. These would also fall into the Type II class of novelties in our classification. Müller's (2010) characterizations of the three classes of novelty were elaborated by Peterson and Müller (2016), including an improved definition of novelty based on the different types, without explicit mention of the homology term that had disturbed some commentators: "Phenotypic novelty refers to a primary body plan (Type 1), a new constructional element (Type II), or a newly individualized character (Type III), that is qualitatively discontinuous from the ancestral state."

With respect to homology, it is noteworthy that in Wagner's (2014) usage a novel homologue is defined by its assuming a distinct "character identity" provided by a unique gene regulatory configuration. By contrast, in our usage, a novel homologue is defined by assuming regulatory autonomy, i.e., relative independence from the control by unique genetic and developmental mechanisms that are involved in their realization (see chapters ▶ "Developmental System Drift" and ▶ "Evolution of Skeletal Tissues"). In the latter view, homologues are stabilized not due to genetic individuation but because of their organizing roles in developmental and structural compositions of the phenotype and, consequently, also of the genotype, as formulated by the organizational homology concept (Müller 2003).

In order to eliminate the confusion about enumerated types of novelty, I will introduce descriptive distinctions and rename my previous categories. Based on the previous characterizations, I distinguish *constituting novelty* (former Type I), *discretizing novelty* (former Type II), and *individualizing novelty* (former Type III). The rationale for keeping the category of constituting novelties separate is that during the early phases of multicellularity, no "development" in the strict sense of the term, i.e., the reconstitution of every new individual from a single cell, was likely to have existed. Following this classification, constituting novelties include all the different kinds of spherical and/or hollow, layered, elongated, or segmented shapes of first multicellular body assemblages. Discretizing novelties comprise cases such as new skeletal elements, insect wing hearts, or the firefly lantern, i.e., new characters added to

existing body plans. Individualizing novelties are represented by uniquely specialized characters that developed from elements of existing body plans, such as beak shapes in Darwin finches, the narwhal tusk, or nasal appendages in star-nosed moles. More examples for phenotypic novelties that can be assigned to this categorization can be found in West-Eberhard (2003), Müller (2010), Wagner (2014), Peterson and Müller (2016), and Erwin (2019).

The focus on morphology as a way of classifying the kinds of novelties realized in evolution does not mean that their formation is restricted to a single class of evo-devo mechanisms. Rather, a host of different developmental and evolutionary processes can be involved in their formation. The origination of constituting novelties established the morphological infrastructure to which all other novelties have been additions. This includes key "inventions" like the egg cell and other major phenotypic transitions in the evolution of life, such as the first multicellular assemblies and their diverse constructional solutions. Besides the necessary establishment and refinement of genetic regulatory mechanisms, these constructs point to the centrality of chemico-physical properties of the cells and tissues involved as well as their autocatalytic activities. Discretizing novelties, the origin of structural elements that have no counterpart in the ancestral species, are intimately associated with developmental interactions among cells and tissues, the genetic subroutines controlling cell behaviors, as well as the chemico-physical context in which these processes play out. This involves the rearrangement of existing gene regulatory circuitry and equally involves suites of different developmental routines, which will be discussed below. Individualizing novelties, the refinement and super-individuation of existing structural elements, sometimes to variational extremes, are usually associated with more conventional mechanisms of developmental variation but raise important issues regarding the dimensions of variation (Hallgrimsson et al. 2012).

Developmental Innovation

Since development consists of processes of systemic interaction among genes, cells, and tissues, with numerous feedbacks between these levels of realization as well as with the complete organism and its environment, all involved processes can become sources of novelty formation. I will sequentially treat genetic, cellular, and tissue level processes, as well as the physical phenomena they activate. Due to the limited number of references allowed for the chapters of this compendium, citations can be provided only for selected cases. Additional references can be found in Peterson and Müller (2016) and in other chapters of this Reference Guide.

Genetic Processes

Following the long ancestry of the mutation-first argument, several variations of this scheme have been associated with novelty formation. The emphasis has shifted from the early notion of cumulative single gene mutations to larger-scale genomic events

affecting development, formerly stigmatized by the "hopeful monster" metaphor. These include gene duplication, horizontal transfer, and regulatory network modifications. Genetic innovation is often treated purely within the realm of genetic evolution (Wagner 2011) and is not expanded to phenotypic novelty. But ever since Britten and Davidson's (1971) seminal paper, in which they specifically address the origin of novel organs, rearrangements of existing gene regulatory relationships have been central to the discussion of the generation of new morphological structures. In particular, this includes the idea of regulatory gene duplication with subsequent co-option and redeployment of genes at new locations (Shubin et al. 2009), whole genome duplications (Moriyama and Koshiba-Takeuchi 2018), and function change in developmental pathways (Ganfornina and Sánchez 1999). Ganfornina and Sanchez provide one of the first proposals of a unified conceptual framework for different kinds of relations between gene duplication, co-option, and selection in the origin of novelties. The authors emphasize that in these domains natural selection must act in distinctly different manners, depending on the historic succession of co-option and duplication events. Since all of these modes of genetic innovation gain their phenotypic specificity only within the developmental context in which they are deployed or re-deployed, from an evo-devo perspective, they rather serve the role of initiating conditions for novelty formation.

Cell and Tissue Level Processes

Here belong innovations realized through developmental processes that affect relative size, shape, composition, pattern, timing, or topological arrangement of morphogenetic entities, in particular in their capacities to disrupt prior homeostatic relations or organizational routines. *Differential proliferation*, often found in individualizing novelties, can lead to newly individuated beak shapes in finches, progressive and rotational growth of mammalian canines and tusks, new variants in the pronotum of treehoppers, nasal appendages of the star-nosed mole, and other forms of discriminating variation. Several forms of new *cell differentiation* were shown to be responsible for novel histological areas in the vertebrate brain, novel appendages formed from histoblasts in sepsid flies, novel tissue types permitting the formation of dermal bone in the turtle carapace, or the origin of new vertebrate tooth types. *Fusions* of previously separate developmental entities underlie the formation of novel horns in dung beetles, the formation of the lower beak in birds, or the origin of the carpel from cupule tissue in flower development. Such fusions often take place at transition points in development (Müller 1990), for instance, when the process of mesenchymal condensation switches to chondrogenesis and subsequently to osteogenesis in vertebrate skeletal development. Various forms of symbiosis can also be interpreted as fusions that lead to novelty (Margulis and Fester 1991). *Separation and compartmentalization* are yet another mode of generating new entities in previously uniform developmental regions, as seen in the differentiation of serial elements, in the patterning of butterfly wings (Nijhout 2001), or the evolution of developmental compartments in the heads of vertebrates (see chapter ▶ "History and

Current Theories of the Vertebrate Head Segmentation"). *Relative shifts* of inductive regions can elicit new differentiations, as is the case in the switch from external check pouches to fur lined internal pouches in rodents or in shifts along the lateral mesodermal divide in vertebrate limb bud initiation (Nuño de la Rosa et al. 2014). *Spatial constraints* in development are equally powerful initiators of novelty as demonstrated by the wing hearts in insects that arose as a consequence of spatial changes resulting from rearrangements of the flight apparatus. Another example is the branching of shoot axes in plants, in which different cellular processes – often based on very similar molecular pathways – result in a limited number of branching modes (see chapter ▶ "The Evolution of Branching in Land Plants: Between Conservation and Diversity"). Furthermore, shifts in developmental timing, *heterochrony*, are a well-known factor in initiating cell and tissue rearrangements that can result in novelty, with numerous examples in the literature (see chapter ▶ "Heterochrony"). An extreme form of heterochrony, the retention of juvenile structures into adulthood, can lead to the appearance of developmental novelties in the adult phenotype, as demonstrated by many so-called caenogenetic features.

Emergent Behaviors

A significant source of novelty formation is rooted in emergent behaviors that are characteristic of all multicomponent and multiscale organizing systems. This applies also to developmental systems that span molecular, cellular, tissue, and organ levels of internal organization as well as external interactions with the environment. The formation of multicellular aggregates during developmental processes includes stochastic behaviors, short-range and long-range signaling, reaction-diffusion systems, oscillatory systems, and other pattern-forming processes that all elicit autonomous cell behaviors leading to cell clustering, cell sorting, tissue layering, cavity formation, and a suite of further morphogenetic consequences (Newman and Bhat 2009). Many of these systems have been studied from the point of view of their evo-devo effects, in particular with regard to novelty formation such as seen in the integument patterns of fish and insects, or the skeletal patterns of vertebrates.

Digit formation in vertebrate limbs, for instance, is based on cell aggregation mechanisms that define digit condensations in the growing limb bud, a process that is influenced by any factor that affects cell proliferation rates or cell number. Mutational, selectional, or experimental perturbations of these parameters lead to the addition or loss of digits via critical threshold numbers of cells required for a condensation to form. A model based on the random bistability of individual cells in the limb bud field suggests that two kinds of mapping events are involved in transforming, for instance, a genetic mutation affecting cell number into discrete character states (individual digits) by first generating a continuous distribution of affected cells and, second, by a transformation of the continuous distribution into individual digit condensations via cellular threshold effects (Lange et al. 2014). Thus, the gain or loss of digits in development and evolution can be interpreted as a consequence of the autonomously pattern forming cell aggregation system

established in the limb, which is able to produce emergent, yet predictable, phenotypic outcomes from different initiating conditions. Significantly, the same kinds of effect appear in mutational, phylogenetic, experimental, and simulated cases. Emergent effects have also been shown to result from varying the boundaries of the patterning system. Simulations of the core chondrogenic mechanism, depending on shape and size variations of the limb bud geometry, are able to predict a wide range of skeletal morphologies and novelties seen in normal, fossil, and pathological limbs (Zhu et al. 2010).

According to this evo-devo-based perspective, emergent developmental behaviors are elicited by all initiating conditions that affect developmental systems, whether through genetic mutation, natural selection, or environmental induction. Emergent effects contribute to the variational repertoire that can be generated in a population and would initially be fitness neutral. The maintenance of the resulting phenotypic features will depend on opportunities for natural selection to act and for genetic stabilization to occur (see chapter ▶ "Developmental Plasticity and Evolution"). For these reasons, how emergent variation, evolving genomic architecture, and adaptive fixation are linked is one of the critical questions in evolutionary biology (Badyaev 2011).

Chemico-Physical Properties

A consideration of the chemistry and physics of development increasingly informs the various domains of novelty generation described above (see also chapter on ▶ "Inherency"). Cellular properties such as adhesivity, elasticity, tensegrity, phase separation, etc. represent the mechanistic links between genetic change and phenotypic realization. Besides the physical processes mentioned in the previous section, biomechanics has a particular role in triggering developmental reactions, especially in mechanosensitive tissues like the ones involved in skeletogenesis. Mechanotransduction pathways are known to be able to affect gene expression and tissue responses, for instance, via compressive force or tension. Such effects have been studied, among others, in anuran and snake jaws, in the formation of sesamoids and their derived structures in birds and mammals, as well as in the pharyngeal jaw apparatus of fish. In the latter, Cichlidae and Labridae independently evolved a novel synovial joint between the upper pharyngeal jaws and the ventral surface of the neurocranium. Finite element modeling has shown that a decoupling of epibranchial elements, changes in muscle vector orientations, and the resulting increase of pressure forces on the neurocranium were critical in eliciting new cartilage and joint formation (Peterson and Müller 2018). Another example shows how emergent color patterns in vertebrates are related to motile pigment cells embedded in a viscoelastic mesenchymal matrix and forming tension tracks along which cell and pigment arrangements take place. Overall alterations in body shape and tension areas influence the formation of novel color patterns, which can be predicted from a biomechanical model (Caballero et al. 2012).

Evo-devo studies of the kind discussed in this section highlight the fact that the evolutionary modifications of gene regulatory circuits all mobilize autonomous cell and tissue behaviors that define the phenotypic outcome. In particular, the continuous variation of genetic and developmental processes can lead to nonlinear effects at the level of morphological novelty. Multiscale feedbacks between alterations at different levels of development and their emergent consequences further enhance the complexity of the processes involved and are often easier to investigate by modeling approaches.

Models of Novelty Formation

A range of models have been proposed for selected aspects of novelty formation based on biometrics, multivariate statistics, computational simulations, and the quantification of gene, cell, and tissue interactions (see chapters in the section). In addition, qualitative and diagrammatic models address novelty generation from a more conceptual perspective.

A class of models related to the idea of a morphospace of possible variation indicates that only a limited number of phenotypic solutions can be obtained from a given developmental system, thus channeling the specific opportunities for novelty generation. A well-studied case is tooth formation in vertebrates, in which the modeling of differential gene activation and gene products is shown to influence morphogenesis and novelty of tooth shape. This type of approach demonstrates that variation of simple phenotypic structures tends to be gradual, whereas variation of complex phenotypes is characterized by more punctuated forms of change and innovation rate (Salazar-Ciudad and Jernvall 2005). Complexity also has an influence on the patterns of innovation rate, promoting early accelerations and late decelerations in a clade (see chapter ▶ "Evolution of Complexity"). Such findings have motivated a recategorization of the types of developmental pattern formation in terms of their capacities to produce morphological novelties, distinguishing morphostatic from morphodynamic modes of development (see chapter ▶ "Mechanisms of Pattern Formation, Morphogenesis, and Evolution"). In morphodynamic mechanisms the phenotype of temporary stages of development becomes a causal factor in directing further development. Subsequent evolution may replace morphodynamic mechanisms by morphostatic ones, which usually require more deterministic gene interactions. Through this kind of developmental entrenchment (see chapters ▶ "Canalization: A Central but Controversial Concept in Evo-Devo" and ▶ "Concept of Burden in Evo-Devo"), certain phenotypes will become more difficult to modify, allowing only slight and gradual forms of variation, whereas a simplification of circuitry or a breaking of constraints would be required for phenotypic novelty to occur. Therefore, computational models can be predictive of evolving genotype-phenotype relations (see chapter ▶ "Computational Modeling at the Cell and Tissue Level in Evo-Devo").

A model of combinatorial pattern forming modules illustrates the potential role of cell-autonomous behaviors that underlie the formation of basic body plan

features during the origin of the metazoa. The products of a set of developmental toolkit genes already present in unicellular forms are thought to have given rise, via the mobilization of physical behaviors in new multicellular contexts, to a number of collective cell behaviors such as differential adhesion, lateral inhibition, or cell sorting. Together, these constitute a combinatorial multicellular "pattern language" that has the capacity to generate basic body arrangements, such as spherical, elongated, segmented, or branched forms (Newman and Bhat 2009). This model implies that initial metazoan forms were phenotypically plastic, interchangeable, and environment-dependent multicellular assemblies that acquired developmental stability and evolutionary robustness only during subsequent rounds of stabilizing selection, such that an early "pre-Mendelian" phase of organismal evolution may have preceded the later Mendelian phase in which a much closer association between inheritance and phenotype would prevail (Newman and Müller 2000).

This idea resonates with the concept derived from plant biology proposing that different combinations of generic developmental motifs underlie the evolution of multicellular organisms (Niklas et al. 2013). Because natural selection typically acts directly not on the generative mechanisms themselves but on the behavioral or functional traits they underlie, radically different variants of a developmental motif can lead to functionally similar novelties. In the case of plants, the different developmental-genetic modules thought to be involved in early specifications of body construction can be mapped into the morphospaces of four major body plans (Niklas et al. 2013). This model also supports the notion that a developmental utilization of substantially different kinds of molecular systems can result in the production of very similar phenotypic effects, indicating a significant imprint of development on phenotypic trait formation.

Another class of models concerns environmentally induced novelties, in particular via different kinds of stresses that act on developmental processes directly. According to one approach, new cell types can originate from stress responses. In this case, the decidual stroma cells of the human uterus, a cell type critical for embryo implantation and maintenance of pregnancy, are suggested to have evolved from a cellular stress reaction. Stress reactions are thought to have been elicited through the slight tissue inflammation caused every time by embryo attachment and uterine tissue invasion (see chapter ▶ "Devo-Evo of Cell Types"). These authors propose that stress-induced novelties represent a distinct form of plasticity relevant for evolutionary change, because it leads to the origin of novel structures rather than the adaptive variation of a preexisting character. In general, stressful environments are known to facilitate the developmental expression of novel genetic variation that may have been phenotypically neutral under a normal range of environments, and evolving organisms have developed a wide range of mechanisms for coping with these environmental challenges. How stress-induced developmental innovation is accommodated by changes in an organism's integration, and how it becomes heritable and adaptive, in turn is the subject of several models (see chapters ▶ "Developmental Exaptation" and ▶ "Modeling Evolution of Developmental Gene Regulatory Networks"). Experimental results show that environmental stress

can induce novel patterns of development that remain stable across multiple generations (Stern et al. 2012).

The *Epigenetic Innovation* model expounded on previous occasions (Müller 2010) is a conceptual model of novelty formation later expanded by Peterson and Müller (2016). "Epigenetic" then referred to the context-dependent nature of developmental innovation processes, as understood and continued in the present text. However, since today "epigenetic" is almost exclusively used in the sense of heritable phenotypic changes that do not involve alterations of DNA sequence and thus gives rise to misunderstandings, I will hereafter refer to the model as *developmental innovation*. The model relates different modes of novelty introduction to standard forms of variation and the spread of novelties in a population. Phases of novelty introduction alternate with phases of quantitative variation. Here, *constituting novelties* arise from combinatorial modules early in the origin of multicellularity and will be followed by multiple cycles of variation and selection, stabilizing first rudiments of body structure. *Discretizing novelties* emerge at threshold points of ongoing continuous variation, defined by limitations of developmental buffering capacity, producing kernels of new traits that can be further refined by successive rounds of adaptation. *Individualizing novelties* arise from extreme forms of continuous variation (Peterson and Müller 2016). In this model, increasing complexity is a result of the permanent interplay between the different forms of novelty formation, variation, and stabilizing selection which leads to an increased routinization and overdetermination of the genetic circuitry involved in the development of the respective traits. It follows that the genes regulating developmental processes in extant model organisms are not necessarily the ones that were responsible for the first origination of the characters whose development they control today. According to this reasoning, Wagner's (2014) concept of gene regulatory identity formation would belong here, providing genetic stabilization rather than causing the origination of novelties per se. In conclusion, the developmental innovation model emphasizes that morphological novelties that emerge through developmental modes are neither arbitrary nor the result of adaptive optimization. Instead, they result from generic motifs inherent to developing systems of cell and tissue organization (Müller and Newman 2005).

Innovation Theory

Evo-devo offers a toolkit for the study of innovation in biology and other areas, such as cultural evolution, the social sciences, economics, and technology. Various attempts toward a general theory of novelty have been made (Callebaut 2010). Due to the multitude of factors and the complex dynamical nature of the processes involved in innovation and novelty formation, a general formal model of novelty that applies to all domains of evolution may not be possible (Erwin 2019), although network control theories have the potential to go a long way (e.g., Fontana 2001). Regarding the more circumscribed problem of phenotypic novelty, evo-devo has devised a multitude of empirical and theoretical approaches and has thus shifted the

attention from the study of the well-known phenomena of the gradual variation of traits toward the conditions that enable the origination of these traits. The majority of the evo-devo approaches discussed above explicitly or implicitly recognize novelty as a separate evolutionary problem and, hence, make a distinction between variational and novelty producing forms of change. Whereas in practice these forms may not always be easy to distinguish, this attitude marks an epistemological contrast to the population theoretical approach in evolutionary biology, which assumes that gradual, continuous, and incremental genetic variation sufficiently explains all phenomena of phenotypic evolution. Although evo-devo recognizes that genetic variation accompanies every kind of heritable phenotypic variation, it places the explanatory weight for specific morphological solutions on development, especially in those instances in which novelties are generated.

Also with regard to the role of natural selection, novelty research in evo-devo represents a shift of epistemic attitude. Whereas in the orthodox view all forms of phenotypic change had to be consistent with the variation-adaptation paradigm, in which natural selection represents the sole factor responsible for the specific reification of a phenotypic trait, in evo-devo novelties depend only indirectly on natural selection, in the sense that selection serves to release generative potential that is inherent to any developmental system. But the morphological specificity of the traits produced will be determined by the dynamical and material properties of development. This position assigns the key explanatory role to internal causation and thus liberates theoretical accounts of phenotypic evolution from the requirement to rely on purely external causes. The alternative to the externalist position is formulated by the concept of inherency. Inherency summarizes the intrinsic propensities of a developmental system. It characterizes what a developmental system is able to generate at the phenotypic level, regardless of whether the initiating impulse comes from mutation, selection, or environmental induction (see chapter ► "Inherency").

As Fontana (2001) has aptly pointed out, the explanandum of innovation theory is not optimization but organization! Evo-devo adds an organizational component to evolutionary theory, which begs the integration with genetic evolution and the population theoretical account. Although several attempts toward theoretical integration have been made (Wagner 2011; Erwin 2019), these proposals largely remain focused on the relationship between genetic variation and natural selection, without taking development causally into account. An exception is West-Eberhard's (2003) approach. In her comprehensive scenario, she provides a profound analysis of different forms of novelty generation, emphasizing the importance of environmental induction and development. Still the evolutionary contribution of development is limited to accommodating plasticity. Selection remains the ultimate explanans. Wagner (2014) also pays close attention to developmental factors in his detailed theory of the evolution of homology. In his account, genetic individuation of a novel character is the key to an integrated understanding of novelty evolution, whereas scant importance is assigned to the morphogenetic rules that define which characters become available for individuation. Pavlicev addresses the integration of evolutionary theory with developmental theory via genetic pleiotropy, but does not deal with

novelty specifically (see chapter ▶ "Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics").

As emphasized by numerous publications, developmental plasticity is an essential ingredient of evolutionary innovation (see chapter ▶ "Developmental Plasticity and Evolution"). This is especially true for individualizing novelty, and it is safe to assume that plasticity and its genetic underpinnings are also involved in the other modes of novelty formation. But plasticity is not the only way to conceptualize novelty formation. Neither for constituting novelty nor for discretizing novelty developmental plasticity represents the decisive causal factor. Plasticity, much like evolvability, is an import of the population theoretical discourse structure that usually impedes the recognition of the contributions of development to innovation and novelty formation. The principle of inherency advocated in this chapter takes the conceptualization of novelty beyond plasticity. It privileges generative developmental factors over ubiquitous genetic variation in the explanation of phenotypic evolution.

Based on its capacity to address phenomena of novelty, nonadaptive traits, variational discontinuity, structural organization, and other aspects of the evolution of organismal complexity, evo-devo contributes to a reformed framework of evolution (see chapter ▶ "Evo-Devo's Contributions to the Extended Evolutionary Synthesis"). In contrast to the traditional focus on variation and population dynamics, its theoretical focus is on the phenotype. This complements the study of genetic evolution and gene regulatory mechanisms with generative principles resulting from cellular organization, modularity, generic physical behaviors, and process dynamics of development. The inclusion of developmental innovation theory not only expands the explanatory reach of evolutionary biology to domains beyond population theoretical phenomena, but it also brings new predictive capacities to evolutionary theory. Although novelties often arise in emergent ways, the phenotypic results are not arbitrary. Knowledge of the rules of developmental systems permits predictiveness of specific phenotypic outcomes. This characteristic capacity of evo-devo opens up a wide range of empirically testable research questions in the study of developmental innovation and phenotypic novelty.

Cross-References

▶ Computational Modeling at the Cell and Tissue Level in Evo-Devo
▶ Developmental Homology
▶ Developmental Plasticity and Evolution
▶ Developmental System Drift
▶ Devo-Evo of Cell Types
▶ Evo-Devo's Contributions to the Extended Evolutionary Synthesis
▶ Evolution of Complexity
▶ Evolution of Skeletal Tissues
▶ Heterochrony
▶ History and Current Theories of the Vertebrate Head Segmentation

- ▶ Inherency
- ▶ Interdisciplinarity in Evo-Devo
- ▶ Mechanisms of Pattern Formation, Morphogenesis, and Evolution
- ▶ Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics
- ▶ Proximate Versus Ultimate Causation and Evo-Devo
- ▶ The Evolution of Branching in Land Plants: Between Conservation and Diversity

References

Badyaev AV (2011) Origin of the fittest: link between emergent variation and evolutionary change as a critical question in evolutionary biology. Proc Biol Sci 278:1921–1929. https://doi.org/10.1098/rspb.2011.0548

Brigandt I, Love AC (2012) Conceptualizing evolutionary novelty: moving beyond definitional debates. J Exp Zool B Mol Dev Evol 318:417–427. https://doi.org/10.1002/jez.b.22461

Britten RJ, Davidson EH (1971) Repetitive and non-repetitive DNA sequences and a speculation on the origins of evolutionary novelty. Q Rev Biol 46:111–133

Caballero L, Benítez M, Alvarez-Buylla ER, Hernández S, Arzola AV, Cocho G (2012) An epigenetic model for pigment patterning based on mechanical and cellular interactions. J Exp Zool B Mol Dev Evol 318:209–223. https://doi.org/10.1002/jez.b.22007

Callebaut W (2010) Innovation from EvoDevo to human culture. In: O'Brien MJ, Shennan SJ (eds) Innovation in cultural systems. MIT Press, Cambridge

Erwin DH (2019) Prospects for a general theory of evolutionary novelty. J Comput Biol 26:735–744. https://doi.org/10.1089/cmb.2019.0089

Fontana W (2001) Novelty in evolution. In: Green Paper for Bio-Evolutionary Advanced Concepts for NASA, pp 1–21

Galis F (2001) Key innovations and radiations. In: Wagner GP (ed) The character concept in evolutionary biology. Academic Press, San Diego, pp 1–13

Ganfornina MD, Sánchez D (1999) Generation of evolutionary novelty by functional shift. BioEssays 21:432–439. https://doi.org/10.1002/(SICI)1521-1878(199905)21:5<432::AID-BIES10>3.0.CO;2-T

Hallgrimsson B, Jamniczky H, Young NM, Rolian C, Schmidt-Ott U, Marcucio R (2012) The generation of variation and the developmental basis for evolutionary novelty. J Exp Zool B Mol Dev Evol 318:501–517. https://doi.org/10.1002/jez.b.22448

Lange A, Nemeschkal HL, Müller GB (2014) Biased polyphenism in polydactylous cats carrying a single point mutation: the Hemingway model for digit novelty. Evol Biol 41:262–275. https://doi.org/10.1007/s11692-013-9267-y

Margulis L, Fester R (1991) Symbiosis as a source of evolutionary innovation. MIT Press, Cambridge

Moczek AP (2008) On the origins of novelty in development and evolution. BioEssays 30:432–447. https://doi.org/10.1002/bies.20754

Moriyama Y, Koshiba-Takeuchi K (2018) Significance of whole-genome duplications on the emergence of evolutionary novelties. Brief Funct Genomics 17:329–338. https://doi.org/10.1093/bfgp/ely007

Müller GB (1990) Developmental mechanisms at the origin of morphological novelty: a side-effect hypothesis. In: Nitecki MH (ed) Evolutionary innovations. University of Chicago Press, Chicago, pp 99–130

Müller GB (2003) Homology: the evolution of morphological organization. In: Müller GB, Newman S (eds) Origination of organismal form. MIT-Press, Cambridge, pp 50–69

Müller GB (2010) Epigenetic innovation. In: Pigliucci M, Müller GB (eds) Evolution - the extended synthesis. MIT Press, Cambridge, pp 307–332

Müller GB, Newman SA (2005) The innovation triad: an EvoDevo agenda. J Exp Zool B Mol Dev Evol 304:487–503. https://doi.org/10.1002/jez.b.21081

Müller GB, Wagner GP (1991) Novelty in evolution: restructuring the concept. Annu Rev Ecol Syst 22:229–256

Newman SA, Bhat R (2009) Dynamical patterning modules: a "pattern language" for development and evolution of multicellular form. Int J Dev Biol 53:693–705. https://doi.org/10.1387/ijdb.072481sn

Newman SA, Müller GB (2000) Epigenetic mechanisms of character origination. J Exp Zool 288:304–317. https://doi.org/10.1002/1097-010X(20001215)288:4<304::AID-JEZ3>3.0.CO;2-G

Nijhout HF (2001) The development and evolution of butterfly wing patterns. Smithsonian Institution Press, Washington, DC

Niklas KJ, Cobb ED, Crawford DR (2013) The evo-devo of multinucleate cells, tissues, and organisms, and an alternative route to multicellularity. Evol Dev 15:466–474. https://doi.org/10.1111/ede.12055

Nuño de la Rosa L, Müller GB, Metscher BD (2014) The lateral mesodermal divide: an epigenetic model of the origin of paired fins. Evol Dev 16:38–48. https://doi.org/10.1111/ede.12061

Peterson T, Müller GB (2016) Phenotypic novelty in EvoDevo: the distinction between continuous and discontinuous variation and its importance in evolutionary theory. Evol Biol 43:314–335. https://doi.org/10.1007/s11692-016-9372-9

Peterson T, Müller GB (2018) Developmental finite element analysis of cichlid pharyngeal jaws: quantifying the generation of a key innovation. PLoS One 13:e0189985–e0189923. https://doi.org/10.1371/journal.pone.0189985

Salazar-Ciudad I, Jernvall J (2005) Graduality and innovation in the evolution of complex phenotypes: insights from development. J Exp Zool B Mol Dev Evol 304B:619–631. https://doi.org/10.1002/jez.b.21058

Shubin N, Tabin C, Carroll S (2009) Deep homology and the origins of evolutionary novelty. Nature 457:818–823. https://doi.org/10.1038/nature07891

Stern S, Fridmann-Sirkis Y, Braun E, Soen Y (2012) Epigenetically heritable alteration of Fly development in response to toxic challenge. CELREP 1:528–542. https://doi.org/10.1016/j.celrep.2012.03.012

Wagner A (2011) The origins of evolutionary innovations: a theory of transformative change in living systems.. OUP Oxford

Wagner GP (2014) Homology, genes, and evolutionary innovation. Princeton University Press, Princeton

West Eberhard MJ (2008) Toward a modern revival of Darwin's theory of evolutionary novelty. Philos Sci 75:899–908. https://doi.org/10.1086/594533

West-Eberhard MJ (2003) Developmental plasticity and evolution. Oxford University Press, Oxford

Zhu J, Zhang Y-T, Alber MS, Newman SA (2010) Bare bones pattern formation: a Core regulatory network in varying geometries reproduces major features of vertebrate limb development and evolution. PLoS One 5:e10892–e10811. https://doi.org/10.1371/journal.pone.0010892

Developmental Homology

James DiFrisco

Contents

Abstract

Homology is the fundamental determinant of the sameness of biological characters or traits. When two characters stand in a relation of homology, they belong to the same character kind. For example, the eyes of humans and birds are homologous as vertebrate eyes – that is, they are the same kind of character: vertebrate eyes. Although the concept of homology originated in pre-Darwinian comparative anatomy, it was subsequently revealed to be an evolutionary phenomenon caused by common descent. Contemporary investigators work roughly within the following generic evolutionary conception of homology:

Homology: Two characters in distinct organisms or taxa are homologous if they are genealogically connected by continuous descent from a common ancestor that had the same character.

J. DiFrisco (✉)
Institute of Philosophy, KU Leuven, Leuven, Belgium
e-mail: james.difrisco@gmail.com

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_74

Keywords

Homology · Developmental homology · Biological characters · Character identity

Introduction

Homology is the fundamental determinant of the sameness of biological characters or traits. When two characters stand in a relation of homology, they belong to the same character kind. For example, the eyes of humans and birds are homologous as vertebrate eyes – that is, they are the same kind of character: vertebrate eyes. Although the concept of homology originated in pre-Darwinian comparative anatomy, it was subsequently revealed to be an evolutionary phenomenon caused by common descent. Contemporary investigators work roughly within the following generic evolutionary conception of homology:

Homology: Two characters in distinct organisms or taxa are homologous if they are genealogically connected by continuous descent from a common ancestor that had the same character.

So, human and bird eyes are homologous because every one of their evolutionary ancestors had eyes, up to and including their most recent common ancestors (the first amniotes). By contrast, human eyes are *not* homologous with insect eyes because their most recent common ancestor (urbilaterians) did not have eyes. Instead, the relation between human eyes and insect eyes is thought to be as a *homoplasy* – a similarity that is due to convergent evolution rather than inheritance from an ancestor.

The concept of homology plays many key roles in biological research, but the following are among the most important.

R1. Homology provides a system of descriptive classification for biological characters.
R2. Homology determines the extent of what is conserved in evolution and thus serves to identify, contrastively, what constitutes evolutionary change or novelty.
R3. Judgments of homology enable phylogenetic inferences that place characters and taxa on phylogenetic trees.
R4. Judgments of homology enable inferences about potential similarities in the genetic and developmental production of homologous characters as well as the propensities of those characters to vary in certain directions.

To fulfill these roles, investigators need more than just the generic evolutionary conception of homology. The latter functions as a reference point so that biologists refer to roughly the same biological phenomenon with the term “homology.” But it

does not specify how to determine genealogical continuity in the absence of direct observation. Moreover, most characters of developing organisms are typically only present for part of their life cycle, and thus their continuity has to be reconstituted across generations. Over time and across species, characters gain and lose features while still remaining homologous, thus we need ways of separating the features of homologues that can change from those that cannot.

To meet these challenges, we need robust *criteria* for individuating homologues. Among the most important criteria for homology that have been proposed are the following (partially corresponding to the above roles R1–R4):

C1. Similarity in descriptive properties of the character, especially complex properties that are unlikely to be independently evolved homoplasies (Riedl 1978; Remane 1956).
C2. Similarity or sameness in the topological position of the character relative to other characters on the body and in the relative positions of internal components of the character (Owen 1843; Jardine 1969);
C3. "Congruence" or agreement with the most probable placement of other characters on a phylogenetic tree, such that homologies are *synapomorphies* or characters that define a monophyletic group (Remane 1956; Bock 1974; Patterson 1982)
C4. Similarity or sameness in the genetic and/or mechanistic generation of the character during development (Van Valen 1982; Roth 1984, 1988; Wagner 1989a, b, 2014).

Roles and criteria (1) and (2) are generally accepted aspects of homology. From the other roles and criteria we can trace the outline of the two main different approaches to homology in the life sciences: a phylogenetic, cladistic, systematic, or "historical approach" (3) versus a developmental or "biological" approach (4).

Phylogenetic Versus Developmental Approaches to Homology

The two main different approaches to homology emphasize different criteria because they have different investigative goals. The main objective of phylogenetic approaches is to discover distributions of characters and patterns of taxa on the evolutionary tree of life. To meet this objective, candidate homologues are identified using C1 and C2 and tested as to how well they fit into known patterns of phylogeny (C3), which are in turn based on relatively more well-confirmed homologies. In the cladistic approach specifically, all homologies are synapomorphies, or characters that are present exclusively in a monophyletic group (an ancestral species together with its evolutionary descendants) (Patterson 1982). Criterion 4 is generally not taken to be a defining feature of homology in this approach, though it may provide supporting evidence for cladistic hypotheses about homology since genes and mechanisms are themselves characters that can be homologized.

The more recent developmental approach to homology – sometimes also called the "biological basis of homology" (Roth 1988; Wagner 1989b) – is oriented toward explaining patterns of conservatism of phenotypic characters and identifying which kinds of phenotypic features tend to get conserved in evolution. Proponents of this approach hold that phylogenetic methods need to be supplemented by a developmental criterion of homology, according to which characters are homologous only if they share the same developmental causes. The nature of these developmental causes is a source of ongoing discussion. They have been identified with (genetic) information (Van Valen 1982), embryological origin or developmental pathways (Roth 1984), developmental constraints (Wagner 1989a), and recently, classes of gene regulatory networks called "Character Identity Networks" (Wagner 2014). Whatever the proposed nature of the developmental basis of homology, the primary rationale is the same. The presence of developmental constraints or dedicated regulatory controls for a given phenotypic character can often explain why that character remains stable over evolutionary time and in different species or why it changes in the way it does. In view of this explanatory goal, phylogenetic approaches are limited to describing and recording character stasis and change without being able to explain them mechanistically. The developmental conception of homology is nonetheless not proposed as a total replacement to the older phylogenetic one, but as a supplemental resource. To have "the same" developmental cause (genes, pathways, constraints, etc.) is to have homologous causes, and the latter notion of homology must be cashed out in phylogenetic terms.

Developmental views of homology have been advanced as a central part of the emerging theoretical structure of evo-devo (see Amundson 2005, Wagner 2014). Although they are not consensus views in the field, they are the most recent innovation in the longstanding discussion on homology and will be the main focus of this chapter. In neo-Darwinian evolutionary theory, by contrast, homology had a less important role. There it was largely viewed as a concept of pre-evolutionary biology that had been successfully explained by Darwinian evolution (Mayr 1982; see Amundson 2005).

One of the main reasons for this disparity is that the neo-Darwinian picture of evolution attributed explanatory primacy to the sorting of variation through selection rather than to the generation and structuring of variation through development. Since evo-devo focuses more on the latter, it affords a more important role for homologues as structural units of variation and variability. Another reason is that the neo-Darwinian perspective on evolution had little to say about organismal or phenotypic evolution, drawing as it did largely from the theoretical resources of evolutionary genetics. Since evo-devo aims to describe, explain, and predict phenotypic evolution, it has more use for homologues as "organizers of the phenotype" (Müller 2003; see Müller 2007). Finally, evo-devo has opened up new possibilities for comparative generalizations across phylogenetic boundaries, and homology is the central concept of comparative biology (Wake 1994). In the neo-Darwinian picture, by contrast, evolution does not occur above the level of populations because selection does not act across reproductively isolated groups. The potential for comparative work to generate insights about *current* evolution is accordingly highly

limited, unless the comparisons are about convergence, which is based on selection. Because it never recognized the important role of development in evolution, traditional evolutionary theorizing failed to predict that a widely shared set of developmental resources, including core regulatory genes and pathways, gets repeatedly re-deployed in developmental evolution. The existence of this well-conserved repertoire, highlighted most dramatically with the discovery of the Hox genes, makes the prospects for comparative work much brighter – and with it a more important role for homology opens up in evolutionary theorizing. Developmental approaches to homology have originated as part of this broader shift in evolutionary theory.

In the remainder of this chapter, I will examine more specific aspects of the homology problem with an emphasis on current approaches and open problems.

Characters: The Relata of Homology

So far homology has been treated as a relation between characters, but what is a character? Can any organismic part, property, or activity whatsoever be homologous with something else? In nineteenth century comparative anatomy, where the concept of homology originated, homology was treated primarily as a relation between anatomical body parts – i.e., organ systems, organs, and their observable components. Since then, the relata of homology have been steadily extended to increasingly diverse features of organisms at multiple levels of organization. Homologues can be organs, but also, tissues, cells, organelles, genes, gene networks, and properties of each. In addition to homologies of structure, theorists have recognized that developmental processes (Gilbert and Bolker 2001), organismic behaviors, and activities of parts can be homologous. With each extension of homology, the guiding rationale is typically to organize the domain of study under a comparative evolutionary framework. From the perspective of the generic evolutionary conception of homology, there is no reason to restrict the relata of homology as long as the general condition of common descent is met, though of course not every judgment of homology is guaranteed to be biologically interesting.

Approaches to homology from systematics and cladistics tend to share this permissive view of the relata of homology, but with an additional pragmatic restriction. Homologues or synapomorphies should be "good" characters for purposes of cladistic analysis: they should be readily identifiable and should be able to provide operational identifying features of monophyletic groups. In general, structural features are more likely than functional features to be good cladistic characters, due primarily to their greater stability over organism lifetimes and their potential to leave behind fossil traces.

Developmental approaches to homology are more restrictive about what can be a homologous character. In Wagner's (1989a, b, 2014) approach, genuine characters should be "developmentally individualized": there should be a dedicated regulatory module that controls a character's development and constrains it against variation. Organism features that do not meet this requirement include pseudo-characters and character states. Pseudo-characters are those that arise merely as by-products of

interaction, adjacency, or overlap between individualized characters. An example is the bone protrusion of the human chin, which is a structural by-product of the differential regression of parallel growth fields of the jaw due to ancestral selection for smaller teeth (McKinney and McNamara 1991, 232; Gould and Lewontin 1979). Character states include properties of characters such as size, shape, and color (Müller 2003). Both kinds of noncharacter lack the key feature of variational independence in the process of developmental evolution (Brigandt 2007) and are expected to be much more evolutionarily labile than individualized characters. Accordingly, they are less relevant to the phenomenon of morphological stasis that developmental approaches seek to explain. The evolutionary lability of character states also makes it more difficult to determine if they are homologous or homoplastic than it is with individualized characters. On this more restricted construal, characters will generally be body parts and cells. But the requirement of developmental individualization also means that the underlying developmental causes (modules and genes) must be homologized – under a phylogenetic definition.

Systematists such as Bock (1974) and Patterson (1982) reject the idea that the distinction between characters and character states has general evolutionary or phylogenetic significance beyond the fact that character states are homologues within a narrower taxonomic group. Moreover, something that is a pseudo-character from the perspective of development (like the human chin) could nonetheless be a good character for taxonomic purposes. Another difference between approaches on the character issue is that phylogenetic relatedness of characters can diverge from developmental or mechanistic sameness, especially when we are comparing an ancestral character with a highly derived one. For example, the remarkable homology between the jaw joint of reptiles and the ear ossicles of mammals captures a relationship of phylogenetic transformation, but these characters are not homologous or the same from a developmental or mechanistic point of view (Bock and Cardew 1999, 21). Individualized characters produced by the same developmental processes may turn out to be sympleisomorphies – i.e., shared ancestral characters not definitive of monophyletic groups – more often than synapomorphies (Roth 1991, 173).

Serial Homology

In classical approaches such as that of Richard Owen (1843), homology was not just a relationship between characters in different organisms but could also be a relationship between characters in the same organism. The former is traditionally referred to as "special" homology, whereas the latter is "serial" or "iterative" homology. Examples of serial homology include vertebrae in the vertebral column, insect body segments, tree leaves, moth and butterfly wing spots (see Fig. 1), cells belonging to the same cell type, and right-left symmetric organs such as kidneys, hands, or eyes (sometimes called "antimeric" homologies).

The criteria used to identify serial homologues are descriptive similarity (C1) and similarity or sameness in genetic and/or mechanistic generation (C4). Because serial homologues are different particular characters within the same organism, they

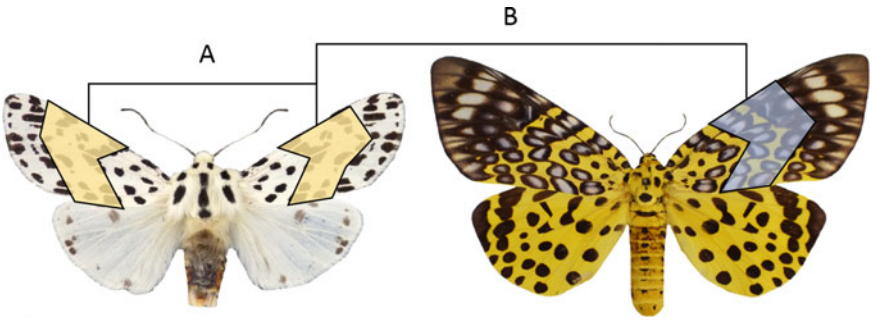


Fig. 1 Serial (**a**) and special (**b**) homologies between the central symmetry systems (highlighted) of lepidopteran wing patterns. In *Hypercompe permaculata* (pictured left), the central symmetry system on the right forewing is serially homologous with the same character on the left forewing, and specially homologous with the central symmetry system on the right forewing of other species such as *Sebastia argus* (pictured right). Individual spots within each central symmetry system are also serial homologues. (Photo credit: Richard Gawne. Specimens are from the collection of the Natural History Museum of Vienna)

cannot occupy the same topological position (C2) without modification of that criterion, and phylogenetic methods don't apply at all (C3). Accordingly, serial homology can play similar theoretical roles as special homology except for enabling phylogenetic inference (R3). Many of the same arguments concerning characters and relata can be applied to serial homology as well. Serial homologues can include many kinds of organism features such as genes and behaviors, but only if they can be partitioned into countable units. In the case of special homology, by contrast, noncountable features like color and shape can be homologized more easily because they inhere in countable organisms. The requirement of developmental individualization therefore has an additional motivation in the case of serial homology.

As might be expected, proponents of the phylogenetic approach do not consider serial homology to be a genuine case of homology (Patterson 1982). Since congruence (C3) is taken to be the ultimate test of homology in this approach, and is inapplicable here, serial homology is viewed as untestable, ill-defined, and potentially arbitrary. Serial homologues are not synapomorphies and have no role to play in phylogenetic inference (except to the extent that having iterated parts of a certain sort is a special homology that could be a good cladistic character).

Proponents of developmental or biological approaches have, in turn, interpreted the lack of a phylogenetic definition of serial homology as a strike against the phylogenetic view (Roth 1984; Wagner 1989b; Ramsey and Peterson 2012). Clearly, there is an important sense in which a human's vertebrae are repetitions of the same character, and so purely phylogenetic approaches to homology seem to miss an important aspect of character sameness. Since phylogenetic methods and criteria don't apply, the reasoning goes, a developmental, genetic, or organizational criterion must be constitutive of homology. Serial homology also has an important role to play in evolution. Duplication and subsequent divergence of characters is a key evolutionary mechanism for generating phenotypic complexity and functional

specialization. This is particularly true of gene duplication, where serially homologous genes (*paralogues*, as opposed to *orthologues*, which are specially homologous genes) can acquire new functions fairly rapidly.

Parties to the debate about serial homology have not provided much justification for the guiding assumption that serial homology, if it is to exist, must fall under the same definition as special homology. Developmentalists assume that a unified definition is a desideratum that phylogeneticists are unable to satisfy (Roth 1984; Wagner 1989a, b). Phylogeneticists are under pressure to reject serial homology in order to maintain their definition, but only on the assumption that the phylogenetic conception of special homology is threatened by serial homology. It is worth asking what would be lost, aside from simplicity, if we were to maintain different definitions, such as a phylogenetic definition of special homology and a developmental definition of serial homology, under the recognition that they represent different phenomena of character sameness. The resolution of this issue depends on whether special and serial homologues have similar roles to play in developmental evolution, which is an open question.

Homology, Homoplasy, Parallelism, and Deep Homology

Homology as the determinant of character sameness is standardly contrasted with homoplasy and parallelism. *Homoplasy*, we saw, is character similarity due to convergent evolution rather than common origin. *Parallelism* is similarity due to convergent or independent evolution of characters *that share the same (homologous) developmental basis* (Hall 2003). The developmental homology underlying parallel characters means their evolution is not totally independent, thus parallelism is distinguished from "true" convergence. A term that is no longer commonly used in technical discussions is *analogy*, which is functional similarity of any sort, considered without reference to phylogeny. Whereas homology and homoplasy are dichotomous, homologous characters can be analogous if they have similar functions.

Although the distinctions between homology, homoplasy, and parallelism are clear conceptually, they are often difficult to distinguish in practice in real biological systems (Wake 1991). One general issue concerns how to determine homology of developmental causes. The development of morphological characters typically involves interactions between networks of regulatory genes. But the conditions for homology of gene regulatory networks are not well-established (Abouheif 1999). Do homologous networks need to share all the same genes and interactions, or just some, and if it's the latter, how many? One way to handle these questions is to say that network homology can be partial rather than being an all-or-nothing affair (Abouheif 1999; Minelli 1998). Allowing this move has important consequences, however. If homology between developmental causes can be partial and is also required for homology between morphological characters – as in developmental approaches – then homology between morphological characters should also be allowed to be partial. That means characters can be partly homologous and partly

parallel, or partly parallel and partly homoplastic, depending on the degree of homology of their developmental causes. The clear-cut distinctions between homology, parallelism, and homoplasy become somewhat blurred. One way of resisting this conclusion and maintaining that homology between gross morphological characters is all-or-nothing is to hold that only a core subset of regulatory networks needs to be the same in order for the characters to be homologous (Wagner 2014).

A new reconfiguration of the distinctions between homology, homoplasy, and parallelism has arisen from the discovery from developmental genetics that the same "toolkit" of regulatory genes and circuits is widely shared across very different taxonomic groups. A famous example of this came when Gehring and colleagues discovered that homologous regulatory genes (*eyeless*/*Pax-6*/*Aniridia*) control the morphogenesis of eyes in *Drosophila*, mice, and humans, respectively (Quiring et al. 1994). Traditionally, the similarity between compound insect eyes and camera vertebrate eyes had been considered a paradigm case of convergent evolution – as expressed in our opening example above – demonstrating the power of natural selection to produce similar outcomes from independent starting points. But in fact, even though vertebrates and insects diverged over 500 million years ago, their starting points are not independent because they share much of the same regulatory circuitry. This evolutionary pattern, in which characters that have diverged deep in the evolutionary past share the same toolkit genes or networks, has been called *deep homology* (Shubin et al. 1997). Deep homology does not mean that the characters are homologous (though they could be) but that they share homologous genes and/or networks. Deep homologies have been discovered for many other characters besides eyes, such as between tetrapod limbs and fish fins, and between beetle horns and insect legs (Shubin et al. 2009), and may prove to be extraordinarily common.

These recent developments have a number of empirical and conceptual implications for our understanding of homology and evolution. The occurrence of deep homology suggests that novel phenotypic structures need not arise from de novo genetic changes but instead can result from the re-deployment of existing regulatory circuitry. In turn, this throws some light on the apparent ease and speed with which evolutionary novelties can arise and become adaptive. But it also means that the conceptual distinction between homology and novelty (R2) may become more difficult to parse whenever the new is produced by a modification of the old. Deep homology also explains the common occurrence of multiple gains and losses of characters in related taxa by pointing to the persistence of developmental potentials that are not always expressed. This raises the conceptual question of whether characters that lack genealogical continuity because they are "blinking" on and off in evolution should nonetheless count as homologous in virtue of the continuity of their developmental causes (Ramsey and Peterson 2012). The same question can be asked about atavisms and ectopically expressed characters. Finally, the occurrence of deep homology suggests that standard methods have overestimated the extent of convergent evolution, and parallelism may be much more common than has been assumed (McGhee 2011). More generally, the role of development may have to be stronger and the role of selection weaker, in explaining patterns of phenotypic evolution.

Challenges and Outlook

An ongoing challenge for work on homology is to understand how phylogenetic and developmental approaches ultimately relate to each other. One perspective on their relationship is that the approaches are incommensurable and retain different definitions because they have different scientific goals. There is some truth to this idea, but it is only part of the story. As we saw earlier, developmental approaches are parasitic on phylogenetic approaches when it comes to determining the homology of developmental causes for a morphological character. But phylogenetic views also stand to be enriched by developmental considerations. Congruence tests (C3) typically operate with a differential weighting of characters according to their probabilities of independent loss or gain, or modification. Clearly, these probabilities will be influenced by the causal structure of development. Ontogeny also plays a major role in determining the "polarity" of character transformation series – i.e., deciding which homologies are primitive and which are derived – often on the basis of recapulationist principles (Patterson 1982). In general, if evolution is the evolution of development, then the biological study of development will tell us something about the historical course that evolution has actually taken. The relationship between approaches to homology is therefore more of a mutualism than parasitism or independence (Roth 1991). What exactly is required for a productive integration of approaches is less clear. For example, does the coordination of their descriptive and explanatory resources require a unified definition of homology, or some weaker kind of linkage?

A major challenge specific to the newer developmental approaches concerns the idea of a one-one mapping between developmental causes and phenotypic characters. Among other things, this mapping is supposed to provide the element of continuity for characters that must be rebuilt across generations, i.e., most characters (Van Valen 1982; Roth 1984). But is this one-one mapping requirement actually satisfied in evolution? Embryologists have long been aware that homologous morphological characters can have nonhomologous developmental origins, and similar dissociations have been recorded at all levels of organization since then (Wray and Abouheif 1998). Proponents of the developmental approach have kept pace with these conflicting data by specifying increasingly narrow types of developmental causes that carry the thread of character identity, with Wagner's (2014) "character identity networks" being the most recent model. However, a challenge for all genetic models of developmental homology comes from cases of "developmental system drift" (DSD) (see chapter ▶ "Developmental System Drift"). DSD occurs when homologous characters accumulate differences in the genes or networks that control them. It can be caused by neutral drift, selection on characters controlled by pleiotropic genes, or a combination of selection and drift. DSD presents a picture of evolution in which the thread of character identity doesn't always lie at the level of specific genes or even gene networks. This picture of character identity fits more readily with developmental theories that stress the autonomy of morphological homology from homology at lower levels of organization (Müller 2003; Newman and Müller 1999; Scholtz 2005). DSD as well as genetic models of homology are nonetheless active and ongoing topics of research, and their relationship constitutes

an important unresolved problem of homology. Different responses in evo-devo to the dissociation of homologues across levels of organization are discussed in more detail in Nuño de la Rosa and Etxeberria (2009).

Finally, homology is a problem-area where philosophical resources can be – and have been – productively deployed. The topics raised by reflection on homology include definitions of scientific concepts, character individuation and decomposition of biological systems, typological versus population thinking, biological classification and natural kinds, conceptual change in science, explanatory pluralism and integration of perspectives, and more. To take the first of these, it is important to ask what sort of definition and criteria of individuation should be sought. With homology, biologists are attempting to ascertain character sameness in spite of the many variations in form, growth pattern, function, and mode of life that are found in divergent taxonomic groups. If no constitutive feature of homology (e.g., sameness of topological position) is immune to the contingencies of evolutionary change, then we should not expect to find a definition of homology that provides universally valid necessary and sufficient conditions for homology and that is at the same time theoretically adequate and practically useful. A natural response to this situation is to shift towards pluralism about definitions or a context-sensitive framework in which homology may be determined by different combinations of factors in different cases (Minelli 1998). A challenge for such alternatives is to offer directives that are not too vague to be concretely implemented and that can successfully deliver judgments of homology in practical contexts. For this task, it will be essential to keep the major biological roles for homology (R1-R4) in focus, while mapping out the classes of contexts where criteria of homology (C1-C4) tend to break down. Then workers can identify how to use the criteria of homology in specific biological contexts in a way that accesses the considerable utility of the concept.

Cross-References

- ▶ Convergence
- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Developmental System Drift
- ▶ Form and Function in Evo-Devo
- ▶ Inherency
- ▶ Typology and Natural Kinds in Evo-Devo

References

Abouheif E (1999) Establishing homology criteria for regulatory gene networks: prospects and challenges. In: Bock GR, Cardew G (eds) Homology, novartis foundation symposium, vol 222. Wiley, Chichester, pp 207–225

Amundson R (2005) The changing role of the embryo in evolutionary thought: roots of evo-devo. Cambridge University Press, New York

Bock WJ (1974) Philosophical foundations of classical evolutionary classification. Syst Zool 22:375–392

Bock GR, Cardew G (eds) (1999) Homology, novartis foundation symposium, vol 222. Wiley, Chichester
Brigandt I (2007) Typology now: homology and developmental constraints explain evolvability. Biol Philos 22:709–725
Gilbert SF, Bolker JA (2001) Homologies of process and modular elements of embryonic construction. In: Wagner G (ed) The character concept in evolutionary biology. Academic, San Diego, pp 437–456
Gould SJ, Lewontin RC (1979) The Spandrels of San Marco and the Panglossian Paradigm: A Critique of the Adaptationist Programme. Proceedings of the Royal Society of London B, 205:581–598
Hall BK (2003) Decent with modification: the unity underlying homology and homoplasy as seen through an analysis of development and evolution. Biol Rev 78:409–433
Jardine N (1969) The observational and theoretical components of homology: a study based on the morphology of the dermal skull-roofs of rhipidistian fishes. Biol J Linn Soc 1:327–361
Mayr E (1982) The growth of biological thought. Cambridge UP, London
McGhee G (2011) Convergent evolution: limited forms most beautiful. Cambridge, MA: MIT Press
McKinney ML, McNamara KJ (1991) Heterochrony: the evolution of ontogeny. Springer, New York
Minelli A (1998) Molecules, developmental modules, and phenotypes: a combinatorial approach to homology. Mol Phylogenet Evol 9(3):340–347
Müller GB (2003) Homology: the evolution of morphological organization. In: Müller GB, Newman SA (eds) Origination of organismal form: beyond the gene in developmental and evolutionary biology. MIT Press, Cambridge, MA
Müller GB (2007) Evo-devo: extending the evolutionary synthesis. Nature Reviews Genetics 8: 943–949
Müller GB, Newman SA (1999) Generation, integration, autonomy: Three steps in the evolution of homology. In: Bock, G. R. and G. Cardew. (eds.) 65–73
Nuño de la Rosa L, Etxeberria A (2009) Pattern and process in evo-devo: descriptions and explanations. In: H. W. de Regt et al (eds) EPSA Philosophy of Science, Amsterdam
Owen R (1843) Lectures on the comparative anatomy and physiology of the invertebrate animals. Delivered at the Royal College of Surgeons, in 1843. Longman, Brown, Green, and Longmans, London
Patterson C (1982) Morphological characters and homology. In: Joysey KA, Friday AE (eds) Problems in phylogenetic reconstruction. Academic, London, pp 21–74
Quiring R, Walldorf U, Kloter U, Gehring WJ (1994) Homology of the eyeless gene of Drosophila to the small eye gene in mice and Aniridia in humans. Science 265(5173):785–789
Ramsey G, Peterson AS (2012) Sameness in biology. Philos Sci 79(2):255–275
Remane A (1956) Die Grundlagen des natürlichen Systems, der vergleichenden Anatomie und der Phylogenetik. Geest and Portig, Leipzig
Riedl R (1978) Order in living organisms: a systems analysis of evolution (trans: Jefferies RPS). Wiley, Chichester
Roth VL (1984) On homology. Biol J Linn Soc 22:13–29
Roth VL (1988) The biological basis of homology. In: Humphries CJ (ed) Ontogeny and systematics. Columbia University Press, New York, pp 1–26
Roth VL (1991) Homology and hierarchies: problems solved and unresolved. J Evol Biol 4:167–194
Scholtz G (2005) Homology and ontogeny: pattern and process in comparative developmental biology. Theory in Biosciences 124(2):121–143
Shubin N, Tabin C, Carroll S (1997) Fossils, genes, and the evolution of animal limbs. Nature 388:639–648
Shubin N, Tabin C, Carroll S (2009) Deep homology and the origins of evolutionary novelty. Nature 457:818–823
Van Valen L (1982) Homology and causes. J Morphol 173:305–312
Wagner GPW (1989a) The origin of morphological characters and the biological basis of homology. Evolution 43(6):1157–1171

Wagner GPW (1989b) The biological homology concept. Annu Rev Ecol Syst 20(1):51–69
Wagner GPW (2014) Homology, genes, and evolutionary innovation. Princeton University Press, Princeton
Wake DB (1991) Homoplasy: the result of natural selection, or evidence of design limitations? The American Naturalist 138(3):543–567
Wake DB (1994) Comparative terminology. Science 265:268–269
Wray GA, Abouheif E (1998) When is homology not homology? Curr Opin Genet Dev 8:675–680

Developmental System Drift

Eric S. Haag and John R. True

Contents

Abstract

Developmental System Drift (DSD) is an evolutionary phenomenon whereby the genetic underpinnings of a trait in a common ancestor diverge in descendant lineages even as the trait itself remains conserved. Evidence for DSD comes from

E. S. Haag
Department of Biology, University of Maryland, College Park, MD, USA
e-mail: ehaag@umd.edu

J. R. True (✉)
Department of Ecology and Evolution, College of Arts and Sciences, Stony Brook University, Stony Brook, NY, USA
e-mail: john.true@stonybrook.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_83

both interspecies hybridizations and comparative developmental genetic studies. The widespread occurrence of DSD implies that developmental systems are constantly evolving, even in the absence of selection for morphological change. Similar implications have been found in studies of the genetics of hybrid inviability and infertility, which reflect divergence in complex developmental systems that are perpetually under strong selection in all taxa. Gene duplications and compensatory changes in proteins and gene regulatory networks have been proposed to be the key mechanisms that drive DSD. DSD has implications for phylogenetic inference and biological homology, experimental tests of interspecies conservation of gene function, and convergent evolution. The burgeoning data and methods of comparative genomics, genome editing, and systems biology promise to greatly enhance our understanding of the dynamics and mechanisms of DSD.

Keywords

Homology · Hybrid incompatibility · Compensatory evolution · Genetic divergence · Developmental pathways

Introduction

Homologous traits reflect the common ancestry of related taxa. If a trait is invariant in a focal taxon, it may be clear the character in question has remained unchanged in the descendant lineages. For example, none doubt that radius and ulna of vertebrate forelimbs are homologous bones across tetrapods. However, morphological characters are the products of developmental pathways whose components and function can be delineated only with significant experimental effort (see chapter ▶ "Developmental Homology"). The assumption that the developmental genetic mechanisms underlying production of homologous traits are similarly stable is thus a convenient one for both evolutionary developmental biology (evo-devo) and for biomedical research that relies on non-human model organisms. For evo-devo, it would appear to allow one to focus on phenotypic novelty without collateral shifts in conserved traits. For biomedical research, it would indicate that findings from model organisms should consistently translate seamlessly to human development. Surprisingly, however, the assumption that the development of homologous traits does not change substantially appears to often be erroneous.

Developmental system drift (DSD) is defined (True and Haag 2001) as the process by which conserved traits diverge in their developmental genetic underpinnings over evolutionary time. This concept was independently articulated by Weiss and Fullerton (2000) as "phenogenetic drift." Motivated by modern developmental genetics research, neither group was apparently aware that the essence of the process and how it may be recognized was proposed much earlier by I.I. Schmalhausen in his 1949 book *Factors of Evolution: The Theory of Stabilizing Selection* (Schmalhausen (1949); see chapter ▶ "Ivan I. Schmalhausen (1884–1963)":

> The constant cooperation of combinations of factors which are normal for a definite population makes them increasingly integrated in the processes of development. For example, neutral factors, which participate in the general metabolic processes, are incorporated little by little into the general mechanism of individual development and become essential factors. There inevitably arise combinations in which some factors become dependent upon others. . . . There develop balanced genetic systems which may have a limited distribution as local forms but which also may diverge further. . . . Introduction of foreign elements by crossing with other local forms may partly destroy an established system, may cause a sharp increase in variability, and may give rise to numerous hybrid individuals of low viability. (pp. 206–207)

As the above passage suggests, the genetic networks that regulate development may be constantly modified as long as the outcome is viable, and alternative changes in different lineages can create hybrid incompatibility. This notion is related to the evolution of Bateson–Dobzhansky–Muller incompatibilities (BDMIs), in which allelic substitutions restricted to (and well tolerated in) isolated lineages create incompatibilities that can manifest in hybrids. DSD is distinct, however, in that it can include the introduction and loss of genetic elements, or of interactions between them. These sorts of changes presumably take longer than gene-for-gene BDMIs. On a more practical level, DSD is often inferred by perturbing development in organisms too diverged to hybridize, but which maintain homologous traits.

It is also important to distinguish DSD from random genetic drift, which is a completely distinct process. In random genetic drift, allele frequencies change by chance due to sampling errors from one generation to the next. This can happen to all types of alleles and is a function of effective population size. For neutral alleles, random genetic drift and migration are the only ways in which allele frequencies can change. DSD, on the other hand, occurs in the presence of stabilizing selection on a trait. The genes involved in the development of that trait are thus inferred to be subject to continuous natural selection. However, random genetic drift following the origination of certain types of variation may contribute to the occurrence of DSD in diverging lineages over time (see the section "Theoretical Mechanisms of DSD").

Types of Evidence for DSD

Species Hybrids

Closely related species can often be hybridized to some extent, and some interspecies hybridizations show defective development of specific traits that are identical in both parental species. This is not restricted to developmentally trivial cases, such as sterility due to meiotic failure stemming from karyotype evolution, and includes anatomical traits with unambiguous homology. For example, F1 hybrids between the flies *Drosophila melanogaster* and *Drosophila simulans* often lack bristles found in both pure species (Takano 1998). This indicates that the presence in the hybrid cells of incompatible, species-specific allelic variants of one or more loci in the bristle-specifying gene network destabilizes the trait. That developmental networks producing such a

trait would diverge despite the apparent role of stabilizing selection on the phenotype was one of the observations leading to the formulation of the DSD idea.

Embryological Evidence

Cases of DSD are also recognized by comparisons of embryonic development. One noteworthy case is the two distinct ways that the distal limbs (autopods) develop in tetrapods (Shubin and Alberch 1986). While the phalanges of salamanders are clearly homologous to those of other tetrapods, they develop a distinctive preaxial (i.e., anterior-first) mode, whereas all others develop in the postaxial (i.e., posterior-first) fashion. This divergence appears to be very old and is correlated with the unique ability of salamanders to regenerate the autopod in adulthood.

Another example of embryonic DSD concerns the alterations of embryonic cell lineages and of gastrulation in nematodes. These animals have a relatively simple, stereotyped anatomy and embryos that are generally similar in size and yolk content. Nevertheless, they yet often differ greatly in the timing and order of founder cell formation (Schulze and Schierenberg 2011). In some cases, basic morphogenetic movements, such as gut primordium formation during gastrulation, are strikingly different.

Revealing DSD by Perturbing Cells and Genes: Caenorhabditis as a Model Genus

Because of ease of culture, their transparent bodies composed of clearly homologous cells and tissues, and a rich set of tools for functional genomics, nematodes (*Caenorhabditis* and a few other taxa) have made numerous contributions to the characterization of DSD. At the level of individual genes, transgene assays have clarified how stabilizing selection produces constant gene expression patterns in different species of *Caenorhabditis,* even as the regulatory elements that enable them diverge (Barriere et al. 2012). These assays can be seen as related to hybridization, in that they place diverged sequences in the same cell. However, they can be performed even when the transgene source is too distantly related to hybridize, and can be configured with phenotypically neutral reporters that limit abnormalities that would otherwise complicate interpretation.

One of the first cases of DSD to be documented experimentally pertains to the development of the nematode vulva. This opening develops in the last larval stage and allows insemination and oviposition. It is unambiguously homologous across the entire phylum. However, despite the stereotyped nature of the organ, vulval development has diverged in ways that are both overt and cryptic. *Pristionchus* and *Caenorhabditis* are members of the Diplogastridae and Rhabditidae families of the order Rhabditida, respectively, but retain a common starting point for vulval development, the 12 PN.p cells. Comparisons of vulval development within and between these genera have revealed both obvious and hidden variation.

In both *Caenorhabditis* and *Pristionchus,* the same three progenitor cells, P5.p, P6.p, and P7.p, give rise to the vulval cells. Some differences are apparent, however. The orientations of some divisions are altered, the two most central P6.p descendants divide one more time in *Caenorhabditis* than in *Pristionchus,* and the fate of PN.p cells that do not contribute directly to the vulva differs. In *Caenorhabditis,* the vulval founders and three PN.p cells flanking them (P3.p, P4.p, and P.8.p) form a vulval equivalence group, any three of which can form the vulva if necessary (Sternberg and Horvitz 1986). PN.p cells outside of this equivalence group fuse with surrounding hypodermal cells. In contrast, in *Pristionchus* (and other genera as well), all but one of the PN.p cells outside of the three vulval founders die by apoptosis (Sommer and Sternberg 1996). Genetic analysis in *Caenorhabditis elegans* and *Pristionchus pacificus* revealed that the expression of the LIN-39 Hox gene specifies the size of the vulval equivalence group in each species (Clark et al. 1993), and that cells lacking this transcription factor adopt the terminal, nonvulval fates. In summary, *lin-39* is crucial for vulval pattern formation in both systems, but it has diverged in both expression and in the default mode of PN.p development it represses to allow vulval fates.

Given their substantial evolutionary divergence and subtle differences in final adult morphology, perhaps the above differences between *Caenorhabditis* and *Pristionchus* reflect adaptations of some kind. Within each genus, however, no differences in PN.p fates are apparent, so one might expect that in these cases development is wholly congruent. Surprisingly, even here vulval development shows abundant variation, though cryptic. In both *Pristionchus* (Sommer 1997) and *Caenorhabditis* (Delattre and Felix 2001; Felix 2007), cell ablations reveal quantitative variations in the role of intercellular signaling pathways in shaping the final organ. These findings point to a general principle that likely characterizes many developmental systems in early stages of divergence: cryptic differences in genotype are revealed upon perturbation. Even more strikingly, substantial cryptic variation in the relative strength of signaling inputs was revealed within natural variants of one species, *C. elegans* (Milloz et al. 2008). Informative perturbations also include mutations and overexpression of signaling factors, which can reveal previously unappreciated intergenic interactions and polymorphism in factors influencing vulval development (Felix 2007).

Kiontke and colleagues combined data for cell lineages, responses to cell ablations, and characterization of late morphogenesis events across 51 different broad-sense Rhabditid species (including Diplogastrids) (Kiontke et al. 2007). By mapping these developmental attributes on a well-resolved phylogeny, they found evidence for rampant DSD. For example, in *C. elegans,* most of its closest *Caenorhabditis* relatives, and the distant relative *Rhabditoides inermiformis,* a signal from the anchor cell (AC) of the gonad is required to induce the central PN.p cells to adopt the vulval precursor fate. In contrast, in other taxa (including one *Caenorhabditis* species) this signal is produced by several somatic gonad cells, such that AC ablation no longer eliminates vulval development. In one clade (*Mesorhabditis*), vulval induction has become completely gonad-independent. The distribution of these variants (and of others characterized by Kiontke et al.) suggests that evolution has sampled several alternative ways to specify vulval precursors, that the method used is largely decoupled from final morphology and that reversals are possible.

The above work has shown that a single nematode organ, the vulva, is a hotbed of DSD activity at all scales of divergence examined. However, it necessarily only involves a few cells and a subset of the genome. Might it be an exceptional case? One group has provided evidence that it is not. Working with *C. elegans* and its close relative, *Caenorhabditis briggsae*, Verster et al. performed a genome-scale comparison of RNA interference knockdown phenotypes for orthologous genes (Verster et al. 2014). By focusing on roughly 1300 genes whose *C. elegans* orthologs were known to have a strong RNAi phenotype, a large number of phenotypic differences were discovered. After controlling for differences in RNAi efficacy, the authors identified 91 cases in which the *C. briggsae* knockdown had a much weaker phenotype than that for *C. elegans*. Many of these impacted transcription factors and genes restricted to the nematodes. These experiments indicated that both changes in expression and changes in gene interaction networks contribute to the divergent phenotypes.

Theoretical Mechanisms of DSD

Gene Duplication and Subfunctionalization

Subfunctionalization of gene duplicates (Force et al. 1999) in isolated lineages is one simple way in which DSD could occur (Fig. 1a). In this scenario, a regulatory gene is duplicated in the common ancestor of two species and the two duplicates divide the regulatory roles differently in the two descendant lineages. As a result, the regulation of the target gene(s) qualitatively differs between the ancestral and derived states, even though their expression has remained identical. This scenario involves an initial phase of redundancy before DSD has occurred. In principle any case of redundant gene regulation can give rise to such divergence. Over extended evolutionary time, there may be a "churn" of such duplication and subfunctionalization events among regulators in complex developmental pathways, wholly independent of developmental and phenotypic outcomes.

Compensatory Evolution

Gene duplication opens the door to subfunctionalization by creating transient redundancy that can be shed in alternative ways in different lineages. This general mechanism of DSD can be applied to other aspects of molecular and developmental evolution, both as a neutral process and as a reaction to adaptive changes (Haag 2007). At the level of molecular interactions, participants in stable complexes can nevertheless evolve to become incompatible between lineages, even in the absence of directional selection (Haag et al. 2002). At the level of genetic pathways, redundant inputs can shift quantitatively without any perceptible change in phenotype (Fig. 1b). For example, both Wnt and EGF signals are used to induce the primary vulva fate in *Caenorhabditis* nematodes, but both inter- and intraspecific variation in their relative importance appears to be rampant (Felix 2007).

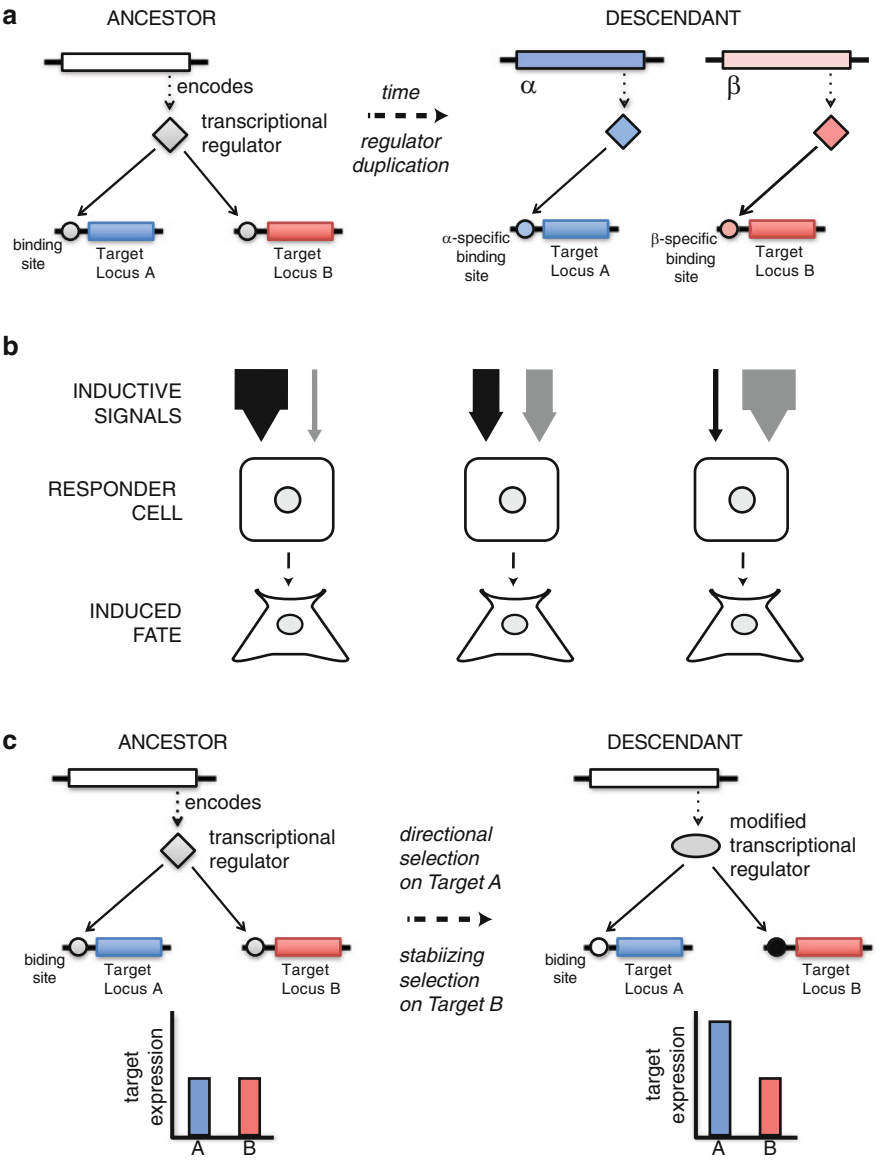


Fig. 1 Three mechanisms of DSD evolution. (**a**) DSD via subfunctionalization of regulatory loci. Ancestrally, a regulatory locus encodes a protein that regulates the transcription of loci A and B by binding to a site that is present in the promoters of both loci. The regulatory locus undergoes duplication into the α and β loci, which evolve specific interactions by which α becomes the regulator of locus A and β becomes the regulator of locus B. (**b**) DSD in cell fate determination via redundant signals. Diagrams depict two signaling pathways that induce a target (squared) cell to differentiate into a particular, conserved cell type. In the center, the two signals are balanced and (at least partially) redundant. In the left scenario, signal A (black) has been enhanced, allowing signal B (gray) to diminish with no impact on phenotype. In the right, the converse has occurred. In

The role of adaptive change in promoting DSD has been explored by Johnson and Porter (2007), who elaborated and tested (via simulations) a gene pathway model in which regulatory evolution leads to DSD and hybrid incompatibility between isolated lineages diverging from a common ancestor (Fig. 1c). The scenario that most frequently led to DSD involved a pleiotropic regulatory gene that controls the expression of two downstream genes. When one undergoes directional selection and the other stabilizing selection, DSD occurs in the latter gene due to compensatory evolution of its promoter. These compensatory changes are needed because the pleiotropic regulator undergoes changes favoring change in expression of the gene that is under directional selection. However, the overall output of the gene under stabilizing selection (the molecular phenotype) does not change. Several bouts of sex-specific compensatory change (favoring increased expression of somatic sexual identity in one sex or the other) have been proposed to underlie the DSD of the somatic sex determination system in animals (Pomiankowski et al. 2004). An important insight from the above is that while DSD may appear to be neutral, it may often be driven or accelerated by adaptation in other traits that share regulatory components.

Implications of DSD

The Practice of Evolutionary Developmental Biology

One of the key goals of evolutionary developmental biology is to understand how novel forms evolve (see chapter ▶ "Developmental Innovation and Phenotypic Novelty"). In searching for such explanations, it would be expedient to be able to assume that aspects of form that are not evolving would retain a constant developmental specification. Indeed, the pioneering molecular evolutionist Emile Zuckerkandl made a comparable prediction about the evolution of macromolecular sequences in "living fossil" taxa that had changed little over hundreds of millions of years:

Fig. 1 (continued) both the left and right cases, the minor pathway can be lost completely, creating a striking case of DSD. (**c**) DSD in the branched pathway model of Johnson and Porter (2007). Ancestrally, a regulatory locus controls expression of loci A and B, each of which have a promoter element (gray) that binds to the regulatory protein with the same affinity, resulting in the optimal expression levels of both genes (here shown as the same level, but they may be expressed at different levels). Directional selection occurs to increase the expression of locus A, which often involves both changes in the A promoter (gray circle in locus A has changed to white) and in the sequence/properties of the regulatory protein (diamond to oval). At the same time, stabilizing selection acts on locus B, necessitating compensatory changes. This is envisioned as occurring via changes in the promoter of locus B (gray to black change). As a result of this process, DSD has occurred in the regulation of locus B. Additional inhibitory factors (not shown) may also be recruited to further attenuate the interaction between the evolved regulatory protein and the B promoter, further magnifying the DSD at locus B.

> My own view is that it is unlikely that selective forces would favor the stability of morphological characteristics without at the same time favoring the stability of biochemical characteristics, which are more fundamental. (Zuckerkandl 1965)

This argument makes sense if we think like human engineers, along the lines of "if it ain't broke, don't fix it." However, life is not the product of engineering, and it now appears that the parts and processes that produce stable, homologous traits (genes, gene interaction networks, and embryological processes that emerge from them) are being "fixed" constantly. Ironically, Zuckerkandl and his colleague and mentor, Linus Pauling, also first demonstrated the utility of a molecular clock (Zuckerkandl and Pauling 1962), whose very existence (and utility) depends on molecular evolution that is largely decoupled from morphology.

Biological Homology and Phylogenetic Inference

A fundamental function of phylogenetic inference is to support the reconstruction of trait evolution. When the trait distribution still leaves the pattern ambiguous, however, details of the trait's development may provide support for homology. The existence of DSD, however, implies that differences in developmental specification do not necessarily rule out trait homology. Wagner has proposed that the resolution lies in not becoming overly dependent on the role of any one gene in generating a phenotype, and to instead search for the more broadly conserved "character identity networks" that create them (Wagner 2007). This proposal helps reformulate the criteria for recognizing homology via development that is robust to DSD.

DSD as Null Hypothesis for Evaluating Conservation

In molecular evolution, a key objective is to delineate and quantify the impact of the forces contributing to sequence change, such as adaptation, constraint, and neutral evolutionary processes caused solely by mutation and drift. In practice, the neutral expectation is used as a powerful null hypothesis that must be rejected in order to accept the role of adaptation and constraint. At a higher order of organization, DSD can be seen as providing a similar sort of null hypothesis. If a comparison of developmental systems reveals incongruities, we must at least consider the possibility that the differences are of no adaptive significance.

Tests of Functional Conservation of Genes

A number of experiments have shown the remarkable ability of homologous sequences from deeply diverged organisms to influence phenotypes in a way that suggests functional conservation (e.g., Halder et al. 1995). However, the existence of DSD suggests these experiments must be interpreted with caution. For example,

while the RNA-binding protein GLD-1 from the nematode *C. briggsae* is highly similar in sequence to that of its *C. elegans* ortholog and can rescue the latter's loss-of-function phenotype completely, the loss-of-function phenotypes in the species are exactly opposite (one causing germline feminization, the other masculinization) (Beadell et al. 2011). This implies conservation of the protein as a biochemical actor, but not conservation of its role in the gene regulatory network that determines sex. Similarly, two interacting nematode proteins with demonstrably identical roles in somatic sex determination, TRA-2 and FEM-3, coevolve so rapidly that inter-species pairing is no longer possible (Haag et al. 2002). Thus, only under a specific set of conditions (free of molecular coevolution and divergent roles in trait specification) are interspecies swaps able to provide reliable inferences of stasis (or lack thereof).

Genetic Bases of Convergent Evolution

The existence of DSD means that there can be many alternative configurations of genes, regulatory networks, and cells that can nevertheless produce a conserved phenotypic output. That there are many routes to the same phenotype also has implications for convergent evolution (see chapter ▶ "Convergence"). Given independent replications of an adaptive walk, DSD leads us to the a priori expectation that superficially identical traits will often be produced by alternative developmental processes (True and Haag 2001). However, the precise extent to which congruent genetic mechanisms will underlie a convergently evolved trait is both constrained by the relatively small set of "toolkit" genes that regulate development across many distinct tissues and influenced by the recency of ancestry of the lineages that are converging. For example, the convergent evolution of pelvic spine loss in distinct threespine stickleback populations seems to be mediated by same *Pitx1* mutation in all populations, perhaps by the same allele selected from ancestral standing variation (Chan et al. 2010). The convergent evolution of self-fertile hermaphrodites in *C. elegans* and *C. briggsae,* two deeply diverged species, however, shows both conserved roles of much of the sex determination pathway (de Bono and Hodgkin 1996) as well as essential lineage-specific genes and alternative roles for conserved factors (e.g., Clifford et al. 2000).

Biomedical Research

Because of ethical limitations on human research, much of biomedical research relies upon nonhuman organisms. Though mice and other mammals are obvious choices due to their relative close relatedness, all manner of eukaryotes are used to model certain aspects of human cells as appropriate. The existence of DSD implies that even when a homologous organ or process is being modeled in a nonhuman organism, incongruencies will inevitably exist. These should not surprise us and will constitute a major obstacle in translating basic research with model organisms to medical applications. Though phylogenetic relatedness can ameliorate this problem

somewhat, even mammalian models (which have organs and cell types that correspond precisely with our own) differ from us in many genetic details (e.g. Bailey and Eichler 2006), and efforts to translate basic research to humans often fail as a result. We therefore cannot relegate DSD to realm of evolutionary curiosity: it has real impact on the biomedical research enterprise and is ignored at our collective peril.

Cross-References

- ▶ Convergence
- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Developmental Homology
- ▶ Evo-Devo and Phylogenetics
- ▶ Micro-Evo-Devo
- ▶ Ivan I. Schmalhausen (1884–1963)
- ▶ Mechanisms of Pattern Formation, Morphogenesis, and Evolution

References

Bailey JA, Eichler EE (2006) Primate segmental duplications: crucibles of evolution, diversity and disease. Nat Rev Genet 7(7):552–564. https://doi.org/10.1038/nrg1895

Barriere A, Gordon K, Ruvinsky I (2012) Coevolution within and between regulatory loci can preserve promoter function despite evolutionary rate acceleration. PLoS Genet 8:e1002961

Beadell AV, Liu Q, Johnson DM, Haag ES (2011) Independent recruitments of a translational regulator in the evolution of self-fertile nematodes. Proc Natl Acad Sci U S A 108(49):19672–19677. https://doi.org/10.1073/pnas.1108068108

Chan YF, Marks ME, Jones FC, Villarreal G Jr, Shapiro MD, Brady SD, Southwick AM, Absher DM, Grimwood J, Schmutz J, Myers RM, Petrov D, Jonsson B, Schluter D, Bell MA, Kingsley DM (2010) Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a Pitx1 enhancer. Science 327(5963):302–305. https://doi.org/10.1126/science.1182213

Clark S, Chisholm A, Horvitz H (1993) Control of cell fates in the central body region of *C. elegans* by the homeobox gene lin-39. Cell 74:43–55

Clifford R, Lee MH, Nayak S, Ohmachi M, Giorgini F, Schedl T (2000) FOG-2, a novel F-box containing protein, associates with the GLD-1 RNA binding protein and directs male sex determination in the *C. elegans* hermaphrodite germline. Development 127(24):5265–5276

de Bono M, Hodgkin J (1996) Evolution of sex determination in *Caenorhabditis*: unusually high divergence of tra-1 and its functional consequences. Genetics 144(2):587–595

Delattre M, Felix M (2001) Polymorphism and evolution of vulval precursor cell lineages within two nematode genera, *Caenorhabditis* and *Oscheius*. Curr Biol 11:631–643

Felix M (2007) Cryptic quantitative evolution of the vulva intercellular signaling network in *Caenorhabditis*. Curr Biol 17:103–114

Force A, Lynch M, Pickett FB, Amores A, Yan Y, Postlethwait J (1999) Preservation of duplicate genes by complementary, degenerative mutations. Genetics 151:1531–1545

Haag ES (2007) Compensatory vs. pseudocompensatory evolution in molecular and developmental interactions. Genetica 129(1):45–55. https://doi.org/10.1007/s10709-006-0032-3

Haag ES, Wang S, Kimble J (2002) Rapid coevolution of the nematode sex-determining genes fem-3 and tra-2. Curr Biol 12(23):2035–2041

Halder G, Callaerts P, Gehring WJ (1995) Induction of ectopic eyes by targeted expression of the eyeless gene in *Drosophila*. Science 267:1788–1792

Johnson NA, Porter AH (2007) Evolution of branched regulatory genetic pathways: directional selection on pleiotropic loci accelerates developmental system drift. Genetica 129(1):57–70. https://doi.org/10.1007/s10709-006-0033-2

Kiontke K, Barriere A, Kolotuev I, Podbilewicz B, Sommer R, Fitch DH, Felix MA (2007) Trends, stasis, and drift in the evolution of nematode vulva development. Curr Biol 17(22):1925–1937. https://doi.org/10.1016/j.cub.2007.10.061

Milloz J, Duveau F, Nuez I, Felix M (2008) Intraspecific evolution of the intercellular signaling network underlying a robust developmental system. Genes Dev 22:3064–3075

Pomiankowski A, Nothiger R, Wilkins A (2004) The evolution of the *Drosophila* sex-determination pathway. Genetics 166(4):1761–1773

Schmalhausen II (1949) Factors of Evolution. Blackiston Company, Philadelphia

Schulze J, Schierenberg E (2011) Evolution of embryonic development in nematodes. EvoDevo 2:18

Shubin N, Alberch P (1986) A morphogenetic approach to the origin and basic organization of the tetrapod limb. In: Hecht W, Wallace B, Prance G (eds) Evolutionary biology, vol 20. Plenum, New York, pp 319–387

Sommer R (1997) Evolutionary changes of developmental mechanisms in the absence of cell lineage alterations during vulva formation in the Diplogastridae (Nematoda). Development 124:243–251

Sommer R, Sternberg P (1996) Apoptosis and change of competence limit the size of the vulva equivalence group in *Pristionchus pacificus*: a genetic analysis. Curr Biol 6:52–59

Sternberg P, Horvitz H (1986) Pattern formation during vulval development in *C. elegans*. Cell 44:761–772

Takano T (1998) Loss of notum macrochaetae as an interspecific hybrid anomly between *Drosophila melanogaster* and *D. simulans*. Genetics 129:1435–1450

True JR, Haag ES (2001) Developmental system drift and flexibility in evolutionary trajectories. Evol Dev 3:109–119

Verster AJ, Ramani AK, McKay SJ, Fraser AG (2014) Comparative RNAi screens in *C. elegans* and *C. briggsae* reveal the impact of developmental system drift on gene function. PLoS Genet 10(2):e1004077. https://doi.org/10.1371/journal.pgen.1004077

Wagner GP (2007) The developmental genetics of homology. Nat Rev Genet 8(6):473–479. https://doi.org/10.1038/nrg2099

Weiss KM, Fullerton SM (2000) Phenogenetic drift and the evolution of genotype–phenotype relationships. Theor Popul Biol 57:187–195

Zuckerkandl E (1965) The evolution of hemoglobin. Sci Am 212:110–118

Zuckerkandl E, Pauling L (1962) Molecular disease, evolution and genetic heterogeneity. In: Kasha M, Pullman B (eds) Horizons in biochemistry. Academic Press, New York, pp 189–225

The Developmental Hourglass in the Evolution of Embryogenesis

Andrew G. Cridge, Peter K. Dearden, and Lynette R. Brownfield

Contents

Abstract

Embryogenesis is the process of transformation of a single fertilized egg into a differentiated, complex organism. This requires coordinated cleavage of the fertilized egg, followed by patterning and cell-fate specification, to establish the adult body plan. Based on morphological studies and, more recently, comparative gene expression analyses, the evolution of embryogenesis in animals, and to some degree in plants, has been proposed to follow a developmental hourglass model. In this model, less conserved early events are followed by a highly conserved phylotypic stage at the narrow waist of the hourglass where species within a phylum have similar morphologies and gene expression patterns.

A. G. Cridge (✉) · P. K. Dearden
Laboratory for Evolution and Development, Genetics Otago and Department of Biochemistry, University of Otago, Dunedin, New Zealand
e-mail: andrew.cridge@otago.ac.nz; peter.dearden@otago.ac.nz

L. R. Brownfield
Department of Biochemistry, University of Otago, Dunedin, New Zealand
e-mail: lynette.brownfield@otago.ac.nz

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_185

Variation in later stages of embryogenesis then follows, providing the diversity of morphologies found in adult forms. As the phylotypic stage is the most conserved, it implies there may be greater evolutionary constrains during mid-embryogenesis compared with the less conserved early and late stages. These constraints may relate to morphological events, and/or the underlying gene regulatory networks, at the different stages of embryogenesis, as well as the requirement for embryogenesis to produce viable offspring adapted to environmental variation.

Keywords

Embryogenesis · Developmental hourglass · Phylotypic stage · Comparative gene expression · Evolutionary constrains · Regulatory networks

Introduction

Embryogenesis is the process where a single fertilized cell goes through cell division and differentiation to form a mature embryo. In sexually reproducing metazoans, embryogenesis starts with the fertilization of the egg cell (ovum) by a sperm cell (spermatozoon), giving rise to a single diploid cell referred to as a zygote. The zygote undergoes mitotic divisions with no significant growth (cleavage), followed by gastrulation as cells differentiate and the basic axes and body plan are established. The body plan is further elaborated upon during organogenesis. Finally, further growth and development produces a mature multicellular embryo. Embryogenesis establishes the basic body plan with organs laid down along body axes. Given the constraint to produce a viable embryo with functional organs, one of the central issues in embryology is how we can formulate the relationships between evolution and the processes that occur during embryo development.

One of the first theories addressing this was the recapitulation theories of (Meckel 1811) and (Serres 1842). These theories became increasingly popular throughout the eighteenth and nineteenth centuries, with Ernst Haeckel (1834–1919) positing that "ontogeny recapitulates phylogeny." That is, as the ontogeny (embryonic development of an organism) of an animal embryo progresses, the embryo's different stages of development represent lower animals' adult forms. According to recapitulation theory, for example, the early human embryos have structures similar to gill slits, and thus that early stage would represent the form of adult fish, which also has gill slits.

The nineteenth-century German embryologist Karl Ernst von Baer (1792–1876) reformulated these recapitulation theories. He noted that there were striking morphological similarities between animal species from the same phyla during periods of their embryonic development (von Baer 1828). Instead of recapitulating other animals' adult forms, von Baer theorized that animal embryos diverge from one or a few shared embryonic forms. In his view, the stages of development in more complex animals never represent the adult stages of less complex animals, rather they resemble the embryos of less complex animals. For example, von Baer noted

that embryos of humans, fish, and chicks look similar to each other in some stages of their embryogenesis but look increasingly different from one another as the embryo matures. Von Baer also noted that animal embryos from related phyla often appear morphologically different in early embryogenesis, and then converge to a similar form during mid-embryogenesis before diverging again in the later stages of embryogenesis (von Baer 1828).

The idea that the early stages of development are conserved among species, with increasing divergence as development progresses, has influenced modern evolutionary and developmental theories. Initially it was proposed that there is a phylotypic period during embryogenesis when embryos look similar and possess a basic body shape (Seidel 1960). This concept was revised and renamed the phylotypic stage, the stage of embryo development when an animal most closely resembles other species in the same phyla (Sander 1983). The observation that the phylotypic stage occurs during mid-embryogenesis formed the basis of the developmental hourglass model for embryo development postulated independently by Elinson (1987), Duboule (1994), and Raff (1996). Here we describe the hourglass model of embryo development and explore how it helps us understand the relationships between evolution and embryo development.

The Hourglass Model of Embryonic Development

The hourglass model was initially formulated based on observations of embryo morphology (Elinson 1987; Duboule 1994; Raff 1996). It divides animal embryogenesis into three stages: early, middle, and late (Fig. 1). The early stage encompasses the multiple types of mitotic divisions of the zygote, gastrulation, and the establishment of the main body axes as well as the broad domains of the adult form. The events and morphology in the early stage vary between species within a phylum, and this stage represents the wide base of the hourglass (Gilbert 2006). The middle stage is when organogenesis occurs, and by the end of this stage the final body plan has been established along the earlier defined axes. In the Hourglass Model this stage is defined as the phylotypic period (Richardson 1995) or phylotypic stage (Raff 1996), as species within a phylum show the maximum similarity at this stage. The phylotypic stage is represented by the narrow waistband of the hourglass. In the late stage, the basic body plan is elaborated upon as the embryo matures and begins to reflect the adult form, resulting in increased variation between different species as indicated by the broad top of the hourglass (Gilbert 2006).

The hourglass model was initially regarded as controversial (Richardson et al. 1997; Hall 1997; Galis and Metz 2001; Bininda-Emonds et al. 2003; Irie and Sehara-Fujisawa 2007; Roux and Robinson-Rechavi 2008; Comte et al. 2010). This was largely due to difficulties in using morphological characteristics, which are usually qualitative in nature, to quantitatively assess the relation between variation and conservation of developmental processes at each developmental stage. In addition, experiments that aimed to identify the developmental stages that are particularly sensitive to mutation, and hence by definition developmentally conserved, were

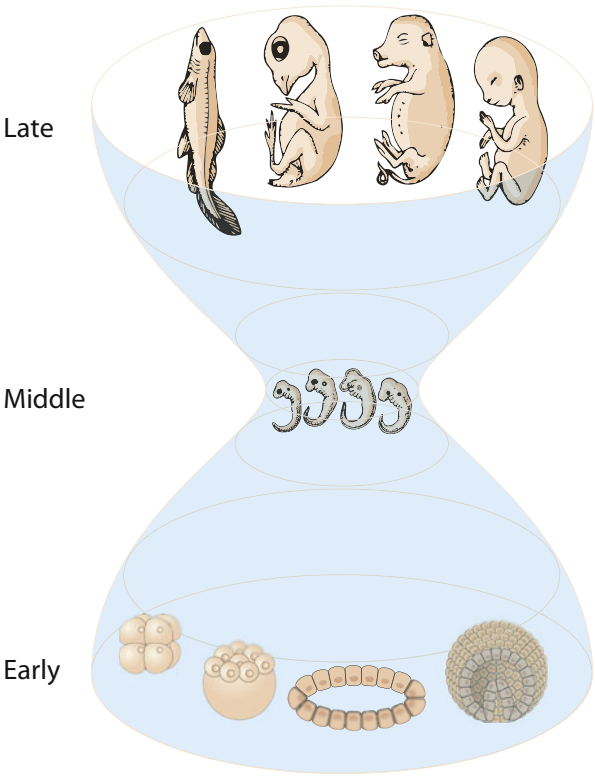


Fig. 1 The hourglass model of embryo development describes how developmental processes are conserved during evolution. According to the model, maximum conservation within a phylum occurs during the middle phylotypic stage, while early and late stages display greater differences.

confounded by the complex mechanisms of action of the teratogens (substances that may cause birth defects) used in these studies (Galis and Metz 2001). However, the development of comparative transcriptome analysis (through RNA-seq analysis) has brought a more quantitative methodology to comparative embryology and has provided further support for the hourglass model.

Comparative transcriptomic studies have used two approaches to assess gene expression patterns in different stages of embryo development. The first approach, a distance-based comparison of transcriptomes in related metozoan species, revealed that orthologous gene expression is the most conserved during the phylotypic stage, and less conserved in the early and late stages of embryogenesis (Kalinka et al. 2010; Domazet-Loso and Tautz 2010; Irie and Kuratani 2011; Yanai et al. 2011; Schep and Adryan 2013; Levin et al. 2012). The second approach was based on transcriptomic indices where genes in a single species are assigned into categories based on phylogenetic age (determined by comparing homologous sequences in related species) and expression pattern during embryogenesis. This indicated that the phylotypic stage is also marked by the expression of the evolutionarily oldest transcriptome set, whereas earlier and later stages (including adult stages) express comparatively younger genes (Domazet-Loso and Tautz 2010; Drost et al. 2015; Quint et al. 2012). Combined, these molecular studies indicate that gene expression

in the morphological phylotypic period is more conserved than in the early or late stages. Thus, they support the morphological observations that evolution of embryo development follows an hourglass-like developmental pattern, with the mid-embryonic phylotypic stages being more conserved than the early or late stages.

The Hourglass Model and the Evolution of Embryogenesis

How does the hourglass model of embryo development help us understand the relationship between evolution and the ontogenetic processes that occur during embryo development?

Similar to animal embryogenesis, flowering plant embryogenesis involves cell division and differentiation to establish a series of tissues along body axes. Interestingly, plant embryogenesis is also proposed to follow an hourglass pattern of development at the molecular level (Drost et al. 2015; Quint et al. 2012). As animal and plant embryogenesis evolved independently, this suggests that the hourglass pattern has evolved at least twice. While this could be coincidental, it is likely that this is an example of convergent evolution. This implies that there are evolutionary constraints that favor an hourglass pattern of evolving embryo development, with the greatest conservation in the middle, phylotic stages (Smith et al. 1985; see chapter "Developmental Constraints"). These constraints are likely governed by the absolute requirement for embryogenesis to generate the tissues and organs necessary to produce a viable embryo, capable of developing into a reproductively fit adult, while still allowing for variation in adult form. To explore the nature of these constraints, it is important to not only consider embryo morphology but also the gene-regulatory networks that underlie morphological development.

Gene-Regulatory Networks

In embryonic development, the establishment of the body plan is coordinated by a complex set of gene regulatory interactions. These interactions produce a cascade of gene activation, from the initial maternal inputs and zygotic genes responsible for establishing the basic body axes to the genes required for the elaboration and development of tissues and organs. Together, the ensemble of gene interactions forms a hierarchical developmental network within which sub networks control the development of particular body parts and regions (Peter and Davidson 2011). Due to the complexity of the developmental interactions in embryogenesis, perturbations to the developmental network may result in substantial phenotypic change, and loss of embryo viability. Evolution of embryogenesis therefore requires a tradeoff between conservation of gene expression networks necessary for the formation of the body plan and the requirement for the establishment of new developmental pathways, which allow the creation of new forms or functions. The developmental hourglass implies that the constraints on the evolution of these gene-regulatory networks differ during the distinct stages of embryo development.

Thus, by comparing morphology and gene regulation at the early, middle, and late stages of embryogenesis, the genetic aspect of the evolutionary constraints on embryo development can be explored.

Early Embryogenesis

The key outcome of early embryogenesis is the establishment of the germ layers as well as the major axes and broad domains of the embryo, without which further development would fail. The diversity observed at both the morphological and gene expression levels at this stage suggests that early embryogenesis has been less constrained during evolution than the middle stage.

The developmental network regulating the establishment of axes in early embryogenesis is composed of a relatively small number of genes and the regulatory interactions of these early axis patterning genes has evolved rapidly. An example of this is axis formation in insects where genes that control patterning in the fruit fly *Drosphila melanogaster* are often missing from the genomes of other insects (Dearden et al. 2006) or have evolved new regulatory interactions (Wilson and Dearden 2011). Specifically, the axis patterning *Orthodentical* (*Otd*) genes display distinct differences. Most insect genomes contain two genes (*Otd1* and *Otd2*) but only a single *Otd1* ortholog, *Ocelliless*, is represented in the *Drosphila* genome with the early patterning role of *Otd2* achieved by *Bicoid*, a hox3-derived transcription factor whose DNA binding domain has evolved to be much like *Otd* (Finklstein and Perrimon 1990).

These changes in gene regulatory networks in insects and other organisms implies that axis formation is a fast-evolving pathway resulting in substantial variation in the wiring of early developmental networks. This indicates that as long as the gene network involved in establishing the major body axes during early development of the embryo remains functional, changes can be tolerated. That is, the expression pattern, function, or sequence of the early acting genes can evolve as long as the main output of the pathways they are involved in do not change, i.e., the axes and body plan are established. This allows for evolution of variation in early embryogenesis on which positive selection can ultimately be applied.

Middle Embryogenesis/Phylotypic Stage

During the middle stages of embryogenesis, the body plan develops further upon the established axes as organogenesis occurs. This process requires cells to differentiate in the correct spatial and temporal pattern. The gene regulatory networks underlying this development become more complex, with regulators, such as transcription factors, expressed in tight domains along the axes established in the early stages. These transcription factors coordinate and modulate gene expression in a concerted manner to allow establishment of the key functional units of the adult form.

Molecular studies have identified that the transcription factors expressed at the mid-embryogenesis stage are conserved and exhibit a similar expression pattern between species of the same phyla (Schep and Adryan 2013). For example, in most metazoans, transcription factors, such as the Hox complex genes, define and regionalize regions of the embryo along the anterior/posterior axis (Garcia-Fernàndez 2004). *Hox* genes were originally discovered in *Drosophila* where functional studies showed that these genes play a critical role in establishing segmental identity along the anteroposterior axis (Lewis 1978). Additional analyses have confirmed the role of *Hox* genes in establishing anteroposterior axis identity is conserved across metazoans (Holland 2012). Subsequently mutations in *Hox* genes have been shown to cause severe body plan alterations (homeotic transformations) and congenital malformations across species (Small and Potter 1993; Emerald and Roy 1997). Thus, the high level of morphological conservation seen in the middle, phylotypic stage of the hourglass model is mirrored by conservation of the underlying gene regulatory networks. This likely relates to the absolute requirement to establish all the organs necessary for the adult form and in the correct pattern during mid-embryogenesis. Therefore, the evolution of gene networks, and the subsequent gene expression profiles, at this time is constrained as even small variations could result in severe developmental defects and loss of viability. This amounts to a negative selection pressure that potentially results in decreased rates of evolution compared to early and late stages, and the characteristic narrow waist of the hourglass model.

Late Embryogenesis

In late embryogenesis, the body plan is further developed as limbs and organs mature and embryos begin to reflect the taxa-specific differences seen in adult forms. Considering the diversity of forms produced during the late stages of embryo development, it is not surprising that the gene-regulatory networks, and their output, differ between taxa. Indeed, molecular studies show that genes expressed at later stages have a greater sequence divergence from closely related genes and are more recently evolved (Schep and Adryan 2013). These changes in gene expression are presumably less pleiotropic than changes during mid-embryogenesis, providing morphological detail rather than the fundamental structural scaffolds on which body plans are built, resulting in tolerance for changes at this late stage. This highlights that positive selection would be acting primarily on this late stage of development to shape and re-shape form to fit the environment. An example of this is the water-walking *Rhagovelia* insects which have evolved a unique propelling fan on the middle leg that is associated with life on fast-flowing streams. Development of the fan structures is controlled by two genes, *geisha* which arose from a duplication of an ancestral gene *mother-of-geisha*, which are only expressed in cells at the tips of the middle legs (Santos et al. 2017). The expression of these two genes leads to the emergence of the propelling fan, which has given the water-walking *Rhagovelia* a selective advantage in its given ecological environment. Thus, a

combination of reduced pleiotropic constraints and positive selection pressure seems to result in increased rates of evolution and the characteristic expansion of the hourglass late in embryo development.

Conclusions

Comparative embryology based on both morphological and molecular data show that the evolution of embryo development by and large corresponds to the hourglass model. In this model, early and late stages of development are less conserved amongst species within phyla, while the middle, or phylotypic, stage is more highly conserved. By comparing morphological events and the underlying gene regulatory networks at each stage of embryo development, it can be concluded that the phylotypic stage indeed represents a central master node in the networks that regulate evolution of embryogenesis. As a result, changes in the phylotypic stage are likely to be detrimental to embryo viability, whereas changes during the late stages may be favored by positive selection on the adult form.

Cross-References

- ▶ A Macroevolutionary Perspective on Developmental Constraints in Animals
- ▶ Concept of Burden in Evo-Devo
- ▶ Convergence
- ▶ Developmental Homology
- ▶ Mechanisms of Pattern Formation, Morphogenesis, and Evolution
- ▶ Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics

References

von Baer KE. (1828) Über Entwickelungsgeschichte der Thiere: Beobachtung und Reflexion. Gebrüdern Bornträger, Königsberg

Bininda-Emonds ORP, Jeffery JE, Richardson MK (2003) Inverting the hourglass: quantitative evidence against the phylotypic stage in vertebrate development. Proc R Soc Lond Ser B Biol Sci 270(1513):341–346

Comte A, Roux J, Robinson-Rechavi M (2010) Molecular signaling in zebrafish development and the vertebrate phylotypic period. Evol Dev 12(2):144–156

Dearden PK, Wilson MJ, Sablan L, Osborne PW, Havler M, McNaughton E, Kimura K, Milshina NV, Hasselmann M, Gempe T, Schioett M, Brown SJ, Elsik CG, Holland PWH, Kadowaki T, Beye M (2006) Patterns of conservation and change in honey bee developmental genes. Genome Res 16(11):1376–1384

Domazet-Loso T, Tautz D (2010) A phylogenetically based transcriptome age index mirrors ontogenetic divergence patterns. Nature 468(7325):815–818

Drost H-G, Gabel A, Grosse I, Quint M (2015) Evidence for active maintenance of phylotranscriptomic hourglass patterns in animal and plant embryogenesis. Mol Biol Evol 32 (5):1221–1231

Duboule D (1994) Temporal colinearity and the phylotypic progression: a basis for the stability of a vertebrate Bauplan and the evolution of morphologies through heterochrony. Development 1994(Suppl):135–142

Elinson RP (1987) Change in developmental patterns: embryos of amphibians with large eggs. In: Raff RA, Raff EC (eds) Development as an evolutionary process. Alan R. Liss, New York, pp 1–21

Emerald BS, Roy JK (1997) Homeotic transformation in Drosophila. Nature 389:684

Finklstein R, Perrimon N (1990) The orthodenticle gene is regulated by bicoid and torso and specifies Drosophila head development. Nature 346:485–488

Galis F, Metz JA (2001) Testing the vulnerability of the phylotypic stage: on modularity and evolutionary conservation. J Exp Zool 291(2):195–204

Garcia-Fernàndez J (2004) Hox, ParaHox, ProtoHox: facts and guesses. Heredity 94:145–152

Gilbert SF (2006) Developmental biology, 8th edn. Sinauer Associates, Sunderland

Hall BK (1997) Phylotypic stage or phantom: is there a highly conserved embryonic stage in vertebrates? Trends Ecol Evol 12(12):461–463

Holland PWH (2012) Evolution of homeobox genes. Wiley Interdiscip Rev Dev Biol 2(1):31–45

Irie N, Kuratani S (2011) Comparative transcriptome analysis reveals vertebrate phylotypic period during organogenesis. Nat Commun 2:248

Irie N, Sehara-Fujisawa A (2007) The vertebrate phylotypic stage and an early bilaterian-related stage in mouse embryogenesis defined by genomic information. BMC Biol 5(1):1

Kalinka AT, Varga KM, Gerrard DT, Preibisch S, Corcoran DL, Jarrells J, Ohler U, Bergman CM, Tomancak P (2010) Gene expression divergence recapitulates the developmental hourglass model. Nature 468:811–814

Levin M, Hashimshony T, Wagner F, Yanai I (2012) Developmental milestones punctuate gene expression in the Caenorhabditis embryo. Dev Cell 22(5):1101–1108

Lewis EB (1978) A gene complex controlling segmentation in Drosophila. Nature 276 (5688):565–570

Meckel JF (1811) Beyträge zur vergleichenden Anatomie. Carl Heinrich Reclam, Leipzig

Peter IS, Davidson EH (2011) Evolution of gene regulatory networks controlling body plan development. Cell 144(6):970–985

Quint M, Drost H-G, Gabel A, Ullrich KK, Bönn M, Grosse I (2012) A transcriptomic hourglass in plant embryogenesis. Nature 490:98–101

Raff RA (1996) The shape of life: genes, development and the evolution of animal form. University of Chicago Press, Chicago

Richardson MK (1995) Heterochrony and the phylotypic period. Dev Biol 172(2):412–421

Richardson MK, Hanken J, Gooneratne ML, Pieau C, Raynaud A, Selwood L, Wright GM (1997) There is no highly conserved embryonic stage in the vertebrates: implications for current theories of evolution and development. Anat Embryol 196(2):91–106

Roux J, Robinson-Rechavi M (2008) Developmental constraints on vertebrate genome evolution. PLoS Genet 4(12):e1000311

Sander K (1983) The evolution of patterning mechanisms: gleanings from insect embryogenesis and spermatogenesis. In: Goodwin BC, Holder N, Wylie CC. (eds) Development and evolution: the sixth symposium of the British Society for Developmental Biology. Cambridge University Press, Cambridge

Santos ME, Le Bouquin A, Crumière AJJ, Khila A (2017) Taxon-restricted genes at the origin of a novel trait allowing access to a new environment. Science 358(6361):386–390

Serres, ERA (1842) Précis d'anatomie transcendante appliquée á la physiologie. Charles Gosselin, Paris

Schep AN, Adryan B (2013) A comparative analysis of transcription factor expression during metazoan embryonic development. PLoS One 8(6):e66826

Seidel F (1960) Körpergrundgestalt und Keimstruktur. Eine Erörterung über die Grundlagen der vergleichenden und experimentellen Embryologie und deren Gültigkeit bei phylogenetischen Berlegungen. Zool Anz 164:245–305

Small KM, Potter SS (1993) Homeotic transformations and limb defects in Hox A11 mutant mice. Genes Dev 7:2318–2328

Smith JM, Burian R, Kauffman S, Alberch P, Campbell J, Goodwin B, Lande R, Raup D, Wolpert L (1985) Developmental constraints and evolution: a perspective from the Mountain Lake conference on development and evolution. Q Rev Biol 60(3):265–287

Wilson MJ, Dearden PK (2011) Diversity in insect axis formation: two orthodenticle genes and hunchback act in anterior patterning and influence dorsoventral organization in the honeybee (*Apis mellifera*). Development 138(16):3497–3507

Yanai I, Peshkin L, Jorgensen P, Kirschner MW (2011) Mapping gene expression in two *Xenopus* species: evolutionary constraints and developmental flexibility. Dev Cell 20(4):483–496

Inherency

Stuart A. Newman

Contents

Abstract

Inherency in development and evolution is the idea that aspects of the phenotype are latent in the organism's material identity and that these features will spontaneously emerge if the conditions are right. This chapter is primarily concerned with inherency of form in the animals (metazoans). Regarding development, inherency means that certain structural motifs (e.g., tissue layers, lumens, segments, appendages) can be readily generated by physical organizing forces acting on tissue masses, with minimal programming by the genome. With respect to evolution, it means that body plans and organ forms will inescapably be characterized by these motifs despite their not having arisen by multiple cycles of selection for improved fitness. The notion of inherency is therefore at odds with the theory of natural selection and its twentieth-century embodiment, the modern evolutionary synthesis. While a recently proposed extended synthesis relaxes the gradualism, gene-centrism, and assumption of unbiased modes of variation of the modern synthesis, it is similarly challenged by inherency, since in most renditions it remains focused on adaptation as the criterion of evolutionary success.

S. A. Newman (✉)
New York Medical College, Valhalla, NY, USA
e-mail: newman@nymc.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_78

Inherency makes generation of form ontologically prior to its uses. It implies that since organisms are limited with respect to potential morphologies, and innovation within these limits may be sudden and unprecedented, the major factor in establishment of new lineages is not competitive struggle in preexisting niches but ingenuity of organisms in using the means at their disposal.

Keywords

Mesoscale physics · Natural selection · Diploblasty · Triploblasty · Segmentation · Macroevolution

Introduction

The notion that biological forms are limited and predictable clashes with the commonly accepted theory of evolution, which favors the idea that morphology is molded by selection for adaption and arrived at opportunistically. In sciences other than biology, it is commonly recognized that a fixed range of forms is inherent to every type of matter and that variability, where it exists, is only expressed within that range. A familiar example is snowflakes, infinitely variable, but always within the sixfold symmetry dictated by the crystal structure of ice. Liquid water has analogous constraints, capable of forming waves and eddies, and exhibiting mathematically describable order even in the approach to turbulence.

The periodic table of the elements provides another case, with protons, neutrons, and electrons capable of generating about a hundred stable, chemically distinct atoms, with molecular weight variants in most atomic species. The elements did not appear all at once at the origin of the universe but "evolved" with cooling and interatomic collisions. This demonstrates that even though forms may be inherent to a type of matter, their emergence can also have a history.

The discrepancy between the most popular theory of the evolution of form, natural selection, and the rest of scientific thought concerning the structure of matter is tied to the era when modern evolutionary thinking first arose. In the nineteenth century, the physical understanding of complex materials of the "middle scale," which includes viscous and viscoelastic substances, but also living tissues and the embryos that give rise to them, did not yet exist (Newman and Linde-Medina 2013). It is only in the past few decades that mesoscale physics has come to be incorporated into the emerging field of evolutionary developmental biology. The twentieth-century version of the theory of evolution by natural selection advanced by Charles Darwin and Alfred Russel Wallace (the modern evolutionary synthesis; MES) had no role for this category of determinants. And whereas the extended evolutionary synthesis (EES) formulated in recent years is much more open to notions of self-organization and biased and "constructive" development (Laland et al. 2015), its theoretical statements do not contend with the implication that major features of animal and plant body plans are inherent and predictable. In this chapter, I focus on

the evidence for inherent forms in multicellular animals and show that their existence diminishes and often invalidates incrementalist and adaptationist accounts of evolutionary change.

Plasticity, Genes of Large Effect, and Developmental Bias

The main objective of the Darwin-Wallace theory and its successors was to provide an account of the observed variety of organismal form and function. The logic of the theory and its postulated mechanism of natural selection was as follows: since biological reproduction is imperfect and external conditions are always changing, organisms can evolve over time if members of a breeding population with features that suit them better to existing or changed conditions (i.e., improved adaptations) contribute relatively greater numbers of progeny with similar features to later populations.

Unlike other scientific theories, which typically aim at prediction, the MES posits no preferred phenotypic outcomes in evolution. Indeed, it makes a virtue of the opportunism of its mechanism – the supposition that nearly anything is possible to living things, given enough time (Vermeij 2015). Even Stephen J. Gould, a transitional figure between the MES and EES and an early proponent of the concept of "developmental constraint" (discussed below), famously asserted that if the "tape of life" recording the last 600 million years were replayed, the outcome (with respect to the types of organisms that appeared) would be entirely different (Gould 1989).

An associated tenet of the MES is that variations in the script of heredity (DNA sequence being the usual candidate) are random with respect to possible directions of phenotypic change. This must be the case if natural selection is to have the power ascribed to it ("the only explanation we have for the appearance of design without a designer" (Levin et al. 2017)). Biased variation implies that organisms, on the contrary, have an intrinsic propensity to change in preferred directions, independently of the external challenges they may face. Appreciation of the prevalence of bias is a distinguishing advance of the EES over the MES (Laland et al. 2015; Müller 2017).

Certain elements that might in principle contribute to a theory of evolution were set aside when the MES was formulated. The most important of these were Lamarckism (inheritance of acquired characteristics), saltationism (abrupt, single-generation phenotypic change), and orthogenesis, the notion that evolution is driven by forces or propensities arising from within the organism itself (see Mayr 1982). The rejection of the first of these became the emblem of the Anglo-American-centered MES due to its illegitimate emphasis (in the form of Lysenkoism) by Soviet agronomists during the Cold War. Lamarckism, however, was less a threat to the theory of natural selection than to the genetic determinism (the notion of genes as the exclusive directors of construction of the phenotype, and later of the "genetic program") to which it was harnessed in the twentieth century (Newman and Linde-Medina 2013). It is well known that Darwin did not consider the inheritance of acquired characteristics to be inimical to his theory and was increasingly open to

Lamarckian modes of variation in the later editions of *Origin of Species*. As concepts of inheritance have been broadened in recent decades to include niche construction and epigenetics, moreover, there has been a greater willingness by experimental and theoretical biologists to acknowledge the evolutionary role of phenotypic changes that occur independently of DNA sequence. Significantly, acquired modifications, now conceptualized as the outcomes of developmental and phenotypic plasticity, can demonstrably be propagated across generations and, with reinforcement by subsequent genetic change, become heritable (reviewed in Laland et al. 2015).

Saltational evolution, another proscribed mode in the mainstream theory, has also gained attention and respectability over recent years, and is also featured in the EES. The main arguments that had been mounted against it in the past were the complexities (due to the many cycles of selection required) of adaptation to an ecological niche, and the resulting intricacies of organismal structure and function. The mathematician R.A. Fisher, a founder of the MS, advanced a geometric argument in which the phenotype of an evolving organism was represented as a point in a multidimensional state space, with the dimensions corresponding to the organism's traits. Fisher showed (in the context of this simplistic model) that small random changes (brought about by mutations in "genes of small effect") were mathematically more likely to move the system a state of improved fitness than large random changes (via "genes of large effect"). Correspondingly, using an argument carried over from the pre-Darwinian zoologist Georges Cuvier, the writer Richard Dawkins stated "[o]rganisms are extremely complicated and sensitively adjusted pieces of machinery. If you take a complicated piece of machinery, even one that is not working all that well, and make a very large, random alteration to its insides, the chance that you will improve it is very low indeed" (Dawkins 1996, 98).

While, for these reasons, the MES has been committed to gradualism (as were both Darwin and Wallace, who saw it as essential to their theory), new findings in ecological and evolutionary developmental biology have provided compelling counterexamples. Epimutations (typically resulting from altered DNA methylation patterns) can change the form of an organism in an abrupt fashion and be propagated over successive generations. A change in floral symmetry from bilateral to radial in the toadflax plant *Linaria vulgaris* was described by Linnaeus in the seventeenth century, who noted that it bred true. Much later the basis of this heritable change was identified as the silencing by methylation of the cycloidea gene within a single generation (Cubas et al. 1999), that is, not by "imperceptibly" (Darwin's term) gradual changes in phenotype over many reproductive cycles due to genes of small effect. This "monstrous form" (so-named by Linnaeus) was no less adapted to its world than the normal variety.

In animals, the gain (e.g., by transposon insertion) or loss (e.g., by deletion) of enhancers for developmental signaling factors can lead to abrupt changes in phenotype. If such cis-regulatory changes affect embryonic morphogenesis in a region- or organ-specific fashion (as occurred with certain skeletogenic BMPs in stickleback fish, and possibly humans (Indjeian et al. 2016)), the resulting novelties can enable their bearers to explore niches adjacent to that of the originating population, in contradiction to the supposed disqualifying role of genes of large effect in the

classical theory. Finally, introgression of even one new gene via conspecific breeding or congeneric hybridization can import a new adaptive function into a lineage despite the function not having evolved in the recipient species (Arnold and Kunte 2017). This appears to be the case with the ornamental head crest in domestic and wild rock pigeons, all of which (even when otherwise distantly related) carry the same gene for a variant growth factor receptor (Shapiro et al. 2013).

The third element banished from mainstream evolutionary theory with the rise of the MS was orthogenesis, the demonstrable occurrence (in the words of the early developmental geneticist A.H. Sturtevant) of "'directive' evolution in characters that can not [sic] be supposed to be of selective value" (Sturtevant 1924). Years before the synthesis was consolidated, Sturtevant acknowledged that "There is probably no evolutionary process about which more obscurity hovers than that of orthogenesis," which "is often held to be incompatible with the view that evolution results from the action of natural selection on random variations" (Sturtevant 1924). Though the MES founders chose to ignore or marginalize such phenomena, they have been embraced by more recent investigators with terminology like "developmental constraint" and "developmental bias" (reviewed in Laland et al. 2015, and Müller 2017).

Developmental constraint has been identified in comparative embryological studies of various systems. A common question is whether unoccupied regions of "morphospace" (all the potential structures that could be generated from the system's components if there were no constraints) are due to natural selection for adaptive advantage or just mechanistic infeasibility. Evidence of directional bias has been discerned in the form of an increase of segment number in centipedes along temperature clines (Vedel et al. 2008) and increase in brain size in primates as a function of the evolution of socialization (Street et al. 2017). Such studies, however, are not accompanied by theories or rules that would allow generalization to other cases.

As with the acknowledgment of plasticity and saltation, incorporation of constraint and bias has broadened the terms of evolutionary discourse (Laland et al. 2015; Müller 2017). Nonetheless, according to one of the main proponents of the EES, "It would seem that contemporary evolutionary biology does not provide us with adequate conceptual tools specifying how to think about the causal role of phenomena like developmental bias" (Laland 2015).

Inherency Is Not an Extension of the Modern Synthesis

Evolutionary developmental biology (evo-devo) emerged in the last years of the twentieth century in response to the historically convergent recognitions that (i) transformations of developmental processes can produce evolutionary innovations, (ii) macroevolution is not straightforwardly attributable to cycles of genetic change, and (iii) organisms have inherent organization (reflected even in "monsters," "forms which lack adaptive function while preserving structural order" (Alberch 1989)). Some versions of evo-devo acknowledge these insights while holding to opportunistic natural selection as the main basis for the origination of biological

form. Others instead advance the view that the major structural motifs of animal and plant body plans are manifestations of the material properties of multicellular entities (Linde-Medina 2010; Müller 2017). This latter perspective implies that physical organizing effects are not only sources of morphological variation, but that the characteristic structural motifs of animals and plants (e.g., segments, layers, lumens, branches, leaves, tooth cusps, digits) are largely inherent and predictable. In this view, natural selection would have, at most, the modest role of culling among a range of quantitative variants of inherent forms. Further, many structures will be "neutral morphologies" with respect to fitness (Bonner 2013), perhaps recruited to functions only after the fact.

Challenged by the defenders of the MES as to how the elaboration of complex forms from simpler ones can be explained, other than by successive rounds of selection on small random variations ever-better adapted to external needs, advocates of the EES reply that the variations are sometimes not small, sometimes nonrandom, and sometimes produced in interaction with the environment. Notwithstanding a focus on expanded sources of variation, however, adaptation as the motor of evolutionary change is still emphasized (Laland et al. 2015).

Inherency inverts the terms of the challenge: finding ways to survive is what organisms do regardless of how they have acquired their traits. Evidence for this can be found in frequent establishment of invasive and introduced species, of new species arising from hybridization in plants and some animals, and of novel morphotypes attributable to introgression of single genes, as mentioned above. In each of these cases forms arise in a way disconnected from cycles of adaptation. Most importantly, they can be passed on because organisms find ways of using them after they appear, or if they are simply unburdened by having them.

If natural selection, biased development, facilitated variation, and so forth (see Laland et al. 2015) do not provide an explanation for why organisms exhibit the actual forms they do (clearly among the most important questions for a theory of evolution), what does? Here the physics of mesoscale materials can provide some answers.

A Brief History of Inherent Forms

Both animals and plants have intrinsic morphogenetic properties that caused them to produce restricted arrays of characteristic (and taxon-specific) morphological motifs over their separate evolutionary trajectories. There is a literature on this phenomenon for each of these groups (Newman and Niklas 2018). Here I will focus on animals and summarize current knowledge of their inherent forms.

The animals (or metazoans) arose roughly 700 million years ago from populations of cells – holozoans – which were also ancestral to present-day unicellular choanoflagellates (reviewed in Newman 2016a). In present-day metazoans, cell-cell attachment is mediated by members of the cadherin family of cell adhesion molecules (CAMs). Metazoan cadherins (but not those of pre-metazoan holozoans) contain a unique transmembrane domain that permits cells to remain cohesive while

they move past one another (reviewed in Newman 2016b) making the resulting cell masses behave like drops of liquid (Forgacs and Newman 2005). Because biological functions (cell-cell adhesion instead of molecular cohesion, undirected cell motility instead of Brownian motion) are responsible for the unique capacity of metazoan cell clusters to have the "generic" (i.e., physically typical) properties of liquids, the resulting category of matter has been referred to as *biogeneric* (Newman 2016a).

All animals, even the "basal" (anatomically similar to the paleontologically earliest, and genetically simplest metazoans (the sponges and the single extant placozoan)), have the requisite cadherins, and their transmembrane linkage has no counterpart in any other sequenced organisms (reviewed in Newman 2016b). The "liquid-tissue" state enabled by metazoan cadherins was thus among the primitive defining conditions of animal life. Liquids have a number of emergent features, none of which could have been a target of selection in the transition (whether gradual or abrupt) between ancestral colonial holozoans and liquid-like protometazoans. Liquids minimize their surface free energy by assuming the geometry with smallest surface-to-volume ratio, a sphere. This is thus the default morphology for embryos and newly formed tissue primordia. In liquids that contain two different kinds of subunits (molecular species, in purely physical examples), one of which has greater affinity for its own type than the other, *phase separation* occurs. In extreme cases, one liquid phase will completely engulf the other, but more generally the interface can be curved or even flat. This is precisely what takes place in co-aggregates of cells in which the homotypic and heterotypic adhesive strengths differ from each other (Forgacs and Newman 2005). The layering during gastrulation in some animal embryos has been attributed to cohesivity differences (reviewed in Newman 2016a).

Another gene product that distinguishes metazoans from all other life forms is the secreted protein Wnt (reviewed in Newman and Bhat 2009). Wnt mobilizes conserved mechanisms of cytoskeletal reorganization that predated the metazoans to make the surfaces of cells nonuniform along their apicobasal axes (A/B polarization). Analogously to polar molecules which spontaneously organize into micelles in water, liquid tissues containing A/B polarized cells will form lumens and interior spaces (Forgacs and Newman 2005). Tissue layering and lumen formation, fundamental features of all animal embryos, are thus inherent forms of the liquid-tissue state of living matter.

Two more molecular systems, both absent in basal metazoans, permitted the emergence of new morphological motifs that defined the body plans of the diploblastic (i.e., two-layered) cnidarians and ctenophores, the simplest of the *eumetazoans*. One of these was an alternative Wnt-activated signaling pathway that caused cells to be polarized in their shapes in addition to the Wnt-induced surface polarization mentioned above. This "noncanonical" Wnt pathway leads cells to align and intercalate, causing the tissue mass to narrow in the direction of intercalation and elongate orthogonally to it. These phenomena, termed *planar cell polarization* (PCP) and *convergent extension* (reviews in Forgacs and Newman 2005), are biogeneric counterparts of the alignment of polymers or anisotropic nanoparticles in liquid crystals, which similarly deviate from the spherical default shape of liquid drops (reviewed in Newman 2016a).

The other morphogenetic functionality that distinguishes eumetazoans from basal metazoans is the planar extracellular matrix (ECM) layer known as the *basal lamina*. This structure is absent in the placozoan and most sponge species but is present in all diploblasts and triploblasts (bilaterians) (reviewed in Newman 2016b). The formation of a basal lamina depends on the cross-linking of subunits of type IV collagen (a protein produced even by basal metazoans) by the enzyme peroxidasin, which is a novelty of eumetazoans. By having basal laminae, eumetazoans exhibit true epithelial issues (reviewed in Newman 2016b). These biogeneric counterparts of elastic sheets, in synergy with convergent extension (the other apomorphic trait of diploblasts, described above), added appendages, tentacles, and tissue ridges, folds and clefts to the repertoire of inherent, essentially inevitable animal forms.

Triploblasty, the three-layered embryonic configuration from which most extant animal species develop, enabled the generation a whole new array of inherent forms. A third tissue layer came to be sandwiched between the two epithelial germ layers in one or more diploblastic ancestors (reviewed in Newman 2016b). The evolutionary appearance of the third layer was dependent on the introduction of additional novel gene products (mainly ECM molecules such as fibronectin) that led to the disaggregation of epithelia to form loosely packed mesenchyme, the embryonic, and presumably ancestral, form of connective tissue. Mesenchyme and connective tissue, lacking the direct integration of cell motility and attachment, are not liquid tissues. They are nonetheless biogeneric materials, variously viscous, viscoelastic, or solid, depending on the composition of their ECMs. Mesenchymes have their own characteristic inherent forms, most prominently *cell condensations*, wherein groups of cells that start out separated by ECM are drawn closer to each other, forming transient focal epithelioid clusters. These can influence the fate of overlying epithelial sheets by a process known as *epithelial-mesenchymal interaction*, and participate in the formation of appendages (reviewed in Forgacs and Newman 2005).

Because of these inherent organizational propensities, triploblasts have more complex body plans than diploblasts and, in contrast to the latter, have true organs. Acoelomate (lacking a body cavity between the body wall and digestive tube) triploblasts such as flatworms have ovaries and testes, and ganglionic clusters of neurons. In coelomate triploblast lineages (e.g., arthropods, mollusks, chordates) organ complexity increased dramatically. The interaction of body surface (ectodermal) epithelia with its underlying mesenchyme produced, in various species, bristles, hairs, feathers, teeth, and limbs, while the interaction of body lining (endodermal) epithelium with its overlying mesenchyme become intrinsic (villi, crypts) and extrinsic (liver, pancreas) elaborations of the digestive tube. Thickening and thinning, invagination and evagination, and folding and branching of composite epithelial-mesenchymal layers in other regions of the developing embryo mediate the formation of the cardiovascular, pulmonary, and urogenital organs, as well as various glands. The formal similarity of the outcomes of these common processes in different triploblasts speaks to the inherency of the generated forms (reviewed in Newman 2016b).

Finally, it should be noted that complex tissues, particularly those of triploblasts, are potential loci of processes of *pattern formation* (processes generating regular

geometric arrangements of cells) that are also inherent, in the sense that they can be brought into existence as emergent effects of slight changes in the relationship of existing components and networks (Forgacs and Newman 2005). For example, certain gene regulatory circuits have a propensity to undergo temporal oscillation, and cells containing such circuits will spontaneously synchronize within a tissue domain. This creates a spatially extended field of cells in identical biochemical states, poised for concerted response to an external regulatory factor. This coordination occurs along the primary axis of vertebrate embryos, for instance, where the periodic expression of the transcriptional coactivator Hes1 acts as a "gate" that allows blocks of synchronized cells to successively coalesce into somites when they grow sufficiently distant from the tail tip as the embryo elongates (Hubaud and Pourquié 2014).

Thus, three processes with independent physiological or developmental origins need to have been mutually tuned for somitogenesis to occur, but the run up to this "sweet spot" could not plausibly have involved selection for any enhanced fitness conferred by axial segmentation. Similar considerations pertain to reaction-diffusion mechanisms like those theorized by the mathematician A.M. Turing as the "chemical basis of morphogenesis" (Turing 1952). These employ ordinary biosynthetic and cell-cell transport or communication processes of animal tissues, which if tuned appropriately can give rise to periodic or quasi-periodic structures, such as pigment stripes on fish skin, mammalian hair follicles, avian feather buds, and the endoskeletal elements of vertebrate paired appendages (reviewed in Kondo and Miura 2010). In the latter case, a subset of interaction parameters was evolutionarily fine-tuned to produce the tandem arrangement of bones, with proximodistal increase in number, of the tetrapod limb (Newman et al. 2018). As with the somitogenesis mechanism, there is no continuous gene-morphology mapping that could have arrived at the pattern gradually. The stasis of the tetrapod limb motif once its generative network emerged, despite multifarious adaptive changes over long periods of evolution, was remarked on (as "similar bones, in the same relative positions") by Darwin himself.

Inherency and the New Evolutionary Theory

The concept of inherency relates to a tradition of evolutionary thought outside of, and parallel to, the Darwinian-Wallacean one. Roots of it can be found in the philosopher Immanuel Kant's (1724–1804) notion of "purposive organization," but not until physics itself moved beyond exclusive Newtonism did evolutionists begin to seek analogies, and eventually explanations, for morphological change in natural processes. Jean-Baptiste Lamarck's (1744–1829) *pouvoir de vie*, an inherent complexifying force (different from his *influence des circonstances*, the "Lamarckism" of the popular imagination), the *Naturphilosophie* of J.W. von Goethe (1749–1832) and his followers, and related ideas on "laws of form" of Étienne Geoffroy Saint-Hilaire (1772–1844), William Bateson's (1861–1926) oscillatory theory of repetitive structures, D'Arcy Wentworth Thompson's (1860–1948) physicalist concepts of growth and form, and Turing's morphogenesis as dynamical

symmetry breaking (Turing 1952) are just a few landmarks in the development of this approach (reviewed in Newman and Linde-Medina 2013).

It can be seen from the foregoing that inherency is not merely complementary to the Darwinian paradigm, but is at odds with it. In contrast to notions of plasticity and nongenetic inheritance, of saltation and genes of large effect, and of developmental constraint and bias, all of which were rejected by the MES at various points but are now cautiously readmitted, or even embraced (Laland et al. 2015), inherency is impossible for the mainstream theory to accommodate while retaining the idea of adaptation as the major driver of evolutionary change. The recognition of inherency goes beyond general inferences, however valid, about development, plasticity, or mutation as sources of variation for selection (reviewed in Laland et al. 2015). It specifies what forms to expect (Newman and Müller 2005), allowing selection only a fine-tuning (i.e., microevolutionary) role. Indeed, in this perspective major transitions in animal evolution are interpretable as a progression of material capabilities rather than (as in the standard narratives) the outcome of opportunistic cycles of adaptation (Newman 2016a).

While this chapter has focused on inherency of multicellular structural motifs, a case also can be made that the functional capabilities of complex organisms have an inherency of their own. Here the intrinsic modes played out over evolution are not those predicted by a set of generic principles (the physics of mesoscale materials), but rather the activities with poorly understood origins that define cellular life. Animal organs – hearts, lungs, intestines, kidneys, skin, glands, bones, muscles, nerves – can be seen as the multicellular embodiment of functions native to individual cells – transport, respiration, digestion, excretion, protection, secretion, support, motility, excitability. To perform these tasks in multicellular organisms, *cell differentiation*, which allocates these ancestral functions (or portions of them), to novel cell types, first had to evolve. It is notable how many of the "master transcription factors" at the apexes of cell differentiation regulatory hierarchies appeared in the immediate unicellular holozoan ancestors of the metazoans, or coincident with their emergence (reviewed in Ruiz-Trillo 2016). Understanding how the inherent functions of multicellular organisms reflected in differentiated cell types came to associate and integrate with the morphogenetic, i.e., structure-generating, processes inherent to these organisms to generate present-day animal bodies and their organs (and their counterparts in plants), may be the next frontier in evolutionary developmental biology.

Cross-References

- ▶ A Macroevolutionary Perspective on Developmental Constraints in Animals
- ▶ Developmental Homology
- ▶ Evo-Devo's Contributions to the Extended Evolutionary Synthesis
- ▶ Mechanisms of Pattern Formation, Morphogenesis, and Evolution

References

Alberch P (1989) The logic of monsters: evidence for internal constraint in development and evolution. Geobios 19:21–57

Arnold ML, Kunte K (2017) Adaptive genetic exchange: a tangled history of admixture and evolutionary innovation. Trends Ecol Evol 32:601–611. https://doi.org/10.1016/j.tree.2017.05.007

Bonner JT (2013) Randomness in evolution. Princeton University Press, Princeton

Cubas P, Vincent C, Coen E (1999) An epigenetic mutation responsible for natural variation in floral symmetry. Nature 401:157–161. https://doi.org/10.1038/43657

Dawkins R (1996) Climbing mount improbable, 1st American edn. Norton, New York

Forgacs G, Newman SA (2005) Biological physics of the developing embryo. Cambridge University Press, Cambridge

Gould SJ (1989) Wonderful life. W.W. Norton, New York

Hubaud A, Pourquié O (2014) Signalling dynamics in vertebrate segmentation. Nat Rev Mol Cell Biol 15:709–721

Indjeian VB et al (2016) Evolving new skeletal traits by cis-regulatory changes in bone morphogenetic proteins. Cell 164:45–56. https://doi.org/10.1016/j.cell.2015.12.007

Kondo S, Miura T (2010) Reaction-diffusion model as a framework for understanding biological pattern formation. Science 329:1616–1620

Laland KN (2015) On evolutionary causes and evolutionary processes. Behav Process 117:97–104. https://doi.org/10.1016/j.beproc.2014.05.008

Laland KN et al (2015) The extended evolutionary synthesis: its structure, assumptions and predictions. Proc Biol Sci 282. https://doi.org/10.1098/rspb.2015.1019

Linde-Medina M (2010) Two "evodevos". Biol Theor 5:7–11

Levin S, Scott T, Cooper H, West S (2017) Darwin's aliens International Journal of Astrobiology:1–9 https://doi.org/10.1017/S1473550417000362

Mayr E (1982) The growth of biological thought: diversity, evolution, and inheritance. Belknap Press, Cambridge, MA

Müller GB (2017) Why an extended evolutionary synthesis is necessary. Interface Focus 7:20170015

Newman SA (2016a) 'Biogeneric' developmental processes: drivers of major transitions in animal evolution. Philos Trans R Soc Lond Ser B Biol Sci 371. https://doi.org/10.1098/rstb.2015.0443

Newman SA (2016b) Origination, variation, and conservation of animal body plan development. Rev Cell Biol Mol Med 2:130–162. https://doi.org/10.1002/3527600906.mcb.200400164.pub2

Newman SA, Bhat R (2009) Dynamical patterning modules: a "pattern language" for development and evolution of multicellular form. Int J Dev Biol 53:693–705. https://doi.org/10.1387/ijdb.072481sn

Newman SA, Linde-Medina M (2013) Physical determinants in the emergence and inheritance of multicellular form. Biol Theor 8:274–285

Newman SA, Müller GB (2005) Genes and form: inherency in the evolution of developmental mechanisms. In: Neumann-Held E, Rehmann-Sutter C (eds) Genes in development: re-reading the molecular paradigm. Duke University Press, Durham, pp 38–73

Newman SA, Niklas KJ (2018) Dynamical patterning modules link genotypes to morphological phenotypes in multicellular evolution. In: Hall BK, Moody A (eds) Cells in evolutionary biology. CRC Press, Boca Raton. (in press)

Newman SA, Glimm T, Bhat R (2018) The vertebrate limb: an evolving complex of self-organizing systems. Prog Biophys Mol Biol (in press)

Ruiz-Trillo I (2016) What are the genomes of premetazoan lineages telling us about the origin of Metazoa? In: Niklas KJ, Newman SA (eds) Multicellularity: origins and evolution. Vienna series in theoretical biology. The MIT Press, Cambridge, MA, pp 171–184

Shapiro MD et al (2013) Genomic diversity and evolution of the head crest in the rock pigeon. Science. https://doi.org/10.1126/science.1230422

Street SE, Navarrete AF, Reader SM, Laland KN (2017) Coevolution of cultural intelligence, extended life history, sociality, and brain size in primates. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.1620734114

Sturtevant AH (1924) An interpretation of orthogenesis. Science 59:579–580. https://doi.org/10.1126/science.59.1539.579-a

Turing AM (1952) The chemical basis of morphogenesis. Philos Trans R Soc Lond B 237:37–72

Vedel V, Chipman AD, Akam M, Arthur W (2008) Temperature-dependent plasticity of segment number in an arthropod species: the centipede Strigamia maritima. Evol Dev 10:487–492. https://doi.org/10.1111/j.1525-142X.2008.00259.x

Vermeij GJ (2015) Forbidden phenotypes and the limits of evolution. Interface Focus 5:20150028. https://doi.org/10.1098/rsfs.2015.0028

Evolvability

Richard A. Watson

Contents

Abstract

Evolvability can be taken to mean just what it says – the ability to evolve. Logically, the ability to change over time, and in particular to change adaptively, via the production and selection of heritable variation depends on the quantity and quality of variation in a population, the heritability of that variation, and the fitness variance it confers. While the ability to evolve belongs properly to a population, the properties of individuals can affect these population-level qualities and quantities. For example, individuals can carry heritable differences that affect the mechanisms of genetic change (e.g., high or low mutation probabilities), or differences that affect the distribution of phenotypic variation (e.g., different developmental organizations). Accordingly, evolvability is itself subject to evolutionary change.

R. A. Watson (✉)
Electronics and Computer Science, University of Southampton, Southampton, UK
e-mail: R.A.Watson@soton.ac.uk

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_184

However, the possibility that natural selection might systematically favor improvements in evolvability is problematic because changes affecting evolvability may not realize fitness benefits in the short-term, i.e., within the timescale where selection is effective. Progress in this area requires, in particular, understanding of the specific ways in which heritable characters affect the quality and quantity of variation. For example, whether characteristics conferring short-term benefit (e.g., robustness) may, or may not, also confer long-term evolvability (e.g., adaptability or innovation). The bidirectional interaction between development and evolution (i.e., natural selection modifies developmental organization, and developmental organization modifies the variation on which natural selection can act), is thus central to the topic of evolvability.

Keywords

Adaptation · Adaptability · Genotypephenotype · Map · Variation · Variability · Constraint · Developmental bias · Modularity · Robustness · Pleiotropy · Mutation rate

Introduction

The ability to evolve, and in particular to exhibit evolutionary adaptation, depends on the presence of suitable variation, heritability, and selective differences. In general, two given populations may exhibit different heritable variation in fitness and hence have different evolvability (see chapter ▶ "Variational Approaches to Evolvability: Short- and Long-Term Perspectives"). For some purposes it is sufficient to assume that the amount of variation, how heritable it is and the fitness differences it confers are empirical matters – quantities that can be measured but need not be explained (or that their explanation is the responsibility of another discipline such as molecular genetics, embryology or ecology). The topic of evolvability, in contrast, asks questions about the factors that affect these quantities, and further, how the process of evolution itself alters these factors. For example, open questions in evolvability include: Is the organization of gene-regulation networks/developmental programs/body plans, etc., like it is because it facilitates evolutionary adaptation? Does increasing canalization, robustness, or heritability oppose or facilitate adaptability? And not least, does natural selection change these properties in a way that improves its own ability to evolve?

Many characteristics of individuals can have an effect on the heritable variation in fitness observed in a population and hence will affect the ability to evolve. It is clear that different populations have diverged in these respects. The *evolution of evolvability* is therefore not, in itself, controversial (Sniegowski and Murphy 2006). What is not so clear is whether these characteristics are present *because of* their effect on evolvability, i.e., that natural selection has favored these characteristics, or in contrast, whether their effect on evolvability is incidental to changes that are favored (or not) by natural selection for other reasons ("evolvability-as-

adaptation" or "evolvability-as-byproduct," respectively, Sniegowski and Murphy 2006; Lynch 2007). This is difficult to ascertain empirically. While some authors reason that evolvability-as-adaptation is highly unlikely (Sniegowski and Murphy 2006), others consider it self-evident (Kirschner and Gerhart 1998).

These differing points of view are perhaps understandable given the range of concepts on which the topic impinges and the lack of established unifying theory or agreed definition (Wagner and Draghi 2010; Wagner and Altenberg 1996; Wagner 2013; Sniegowski and Murphy 2006; Pigliucci 2008; Kirschner and Gerhart 1998; Hendrikse et al. 2007). One issue that has been raised is whether evolvability is a property of populations or individuals. Since the requirements for evolution by natural selection (variation, heritability, and fitness differences) are properties of populations, evolvability is, in a strict sense, a population-level property. Some have interpreted this to mean that the evolution of evolvability requires competition between populations, and thus for populations to have heritable variation in the relevant quantities (Lynch 2007; Pigliucci 2008). However, relevant population-level quantities can be modified by selection on individual traits (Wagner 1981; Wagner and Draghi 2010). Accordingly, while evolvability is sometimes quantified at the population level (see chapter ▶ "Variational Approaches to Evolvability: Short- and Long-Term Perspectives"), a significant literature on evolvability addresses characteristics of individuals and organisms, including body plans, developmental organizations or processes, and individual traits (Kirschner and Gerhart 1998; Wagner and Altenberg 1996; Payne and Wagner 2019; Sniegowski and Murphy 2006; Hendrikse et al. 2007; Pigliucci 2008; Pavličev et al. 2010; Jones et al. 2007; Wagner 2013). For example, Kirschner and Gerhart (1998) start their influential paper by stating that "Evolvability is an organism's capacity to generate heritable, selectable phenotypic variation," Wagner and Altenberg (1996) define it as "the ability of random variations to sometimes produce improvement," and Payne and Wagner (2019) as "the ability of a biological system to produce phenotypic variation that is both heritable and adaptive."

It is thus useful to distinguish between properties of a population and properties of an individual that have an effect on the relevant properties of populations. In particular, Wagner and Altenberg (1996) usefully distinguish between the population-level property of *variation* (the differences among individuals in a population) and the individual-level property *variability* (the capacity to produce variation under new mutations). Notably, whereas selection acting on a population containing individuals with low variability will quickly exhaust any standing variation (see chapter ▶ "Variational Approaches to Evolvability: Short- and Long-Term Perspectives"), a population containing individuals with high variability will have variation that is continuously renewed (Jones et al. 2007). It is thus easy to understand that one population may evolve more easily than another due to the heritable properties of the individuals it contains.

However, if we consider the question of *how* the individuals with those properties have originated, then we must be careful not to mix our levels of explanation. Individual-level natural selection does not favor characteristics because they confer a competitive advantage to the population – individual-level natural selection

explains characteristics that benefit individuals (at best). Since selection is a consequence of fitness differences between individuals that exist now (not groups or future individuals), appeals to naïve group selection ("for the good of the population"), or teleology ("for the good of future generations"), are invalid. This possibly explains some of the impatience that exists in discussions of evolvability (Sniegowski and Murphy 2006; Lynch 2007). While the concrete models for the evolution of evolvability do not make any such appeals, the evolution of evolvability remains a topic that can sometimes be misunderstood (Pigliucci 2008; Sniegowski and Murphy 2006; Hendrikse et al. 2007; Wagner and Draghi 2010). In this chapter, we discuss some of the key models and concepts that resolve the issues involved.

From Genetic to Phenotypic Concepts of Evolvability and the Role of Development

Molecular genetics and population genetics approaches to the topic of evolvability often focus on factors affecting the production of molecular variation without reference to development or phenotypes. This includes genetic mutation rate (Houle et al. 2017; Sniegowski et al. 1997), susceptibility to transcription read-through errors (Masel and Trotter 2010), and recombination rate (McDonald et al. 2016). This most often concerns itself with changes to the amount of variation, rather than changes to the quality, pattern, or structure of that variation. That is, it retains the assumption that the fitness consequences of molecular variation are random. However, if large variations are more likely to be deleterious than small ones (e.g., as per Fisher's geometrical arguments, Fisher 1930), this naturally creates a trade-off such that variations are unlikely to be both large and advantageous, even though this would maximize the rate of adaptation.

Other work emphasizes the *phenotypic* consequences of molecular genetic variation. This often refers to a *genotype-phenotype map*, i.e., how the space of genetic possibilities is mapped into the space of phenotypic possibilities (see chapters ► "Epistasis," and ► "Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics,"). In some cases, this retains a molecular emphasis, e.g., how points in the genetic sequence space map onto the space of protein structure or function (Payne and Wagner 2019). Characteristics of this mapping that are potentially important to evolvability include *redundancy* (how many genotypes result in the same phenotype, i.e., same protein structure), *neutrality* and *robustness* (how many mutational neighbors of a given genotype correspond to the same fitness or same phenotype), and *phenotypic accessibility* (how many different phenotypes are mutational neighbors of a given phenotype). The latter is sometimes taken as a definition of evolvability (without direct regard for their fitness effects). Regions of genetic space "connected" by virtue of giving rise to the same phenotype, or "genotype network" (Wagner 2013) – and by implication, the same fitness (known as "neutral networks") – are observed to be large and thus enable access to many other phenotypes without the need to pass through intermediate phenotypes. This view partly alleviates the tension between the amount of variation and the quality of variation by noticing that such neutral networks allow a greater number of phenotypes to be accessed compared to a

non-neutral mapping. The implication of this is that the more phenotypes are accessible, the higher the likelihood that at least some of that variation may be beneficial. This work generally takes the characteristics of the G-P mapping to be defined by the biophysical properties of sequence transcription and translation, and thus not subject to evolutionary change. But aspects of this mapping, even at this molecular level, are evolvable (Payne and Wagner 2019).

More relevant to this volume, other work on evolvability focuses on how the phenotypic consequences of molecular genetic variation are variously suppressed, amplified, and shaped by the processes of development, and how the organization of these developmental processes is itself subject to evolutionary change (Hendrikse et al. 2007). Significantly, this developmental view makes it easier to imagine how the trade-off between large and advantageous mutations might be alleviated. For example, in addition to affecting the quantity of variation (e.g., through canalization or decanalization of a trait, Hansen 2006), differences in developmental organization can also modify the pattern of that variation (e.g., via the covariation of traits) and hence modify the fitness distribution or "quality" of that variation. A common way to represent a genotype–phenotype map is with an M-matrix, which describes phenotypic heritable variances and covariances produced by new mutations (Hansen 2006; see chapter ▶ "Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics"). Other approaches introduce abstract models of developmental organization using vector spaces (Draghi and Wagner 2008), logic circuits (Kashtan et al. 2007; Parter et al. 2008), or network-based models (Draghi and Whitlock 2012; Kounios et al. 2016; Watson et al. 2014). More realistic modeling approaches that address cell and tissue morphogenesis include lattice modeling, enabling case-specific models of developmental processes.

The topic of evolvability thus emphasizes a potential bidirectional interaction between development and evolution, i.e., the process of evolution modifies the organization of development, "Evo → Devo," and, by modifying the available variation on which natural selection can act, the organization of development modifies the process of evolution, "Devo→Evo" (Houle et al. 2017; Hansen et al. 2006, see chapters ▶ "Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics," ▶ "A Macroevolutionary Perspective on Developmental Constraints in Animals," and ▶ "Developmental Evolutionary Biology (Devo-Evo)").

Devo-Evo: Developmental Characteristics That Affect Evolution

The Amount of Variation: Robustness Versus Adaptability

Evolutionary adaptation requires variation and to start with we might suppose that more variability confers more evolvability, or at least the possibility that some of that variation is adaptive (Payne and Wagner 2019). But if a single change is more likely to be deleterious than advantageous (intuitively, if it is easier to break complex systems than improve them), and multiple changes more so (e.g., Fisher 1930), more variability might confer less evolvability. This applies to genetic mutation rates and also to developmental processes that control phenotypic variation or robustness

(i.e., reduced sensitivity of phenotypes to environmental or genetic change). The inherent tension between the amount of variation and the quality of that variation, and in particular increasing the amount of advantageous variation while minimizing the amount of deleterious variation, is a recurrent theme in evolvability (Wagner 2013; Mayer and Hansen 2017; Masel and Trotter 2010).

It seems natural to suppose that being able to vary in all dimensions equally (isotropic variability) would be best for evolvability, if this were possible. *Developmental constraint* is often used to describe the property that phenotypic variation in a particular trait or traits is limited and, accordingly, that adaptive change is limited. However, the observation that variation in some phenotypic dimensions may be more or less likely to be relevant to selection than others (e.g., changes to the color of skin cells is more likely to be relevant to selection than changes to the color of liver cells) begins to open up a more sophisticated view of evolvability where the type or pattern of variability is important, rather than the amount of variability. Such *developmental bias* is thus not necessarily limiting to adaptation. By recognizing that the organization of development determines the pattern of variation (e.g., the structure of variation and covariation among traits), we see that it also affects the quality of variation, e.g., how likely it is to be beneficial. Evo-devo thus moves the topic of evolvability away from genetic mutation rate and (isotropic) phenotypic robustness and instead requires us to look at how a particular pattern of variation is relevant to the particular pattern of selection in an environment.

Correspondence with the Type or Pattern of Selection

Evolvability is often considered in changing environments where the need to adapt is more obvious (Clune et al. 2013; Draghi and Wagner 2008; Kashtan et al. 2007; Kouvaris et al. 2017; Parter et al. 2008; Pavličev et al. 2010; Watson et al. 2014). In some cases, the variability of the environment may in itself speed-up adaptation compared to a static environment (Kashtan et al. 2007), but in other cases we are interested in how developmental characteristics provide more suitable variation *given* the structure of the variation in the selective environment. In particular, rather than a generic notion of variability (not specific to selective context), this leads us to consider whether the variability of an individual is well matched to the variability in the selective conditions that individual experiences (Clune et al. 2013; Conrad 1979). For example, whether variability is aligned with the direction of selection (Pavličev et al. 2010; Schluter 1996; Houle et al. 2017), i.e., greater variability in the dimensions aligned with greater fitness variance, or exhibits modularity (discussed later), that corresponds to the (spatio–temporal) structure of the selective pressures experienced.

Facilitated Variation

It has been argued that a correspondence between variability and the structure of selection or environmental variation will be beneficial to adaptation (Conrad 1979; Wagner and Altenberg 1996). Using the term *facilitated variation,* Gerhart and

Kirschner (2007) discuss a number of concepts including modularity, robustness, adaptability, weak regulatory linkage, and exploratory behavior that might improve evolvability. Note that focusing variation on certain *dimensions* does not assume that we know the *direction* within that dimension that is beneficial, but may improve the chances of beneficial variations (compared to variability in wholly deleterious or neutral dimensions).

More generally, we can consider the interaction of selection with the mutational distribution, i.e., the distribution of offspring phenotypes created through genetic variation on a parent genotype. The M-matrix is a formal way to characterize pairwise interactions among genetic effects, creating correlations in the mutational distribution. Non-isotropic mutational distributions (a.k.a. bias) can cause evolutionary trajectories to move in a direction that deviates from the path of steepest ascent in the fitness landscape (Schluter 1996; Arnold et al. 2001). This means that the organization of development can change not just how quickly fit phenotypes are evolved but also which fit phenotypes evolve. In multi-peaked adaptive landscapes this can change the long-term equilibrium, i.e., cause a population to approach a different adaptive peak (Melo et al. 2016; Kounios et al. 2016).

Modularity

In simple terms, modular systems are those where the developmental interconnectedness of physiological or anatomical components can be described as subsystems with greater connectivity between components in the same subsystem than between components in different subsystems (Wagner et al. 2007; Clune et al. 2013; Melo et al. 2016). In genetic terms, modularity is a property of the mutational distribution created by the structure of pleiotropic effects of genes (see chapters ▶ "Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics" and ▶ "Variational Approaches to Evolvability: Short- and Long-Term Perspectives"). The effect of modularity on evolution can be both to enable the concerted change of multiple traits within the same module (increased "integration," Wagner and Altenberg 1996) and to localize the effects of change in one module preventing it from causing inadvertent side effects on traits in other modules ("parcelation," Wagner and Altenberg 1996 and Wagner and Laubichler 2004). Modularity can create phenotypic distributions that are multimodal (Watson et al. 2014). This is particularly significant for evolvability as it means that modularity can potentially enable small genetic changes to "jump" between distant points in phenotype space without visiting intermediate phenotypes, e.g., by redeployment of multiple integrated characters (a module) in new contexts (Wagner and Altenberg 1996; Gerhart and Kirschner 2007; Wagner et al. 2007; Melo et al. 2016).

In principle, a given modular structure may or may not be relevant to the properties of a particular selective environment. Suppose that the selection experienced on a number of traits is variable (due to environmental fluctuation or epistatic interactions; see chapter ▶ "Epistasis") and structured such that traits {a,b,c,d} and traits {e,f,g,h} experience correlated selection (but selection on traits in different sets is independent). Consider then two different developmental organizations: *M1,*

where pleiotropic mutations affect traits in one of two subsets of traits, {a,b,c,d} and {e,f,g,h}, and *M2*, where pleiotropic mutations affect traits in one of two subsets of traits, {a,b,e,f} and {c,d,g,h}. Although both developmental organizations are equally modular, one may facilitate evolvability in this environment and the other may not (Wagner and Altenberg 1996). Rupert Riedl's intuition was that developmental organizations that "mirrored" the organization of constraints on phenotypes (like *M1*) would facilitate evolutionary innovation (Wagner and Laubichler 2004; Kounios et al. 2016). That is, if particular combinations of alleles have fitness effects that experience highly epistatic or correlated selection (see chapter ▶ "Epistasis") then changing them individually would be likely to destroy the fitness contribution they confer together. If, however, developmental modularity had the same structure as the fitness epistasis, this would divide the problem at its "natural joints" (Draghi and Wagner 2008), enabling those sets of traits to change as a unit, and independently of other modules. Modularity that mirrors the variability in the selective environment has been investigated in simple matrix/network models (Clune et al. 2013; Watson et al. 2014) and others (Parter et al. 2008; Draghi and Wagner 2008).

Evo-Devo: The Evolution of Developmental Characteristics (That Affect Evolvability)

Much of evolutionary biology takes the structure of trait variance and covariance created by the various characteristics of development discussed above (represented by the structure of the M-matrix) to be parameters that do not change over time – even while recognizing their significance for enabling evolutionary change and adaptation. However, the topic of evolvability also recognizes that these characteristics are themselves evolutionary variables (Conrad 1979; Wagner and Altenberg 1996; Hansen 2006, see chapter ▶ "Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics"). Many studies of the evolution of evolvability have focused on the G and M matrices and how these change over evolutionary time (Jones et al. 2007; Hansen 2006; Hansen et al. 2006). This includes treatments that go beyond pairwise covariation to address higher-order models of variation (Hansen et al. 2006). Much of this work demonstrates that evolvability can increase, often via the alignment of variability with the properties of the fitness landscape or environmental variation, and these approaches have the advantage of being amenable to mathematical treatments that identify general results. A different approach studies the action of selection on more mechanistic models of development; often using simulation methods (Clune et al. 2013; Draghi and Whitlock 2012; Draghi and Wagner 2008; Parter et al. 2008; Watson et al. 2014). Although sacrificing mathematical clarity, this can have the advantage of connecting with some of the mechanistic properties of developmental processes that might be involved.

While many of these works show that the evolution of evolvability is possible under some conditions, important open questions remain: Are the generic conditions identified by the mathematical models true of specific developmental mechanisms? Are the results of the mechanistic examples dependent on ad hoc assumptions? Is the

evolution of evolvability the exception or the rule? Answering such questions requires clarity on how exactly natural selection can affect evolvability (Wagner and Draghi 2010), and also understanding of how developmental mechanisms affect properties of the genotype–phenotype map (see chapters ▶ "Epistasis" and ▶ "Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics").

Mechanisms for the Evolution of Evolvability

We must be careful to address the appropriate level of selection when discussing the evolution of evolvability, i.e., of what unit can evolvability be an adaptation? For example, it might be the case that a population containing individuals with a particular characteristic (e.g., a high mutation rate) evolves faster, or is more likely to survive a change in environment, than a population without such individuals. If the individuals with such characteristics are fitter than other individuals in their own population then there is no problem explaining the evolution of this character. But if those individuals are less fit compared to others in their own population (e.g., have higher mutational load, Sniegowski and Murphy 2006), then the expectation is that competition between individuals will drive this characteristic out of the population (e.g., Sniegowski et al. 1997). Sufficiently strong competition between populations could cause the evolution of individual characteristics that favor population-level evolvability even when selected against at the individual level (Sniegowski et al. 1997). However, competition between populations is generally considered to be a weaker effect than individual selection. Logically, there are two other possibilities – alleles that confer evolvability are themselves neutral or beneficial.

Neutral Modifier Alleles and Lineage Selection

A common way to model the evolution of characteristics that affect evolvability without invoking competition between populations is to consider *modifier alleles* that evolve by competition among individuals within a single population. These are alleles that affect the parameters of the evolutionary process but are in themselves selectively neutral. Naturally, for the topic of evolvability, alleles that modify the rate of increase of mean fitness are particularly pertinent (Wagner 1981). This allele could be fitness neutral, i.e., the survivability of the parent and number of offspring produced (in one generation) could be the same for two individuals with and without this allele. Although the number of offspring that carry the modifier allele in the next generation is the same as those that do not, the frequency of the allele can nonetheless change systematically by selection. This can be understood by considering not the fitness of these two individuals but the average fitness of their offspring, or the number of grandchildren produced and surviving. For example, offspring generated with a low mutation rate may, in some circumstances (perhaps a stable environment), have lower mutational load and hence leave more surviving offspring of their own. Or in other circumstances (perhaps under high selective stress), offspring generated

with a high mutation rate may have greater opportunity to find adaptive variants and hence leave more surviving offspring of their own (Sniegowski et al. 1997). Wagner (1981) quantified the selection on an allele having the effect of modifying the rate of increase in mean fitness – and showed that it increases in frequency under individual selection in an asexual population (for sexual populations see, Wagner and Draghi 2010). This is sometimes referred to as *lineage selection* (Nunney 1999), i.e., the lineage containing the modifier has a differential fitness advantage or disadvantage experienced over a number of generations greater than one.

There are some reasons to suppose that lineage selection may be limited as a mechanism for the evolution of evolvability. First, lineage selection of modifier alleles relies on the neutral modifier hitchhiking through selection on the benefits of the non-neutral effects it has caused at other loci (Conrad 1979). In sexual populations the modifier allele may become separated from the beneficial alleles it produced, and although the latter may increase in the population, the modifier allele may not (Sniegowski and Murphy 2006). This is not necessarily prohibitive though since linkage equilibrium is not restored in one generation even under free recombination (Wagner 1981; Wagner and Draghi 2010). Second, even in asexual populations, lineage selection cannot favor a lineage if it does not produce sufficiently beneficial variants quickly enough, i.e., such that selection can act on it before the lineage is lost due to drift or negative selection. These issues may be partially alleviated by structured populations that maintain lineages long enough for selection to reflect the long-term consequences of differences between them. This tension is beautifully illustrated by the experiments of Leon et al. (2018). This means that lineage selection may be less effective in sexual populations, in small populations, in well-mixed population structures, and when selection is strong.

Short-Term Advantages with Long-Term Consequences

It might be the case that a characteristic that confers immediate or short-term fitness benefits also confers an advantage to future evolvability ("evolvability-as-byproduct," Sniegowski and Murphy 2006). Some of the results from more complex models of the evolution of evolvability show that it is thus possible to evolve characteristics that confer long-term evolvability without lineage selection (e.g., Parter et al. 2008; Kounios et al. 2016; Kouvaris et al. 2017). In these models the benefits of evolvability occur after competition between lineages has been resolved (this must be the case when strong selection weak mutation assumptions are employed, Watson et al. 2014). Parter et al. (2008), for example, demonstrate that genotype–phenotype mappings can evolve that facilitate adaptation to future phenotypic targets that have not been previously selected. Crucially, natural selection cannot act on *potential* benefits that have not yet been realized – the future cannot cause the past – so the reason that these evolvability characteristics evolved cannot be because they were going to enable faster adaptation in the future. In cases where the benefits to evolvability arise after the timescale where selection is effective, this suggests the evolvability-as-byproduct idea as the only remaining option.

Is evolvability-as-byproduct merely fortuitous happenstance? The possibility of evolvability-as-byproduct is often dismissed because if alleles have beneficial direct effects that are unrelated to their effect on evolvability then although this might cause the evolution *of* evolvability, it is not selection *for* evolvability; i.e., it was not favored *because of* its long-term potential for future evolvability. Accordingly, it seems equally plausible, if not more so, that the characteristics conferring short-term benefit might oppose long-term evolvability. This tension between characteristics with short-term fitness consequences (that are easy to select on, Wagner 1981) and characteristics with long-term fitness consequences for evolvability (that are not) hints at a deeper conceptual problem with the evolution of evolvability; an inherent "catch-22." That is, natural selection clearly cannot favor structures for benefits they have not yet produced; and favoring characteristics for benefits that have already been produced is "common garden evolution" that does not require any special explanation. In short, the basic problem with the evolution of evolvability is that selection cannot act on potentials or *abilities* – only on results. It can act on a fit phenotype but not on the ability to produce a fit phenotype per se – but it is precisely the latter and not the former that is pertinent to the evolution of evolvability.

Resolving the "Byproducts Are Just Happenstance"-Problem: Common Cause

The dichotomy that "evolvability is selected for directly" or else "the evolution of evolvability is merely fortuitous happenstance" is too simplistic. A potential middle ground is that although the short-term and long-term benefits are not literally the same, under some conditions they have some systematic, non-coincidental relationship (a.k.a. *congruence*), established by a common cause. In particular, if the effects of mutations are mediated by the same given constraints and biases, such as development, then we expect that their effect in the short-term (changes in phenotypic value) and long-term (e.g., the variance and covariance of traits, and higher-order moments) will be nonindependent (Draghi and Wagner 2008; Draghi and Whitlock 2012). To take a simple statistical example, suppose that decreasing the amount of deleterious variation provides short-term benefits (without producing new adaptive phenotypes) and increasing the amount of non-deleterious variation provides long-term benefits (by sometimes producing new adaptive phenotypes). Decreasing the amount of deleterious variation is not literally the same as increasing the amount of non-deleterious variation (for example, isotropic canalization might decrease both), but in a case where the total amount of variation is constant then the former would entail the latter. Understanding congruence between short-term benefits and long-term benefits requires that we gain a better understanding of the underlying common causes. For example, when thinking in terms of genetic variation (e.g., Sniegowski and Murphy 2006), improving the ratio of beneficial variation without altering the total amount of variation seems rather improbable. But thinking in terms of phenotypic variation and how developmental organization can alter the distribution of fitness effects, this becomes at least plausible (e.g., Gerhart and Kirschner 2007; Kirschner and Gerhart 1998). For this reason, developmental

biology offers insight into the topic of evolvability that molecular genetic treatments of evolvability may overlook.

Differing assumptions about congruence are also apparent in the tension between robustness and evolvability (Gerhart and Kirschner 2007; Payne and Wagner 2019; Wagner 2013; Draghi and Wagner 2008; Mayer and Hansen 2017). If robustness decreases all variation then it opposes evolvability. However, if robustness decreases deleterious variation without decreasing total variation then robustness and evolvability are two sides of the same coin (Wagner and Altenberg 1996; Gerhart and Kirschner 2007).

Mutations that increase the ratio of beneficial to deleterious mutations might seem like wishful thinking but this possibility need not be complicated. For example, it can result simply from increasing the alignment of a phenotypic distribution with the direction of selection. Pavličev et al. (2010) show that an allele controlling the alignment of heritable phenotypic variation with the direction of selection can evolve by short-term (lineage) selection and will also confer long-term fitness advantage (assuming the direction of selection stays the same). Simple mechanistic models, where mutations alter gene-regulatory interactions (e.g., Draghi and Whitlock 2012; Watson et al. 2014) can illustrate such cases. Moreover, a mutation to a regulatory interaction will, in general, have a direct effect on the expression level of the genes involved and also affect the correlation of gene-expression changes observed under subsequent mutations. Such a mutation can therefore be selected because of its immediate phenotypic effect and will also modify evolvability through its (latent) effect on phenotypic correlations (Watson et al. 2014). Crucially, the mutations to regulatory interactions that are directly beneficial (because they move the phenotype in the direction of selection) are also necessarily the mutations that increase the alignment of variability with the current direction of selection thus conferring an increase in evolvability (Watson et al. 2014).

Is such congruence to be expected in general? Conversely, could it be the case that mutations that change the phenotype in the direction of selection (and thus have directly beneficial effects) tend to *decrease* the alignment of variability with the direction of selection (and thus decrease evolvability)? Working in the abstract, all assumptions may be considered. But in a simple mechanistic model where mutations alter regulatory interactions, this is not the case (Watson et al. 2014). When mutations change regulatory interactions, the effect of mutations that increase the alignment of covariation with the direction of selection is necessarily to amplify the effects of beneficial alleles at other loci, allowing them to "reach further" in the direction of selection. Likewise, mutations that decrease the alignment of covariation with the direction of selection reduce the beneficial effects of alleles at other loci and are therefore selected against on average.

The idea of congruence between short- and long-term benefits has earlier been discussed in addressing a different pair of timescales – to explain how selection for within-lifetime phenotypic variability (plasticity or environmental robustness) can result in corresponding changes to genetic variability (evolvability or genetic robustness) (Hansen 2006; Draghi and Whitlock 2012). For example, changes to organismal architecture that are selected for their increased environmental robustness or

adaptability also confer increased genetic robustness or adaptability (Hansen 2006). The evolution of environmental robustness and plasticity may thus be significant drivers in the evolution of genetic evolvability, especially since the fitness consequences of changes to genetic architecture revealed under environmental change can be realized on much shorter timescales than those requiring new mutations (Draghi and Whitlock 2012; Hansen 2006).

Riedl's attention to developmental organizations that mirror the structure of selective pressures experienced in the environment is not explicit about whether such organizations are selected for their long-term or short-term benefits. Conceivably, such an organization might be beneficial under short-term selection because it increases robustness (decreases the production of deleterious variations), and confers a long-term advantage because it increases the probability of beneficial variation (even though it may not yet have produced any beneficial variants in the short term) (Wagner and Laubichler 2004).

However, although changes to developmental organizations with short-term benefits may also cause favorable long-term benefits, this is not necessarily the case (Hansen et al. 2006; Mayer and Hansen 2017). For example, isotropic canalization, reducing variability in all dimensions, may be beneficial in the short term but prevent adaptability in the long term. Whether favorable congruence of short-term and long-term consequences is typical is unknown empirically. (Kirschner and Gerhart 1998; Gerhart and Kirschner 2007) argue in favor; whereas Hansen et al. (2006), for example, suggest the evidence is not clear. Hansen et al. (2006) examine the multilinear epistatic model (free from any mechanistic assumptions of developmental processes). They show that natural selection will increase evolvability if there is *directional epistasis* such that mutations systematically reinforce each other's effects in a particular direction. For example, if each beneficial mutation increases the effect of subsequent beneficial mutations (put differently, immediate benefits increase future benefits), then selection increases evolvability. Alleles that increase the alignment of variability with the direction of selection satisfy this criterion (Pavličev et al. 2010), and the evolution of gene-regulatory connections discussed above provides a mechanistic model of such alleles (Watson et al. 2014). Hansen et al. (2006) also show that this cannot be true in general when considering the space of all possible fitness landscapes (or even all points within a single landscape). Whether it is reasonable to expect this in typical cases (or the regions of a fitness landscape where evolving populations typically reside) depends on the developmental processes that parameterize genetic architecture. This motivates empirical research programs within evo-devo to study how mutations affect developmental organization, pleiotropy, modularity, and constraints and hence genetic architecture (Hendrikse et al. 2007; see chapters ▶ "A Macroevolutionary Perspective on Developmental Constraints in Animals," ▶ "Epistasis," and ▶ "Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics").

One theoretical toolkit that might be useful to describe how developmental organization links short-term benefits with long-term evolutionary consequences in a more formal and general fashion is the theory of learning systems (Watson and Szathmary 2016). Parter et al. (2008) introduce the idea of high-fitness phenotypes

that belong to a "family" of phenotypes sharing common structural regularities (e.g., different combinations of phenotypic modules). In learning theory this is called a *class* (Watson and Szathmary 2016). Characteristics that enable rapid adaptation back-and-fore between a few of these phenotypes in the past also confer rapid adaptation to new phenotypic targets from the same family in the future. In learning systems this is called *generalization* from specific instances to the general class (Watson and Szathmary 2016). It is important to realize that although we are used to thinking of learning systems as "clever," learning systems are just as short-sighted as evolving systems. Learning systems cannot alter the parameters of a model in order to improve the fit to test data they have not yet seen; they can only alter parameters to improve the fit to the training data experienced in the past or present. But learning models make it clear that this "fitting" to the present (and past) can also improve the fit of the model to previously unseen test data in future in non-happenstance ways. Specifically, this is possible when these data (past and future) are drawn from the same distribution or class, and the learning model is capable of representing the underlying regularities of that distribution or class (rather than simply memorizing the examples observed in training). To bring this into the evolutionary domain, Watson et al. (2014) show that selection on the heritable variation in gene-regulatory connections acts in the same direction as simple learning mechanisms, and the ability of gene-regulatory networks to produce novel phenotypes from the same family is functionally equivalent to the ability of neural networks to generalize from past data to respond correctly to novel data (Watson et al. 2014; Kouvaris et al. 2017). This connection offers the potential to transfer extensive existing knowledge from learning theory where such "congruence" is well-understood, into evolutionary theory where it is presently underdeveloped (Kounios et al. 2016; Kouvaris et al. 2017; Watson and Szathmary 2016).

Taken together, these works suggest that although neutral modifiers could evolve by lineage selection, changes to developmental organization (that alter the mutational distribution) are unlikely to be fitness neutral. Accordingly, concerns about whether neutral modifiers can evolve under strong selection or in sexual or small populations, may be less relevant to the evolution of evolvability. It may be more useful to understand how long-term evolvability is affected by characteristics that are selected for their direct or short-term fitness benefits. Theoretical works clarify the logical requirements for this to enable the evolution of evolvability. Whether these requirements are met in natural systems is an empirical matter, of course. But they cannot be understood without some reference to mechanistic assumptions that describe both short-term and long-term effects as consequences of a common cause, such as developmental organization. Both empirical and theoretical developments in developmental biology are thus needed to advance our understanding of the evolution of evolvability.

Cross-References

- ▶ A Macroevolutionary Perspective on Developmental Constraints in Animals
- ▶ Developmental Evolutionary Biology (Devo-Evo)
- ▶ Epistasis

► Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics
► Variational Approaches to Evolvability: Short- and Long-Term Perspectives

Acknowledgments This work was partly supported by a research grant from the John Templeton Foundations (60501). Thanks to Alfredo Rago, Miguel Brun-Usan, Joshua Payne, Frederick Nash, Louis Kounios, Kostas Kouvaris, Jeff Clune, Daniel Weinreich, Gunter Wagner, Eors Szathmary, Lee Altenberg, and Chrisantha Fernando for discussion on these topics. And in particular to Michaela Pavlicev and Laura Nuño de la Rosa for their detailed comments on an earlier draft.

References

Arnold SJ, Pfrender ME, Jones AG (2001) The adaptive landscape as a conceptual bridge between micro- and macroevolution. Genetica 112–113:9–32

Clune J, Mouret JB, Lipson H (2013) The evolutionary origins of modularity. Proc R Soc B 280(1755):20122863

Conrad M (1979) Bootstrapping on the adaptive landscape. Biosystems 11:167–182

Draghi J, Wagner GP (2008) Evolution of evolvability in a developmental model. Evolution 62(2):301–315

Draghi JA, Whitlock MC (2012) Phenotypic plasticity facilitates mutational variance, genetic variance, and evolvability along the major axis of environmental variation. Evol Int J Organ Evol 66(9):2891–2902

Fisher RA (1930) The genetical theory of natural selection. The Clarendon Press, Oxford

Gerhart J, Kirschner M (2007) The theory of facilitated variation. Proc Natl Acad Sci 104(suppl 1):8582–8589

Hansen TF (2006) The evolution of genetic architecture. Annu Rev Ecol Evol Syst 37:123–157

Hansen TF, Álvarez-Castro JM, Carter AJ, Hermisson J, Wagner GP (2006) Evolution of genetic architecture under directional selection. Evolution 60(8):1523–1536

Hendrikse JL, Parsons TE, Hallgrímsson B (2007) Evolvability as the proper focus of evolutionary developmental biology. Evol Dev 9(4):393–401

Houle D, Bolstad GH, van der Linde K, Hansen TF (2017) Mutation predicts 40 million years of fly wing evolution. Nature 548(7668):447

Jones AG, Arnold SJ, Bürger R (2007) The mutation matrix and the evolution of evolvability. Evolution 61(4):727–745

Kashtan N, Noor E, Alon U (2007) Varying environments can speed up evolution. Proc Natl Acad Sci 104(34):13711–13716

Kirschner M, Gerhart J (1998) Evolvability. Proc Natl Acad Sci 95(15):8420–8427

Kounios L, Clune J, Kouvaris K, Wagner GP, Pavlicev M, Weinreich DM, Watson RA (2016) Resolving the paradox of evolvability with learning theory: how evolution learns to improve evolvability on rugged fitness landscapes. arXiv preprint arXiv:1612.05955

Kouvaris K, Clune J, Kounios L, Brede M, Watson RA (2017) How evolution learns to generalise: using the principles of learning theory to understand the evolution of developmental organisation. PLoS Comput Biol 13(4):e1005358

Leon D, D'Alton S, Quandt EM, Barrick JE (2018) Innovation in an E. coli evolution experiment is contingent on maintaining adaptive potential until competition subsides. PLoS Genet 14: e1007348

Lynch M (2007) The frailty of adaptive hypotheses for the origins of organismal complexity. P Natl Acad Sci 104(suppl 1):8597–8604

Masel J, Trotter MV (2010) Robustness and evolvability. Trends Genet 26(9):406–414

Mayer C, Hansen TF (2017) Evolvability and robustness: a paradox restored. J Theor Biol 430:78–85

McDonald MJ, Rice DP, Desai MM (2016) Sex speeds adaptation by altering the dynamics of molecular evolution. Nature 531(7593):233–236

Melo D, Porto A, Cheverud JM, Marroig G (2016) Modularity: genes, development, and Evolution. Annu Rev Ecol Evol Syst 47(47):463–486
Nunney L (1999) Lineage selection: natural selection for long-term benefit. Levels of selection in evolution, pp 238–252
Parter M, Kashtan N, Alon U (2008) Facilitated variation: how evolution learns from past environments to generalize to new environments. PLoS Comput Biol 4(11):e1000206
Pavličev M, Cheverud JM, Wagner GP (2010) Evolution of adaptive phenotypic variation patterns by direct selection for evolvability. Proc R Soc Lond B Biol Sci. rspb20102113
Payne JL, Wagner A (2019) The causes of evolvability and their evolution. Nat Rev Genet 20(1):24–38
Pigliucci M (2008) Is evolvability evolvable? Nat Rev Genet 9(1):75
Schluter D (1996) Adaptive radiation along genetic lines of least resistance. Evolution 50(5):1766–1774
Sniegowski PD, Murphy HA (2006) Evolvability. Curr Biol 16(19):R831–R834
Sniegowski PD, Gerrish PJ, Lenski RE (1997) Evolution of high mutation rates in experimental populations of E. coli. Nature 387:703–705. https://doi.org/10.1038/42701
Wagner GP (1981) Feedback selection and the evolution of modifiers. Acta Biotheor 30(2):79–102
Wagner A (2013) Robustness and evolvability in living systems. Princeton university press, Princeton
Wagner GP, Altenberg L (1996) Perspective: complex adaptations and the evolution of evolvability. Evolution 50(3):967–976
Wagner GP, Draghi J (2010) Evolution of evolvability. In: Pigliucci M, Mueller G (eds) Evolution, the extended synthesis. MIT Press, Cambridge
Wagner GP, Laubichler MD (2004) Rupert Riedl and the re-synthesis of evolutionary and developmental biology: body plans and evolvability. J Exp Zool B Mol Dev Evol 302(1):92–102
Wagner GP, Pavlicev M, Cheverud JM (2007) The road to modularity. Nat Rev Genet 8(12):921
Watson RA, Szathmary E (2016) How can Evolution learn? Trends Ecol Evol 31(2):147–157
Watson RA, Wagner GP, Pavlicev M, Weinreich DM, Mills R (2014) The Evolution of phenotypic correlations and "developmental memory". Evolution 68(4):1124–1138

Macroevolution

Erin E. Saupe and Corinne E. Myers

Contents

Abstract

Macroevolution is the study of patterns and processes associated with evolutionary change at and above the species level, and includes investigations of both evolutionary tempo and mode. Tempo refers to the rate or pace of change, whereas mode refers to how that change occurs. Both the tempo and mode of macroevolution are difficult to predict based solely on the study of populations, organisms, and genes – the realm of microevolution. Important macroevolutionary discoveries include the observation that species rarely accrue net morphological change over their lifespans of millions of years, that episodes of mass extinction substantially modify the evolutionary trajectory of life on Earth, and

E. E. Saupe (✉)
Department of Earth Sciences, University of Oxford, Oxford, UK
e-mail: erin.saupe@earth.ox.ac.uk

C. E. Myers
Department of Earth and Planetary Sciences, University of New Mexico, Albuquerque, NM, USA
e-mail: cemyers@unm.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_126

that variation in rates of speciation, extinction, and morphological change occurs over time, in different habitats, and across groups. The potential disconnect between microevolution and macroevolution suggests different processes may operate at different levels of biological organization, and at different spatial and temporal scales. Thus, macroevolution should be considered in concert with microevolution when determining the processes that have shaped the coevolution of Earth and life.

Keywords

Macroevolution · Microevolution · Tempo and mode of evolution · Rate of speciation and extinction · Mass extinctions

Introduction

Macroevolution is the study of *patterns* and *processes* associated with evolutionary change at and above the species level (i.e., species and clades). Two common examples of macroevolutionary change include the evolution of flowering plants or the transition of tetrapods onto land (see chapters ▶ "The Origin of Angiosperms" and ▶ "Evo-Devo of the Fin-to-Limb Transition"). Macroevolution is distinct from microevolution, which describes the patterns and processes associated with evolutionary change below the species level (e.g., among populations, individuals, and genes). An example of microevolutionary study would be genetic change between island populations of birds, or the appearance of genetic mutations. Although the definition by itself is not controversial among evolutionary biologists, the implications of macroevolution as "scaled up" from microevolution or, alternatively, "decoupled" from microevolution has sparked heated debate for over 80 years (Futuyma 2015).

Evolutionary biology developed rapidly in the mid-twentieth century. For the first time, scientists provided mathematical models linking genes to morphologies and natural selection. This intellectual flowering resulted in the Modern Synthesis, a consensus among geneticists (such as Dobzhansky, Fisher, Haldane, and Wright), systematists (such as Mayr, Stebbins, and Rensch), and paleontologists (such as Simpson) that evolution was governed primarily by natural selection that caused changes in gene frequencies among populations. This process over geological timescales was assumed to explain sufficiently evolutionary patterns at all spatial and temporal scales – including those pertaining to higher taxa (Myers and Saupe 2013).

Breakthroughs in the 1970s, led by Eldredge and Gould (1972), Stanley (1979), and others, challenged this view of evolution. These researchers proposed that macroevolution was governed by different processes to those involved in microevolution, and that differences in spatial and temporal scales of evolution were real, significant, and worthy of study in their own right. Many proponents of this view consider microevolution as nested *within* macroevolution, with processes occurring at both scales having reverberating effects on the other, not unlike sloshing water

wets higher and lower regions of a bucket (Gould 2002; Vrba 1980). While this debate continues (Futuyma 2015), several major advances in evolutionary theory have been uncovered using a macroevolutionary lens. These observations can be grouped broadly as pertaining to the *tempo* (or timeline) of evolution and the *mode* (or processes/mechanisms) of evolution.

Evolutionary *tempos* from a macroevolutionary perspective are long. For example, species are suspected of persisting an average of 1–5 million years (Myrs), and clades, composed of closely-related species that share a common ancestor, can persist for 20 Myrs or more. Consequently, macroevolution documents patterns of evolutionary change that occur on the timescale of thousands to millions of years (kyrs–Myrs). In a similar vein, macroevolutionary processes generating these patterns must also occur over extended timescales. For example, speciation in nature may occur over 5–40 kyrs or longer (Gould 2002). Investigations that have elucidated the *tempo* of evolution on macroevolutionary scales include: gradual versus punctuated evolutionary change, dynamic morphological stasis, community coordinated stasis, evolutionary radiations, and spatiotemporal patterns of biodiversity and disparity through time caused by variable rates of speciation, extinction, and morphological change. These patterns are discussed in more depth in the "Tempo in Macroevolution" section below.

The *mode* of evolutionary change may also vary when viewed from a macroevolutionary versus microevolutionary lens. Natural selection characterizes well the morphological and genetic change observed within and between populations on a microevolutionary scale. However, it is unclear whether organismal-based natural selection is the dominant process that leads to species-level differentiation, or if selection occurs at multiple taxonomic levels (Stanley 1979; Gould 2002). Investigations that elucidate the *mode* of evolution on macroevolutionary scales include: selection at different hierarchical levels, the mechanisms of speciation, the effect of abiotic versus biotic factors in driving changes in diversity and disparity (see chapter ▶ "Morphological Disparity"), the causes of mass extinction events and their evolutionary and ecological effects, the factors responsible for evolutionary radiations, and the mechanisms driving the development of latitudinal diversity gradients (Saupe et al. 2019). These processes are discussed in more depth in the "The Modes of Macroevolution" section below. In reality, the *tempo* and *mode* of evolution are linked, and it is often difficult to disentangle one from the other using current methodologies (Hunt 2012).

Tempo in Macroevolution

Researchers measure the *tempo* of evolution by characterizing rates of speciation, extinction, and morphological change using both fossil and modern biological data.

Speciation and Extinction Rates

Global biodiversity is the result of both the production of new things (speciation) and the removal of existing things (extinction). These processes have been operating

with varying rates since the origin of life and have been studied using fossil data covering life history over the last ~3,200 Myrs. Often, paleobiologists refer to the maxim that 99% of all species that have ever lived are now extinct. Thus, to gain an understanding of the patterns and processes governing *tempos* of speciation and extinction, a macroevolutionary perspective is required.

The rate of speciation is a measure of how many new species appear in an interval of time within a given taxon, habitat type, region, or ecosystem. Although speciation may occur over years to kyrs, geologically speaking speciation is observed as a discrete event in time, defined as the first occurrence of an organism from a given species (first appearance datum, FAD). However, the precise moment when a new species is produced is nebulous. There is no generally agreed upon definition for what constitutes "sufficient" reproductive isolation in combination with genetic, morphological, ecological, and behavioral differentiation to constitute two distinct species during the process of speciation. Speciation is also temporally challenging to define because it tends to occur over timescales for which empirical data are scarce. That is, if speciation occurs over 5–40 kyrs, this timeline is too long to be documented by modern empirical data but is often too short to be preserved in the fossil record (see Wiley and Lieberman (2011) for a review of species delimitation and speciation).

Extinction is no less muddled. The rate of extinction is a measure of how many species disappear in an interval of time within a given taxon, habitat type, region, or ecosystem. Conceptually, extinction is the exact moment the very last individual of a species dies. However, extinction is also inescapable when, for example, the last male in a sexually reproducing population of females dies. There are also known population size limits that indicate destabilization and subsequent demise of a species, and thus extinction could also be counted when such a population size threshold is reached. The future extinction of species due to events in the past is commonly called "extinction debt" (Kuussaari et al. 2009). Like speciation, extinction occurs on a timeline that is difficult to study directly: biologists cannot search everywhere for the last remnant populations or individuals of declining species, just as the fossil record is unlikely to preserve those same few remaining individuals or populations. Thus, on geological timescales extinction is operationally defined as the last occurrence of an organism from a given species (last appearance datum, LAD).

Speciation and extinction rates were traditionally calculated from paleobiological data and relied on FADs and LADs recorded in published compendia or online databases, such as the Paleobiology Database (www.paleodb.org). Many equations have been proposed to quantify speciation and extinction rates from these count records. Most compute instantaneous rates and attempt to correct for incomplete knowledge of fossil occurrences (e.g., Alroy 2015). Time-calibrated molecular phylogenies are also used to provide information about speciation and extinction rates through time (e.g., Silvestro et al. 2018).

Estimating rates from phylogenetic information depends on a model of diversification; the simplest and most widely applied of these assumes a random speciation–extinction process. It should be noted, however, that it is difficult to disentangle diversification rates (speciation–extinction) from speciation and extinction rates

individually. This can be particularly challenging using molecular phylogenies (see Pagel 2020). By quantifying how rates of speciation and extinction vary among taxa, across space, and through time, researchers formulate hypotheses of the processes and factors that initiate evolutionary change (see "The Modes of Macroevolution" section).

The Tempo of Speciation

Investigations into speciation tempos have focused on whether rates have remained constant through time and space, and whether they have varied within and among lineages. Fossil data provide evidence for periods of elevated speciation that have punctuated geological history. At the largest scales, there appear to be periods in the geological past when rates of evolution were particularly high, for example, during the Cambrian radiation when all phyla amenable to fossilization (except Bryozoa) first appeared within a 10–15 Myr interval (Erwin et al. 2011). Similar periods of rapid diversification have occurred since, such as after mass extinctions or after the origin of major evolutionary innovations (Jablonski 2017; Bambach 2006).

The causal factors (*modes*) behind these periods of elevated origination are debated. For example, traditional arguments for post-extinction radiations invoke ecological release and diversification spurred by the emptying of many niches at once – the classic supporting example being the radiation of mammals after the extinction of the non-avian dinosaurs. Based on this observation, palaeontologist and Modern Synthesis scientist G. G. Simpson popularized the term *adaptive radiation*, referring to rapid diversification accompanied by morphological and ecological change. A more agnostic term used to describe rapid diversification when the role of adaption is uncertain is *evolutionary radiation* (Lieberman 2012). Often, it can be difficult to determine whether a specific trait of an organism or group is produced by adaptive selection, or instead is a by-product of the evolution of some other characteristic. This would include both *exaptive* traits (those evolved for a different use than their current one) and traits produced by structural constraints (Myers and Saupe 2013; see also chapters ►"Developmental Exaptation" and ►"A Macroevolutionary Perspective on Developmental Constraints in Animals").

Elevated rates of speciation can also be associated with evolutionary innovation. In this case, innovation allows for occupation of new environments and the creation of new ecospace, which can promote speciation (Jablonski 2017; see also chapter ►"Developmental Innovation and Phenotypic Novelty"). For example, Antarctic notothenioid fishes have evolved an antifreeze protein in their blood. The origination of this novel protein allowed the group to speciate into many of the open niches in the Antarctic region; hence, they are dominant members of these polar ecosystems. This innovation, however, was not tied to ecological opportunity because it occurred *before* the onset of polar conditions in the Southern Ocean (Daane et al. 2019).

Of course, any discussion of elevated speciation rates assumes that some standard or average background rate exists. While there is evidence for spikes in

speciation during certain times in Earth history, debate exists as to whether speciation rates are relatively constant outside of these intervals, or instead exhibit broad-scale secular trends over geological time (e.g., Alroy 2008). The constancy of speciation rates or lack thereof has implications for distinguishing among competing macroevolutionary hypotheses, including those supporting a role for biotic (intrinsic) mechanisms of evolutionary change versus abiotic (extrinsic) mechanisms (Myers and Saupe 2013).

Global and/or within clade diversity dependence is one biotic hypothesis that supports competition and predation as significant controls on speciation and extinction rates – and therefore biodiversity levels. Diversity dependence describes the potential pattern of asymptotic diversity change through time. Diversity in this model is thought to reach a cap due to biotic pressures such as ecosystem filling. That is, once an ecosystem is "full" of species, competition for resources, predation pressure, and lack of open niche space prevent the addition of new species (e.g., Rabosky 2013). Alternatively, others consider that abiotic mechanisms have a larger influence on diversification, such as temperature dependence or geography facilitating speciation and extinction rates (e.g., Condamine et al. 2019).

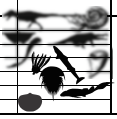
Variation in speciation rates over time may not be surprising given the diversity of life on the planet. However, speciation rates also vary within clades of closely related species, where one might predict they would be more generalizable. These variations seem to be dependent on time, geography, climate, and life-history strategies. Differential rates of speciation in groups living in tropical versus temperate regions has been invoked to explain the classic conundrum of *latitudinal diversity gradients*, in which the number of species increases from the poles to the tropics (Saupe et al. 2019).

One of the central goals of macroevolutionary studies is to quantify how speciation rates vary within and among lineages, and to understand the drivers responsible for rate variations at both levels. Do speciation rates decline throughout the history of a single clade? Are certain clades more apt to undergo evolutionary radiations than others? What is the contribution of intrinsic species characteristics (e.g., breeding behavior, developmental bias, dispersal ability) versus extrinsic factors (e.g., climate, geography)? These are all questions that may be tackled with data spanning long temporal intervals – the realm of macroevolutionary *tempo*.

The Tempo of Extinction

Quantifying extinction rates is equally important to macroevolutionary studies of *tempo* and discerning the dynamics of diversification. Like speciation, rates of extinction have not been constant throughout Earth history. One of the most important contributions of macroevolution is the identification and interrogation of two major scales of extinction: those that occur semi-continuously with mild-to-moderate intensity, described as *background extinction*, and those that occur periodically and with extreme intensity, known as *mass extinctions* (Table 1).

Table 1 Major extinction events in Earth history ranked by their ecological and taxonomic severity. Triggers and kill mechanisms are ranked by confidence, with a large and small "x" indicating high and low confidence in the mechanism, respectively. Data from McGhee et al. (2013), Harnik et al. (2012), Kaiser et al. (2016), Bambach (2006), and Balseiro and Powell (2020)

Time (Ma)	Extinction event	Taxonomic severity (rank)	Ecological severity (rank)	% spp lost	% genera lost	Example taxa lost	Mass Extinction Triggers			Mass Extinction Kill Mechanisms				
							Large Igneous Province	Asteroid Impact	Glaciation	Acidification	Anoxia	Warming	Cooling	Habitat Loss
66	end-Cretaceous	5	2	75	40		x	X		X		x	X	x
201	end-Triassic	2	3	80	73		X			X		X		
251	end-Permian	1	1	95+	83		X			X	X	X	x	X
325	Serpukhovian	6	5		39				X				X	x
359	Famennian	4	7		50		X		x	x	X	x	x	
374	Late Devonian (end Frasnian)	5	4	75	40		x				X	x	X	X
445	end-Ordovician	3	6	85	52				X		x	x	X	X

Distinctions between background and mass extinctions were first noted in the mid-1800s by John Phillips but not quantified until the 1960s by Norman Newell and Otto Schindewolf. In the early 1980s, David Raup in collaboration with Jack Sepkoski identified patterns in both background and mass extinctions – including the observation of a secular decline in background extinction magnitude through time (Raup and Sepkoski 1982). This observation is an area of considerable interest in macroevolutionary science, with researchers debating what evolutionary processes (*mode*) may cause this phenomenon, and whether it is a real signal or artifact of fossil preservation and sampling biases (see Alroy 2008).

Patterns of background extinction were used to formulate one of the more contentious ideas in macroevolution – the *Red Queen Hypothesis*. Using an impressive compilation of diversity data, Van Valen (1973) examined taxon diversity compared to taxon duration across all major clades in the Phanerozoic. He found that paleo-survivorship curves were commonly linear, which indicates the probability of extinction is constant with respect to taxon age (duration), and that extinction probability may be decoupled from taxon duration. Van Valen interpreted this pattern to infer evolutionary *mode*, suggesting that species' extinction is related to constantly changing biotic pressures, such that taxa must continually adapt to keep pace with ever better adapted competing organisms, just as the Red Queen in *Alice in Wonderland* told Alice she must always be running just to stay in place. In contrast, the Court Jester hypothesis (Barnosky 2001) suggests that abiotic forcers, such as climate, are the major cause of species' extinction (more on this in "Modes of Extinction" section below).

Sepkoski (1986) provided the first working definition of a mass extinction, which is still in use today: "any substantial increase in the amount of extinction (i.e., lineage termination) suffered by more than one geographically widespread higher taxon during a relatively short interval of geologic time, resulting in an at least temporary decline in their standing diversity." This definition is necessarily ambiguous in its description of intensity and rate (*tempo*), which largely reflects the difficulties in generalizing among conditions surrounding known mass extinction events. Operationally, mass extinctions are recognized by greater than ~75% extinction of species over an interval of ~2 Myrs or less. Mass extinction events also affect species globally, and the ecological and evolutionary recovery from them takes millions of

years (Erwin 2001). These operational criteria reflect substantial research into the five largest mass extinction events recognized in the Phanerozoic, collectively termed the "Big 5": end-Ordovician, late Devonian, end-Permian, end-Triassic, and end-Cretaceous (Harnik et al. 2012; McGhee et al. 2013). The status of the Devonian as a mass extinction however, has been debated recently (Fan et al. 2020) (Table 1).

Because their extinction rate is very high, mass extinctions substantially modified the evolutionary trajectories of life on Earth, often upsetting patterns and processes that had persisted for hundreds of millions of years. These sweeping evolutionary changes often cannot be predicted from the study of microevolutionary processes alone. In the famous example, the Cretaceous-Paleogene Mass Extinction ended the reign of non-avian dinosaurs – the prevailing large land animals for over 150 Myrs – and led to the rise of mammals that dominate today. Mass extinctions not only have large effects on the composition of species and clades present on Earth, but they also have enormous ecological influence, reorganizing communities and ecosystems (Table 1) (Erwin 2001; McGhee et al. 2013). Although mass extinctions are both devastating and alluring, it is important to acknowledge that background extinction has been the predominant type of extinction observable over the last 541 Myrs, and occurs at a rate of about 5–15% of species per million years (Raup and Sepkoski 1982).

As with speciation, the study of macroevolution can compare and contrast the evolutionary effects of extinction events by delineating trends in extinction rates through time. This may include a potential decline in the global background extinction rate, cycles in rates of extinction, and instances when speciation and extinction rates may be decoupled. Although speciation and extinction represent independent processes, study of their rates through time show they are often correlated positively (Alroy 2008). That is, periods of rapid speciation also tend to include high rates of extinction. The coupling of speciation and extinction has been invoked as evidence for the influence of biotic interactions in driving diversification dynamics: species need to die for new species to proliferate, implying a role for competition and the existence of ecosystem and global biosphere carrying capacities. However, the coupling of speciation and extinction rates could also reflect abiotic change as drivers of diversification: perturbations to the Earth system that cause widespread extinction may simultaneously create opportunities for population isolation, facilitating allopatric speciation (e.g., Vrba 1993; Myers and Saupe 2013; and references therein).

Morphological Change

In addition to rates of speciation and extinction, the study of macroevolution can reveal the rate of morphological change both within and across lineages. Morphological evolution is usually studied by quantifying aspects of a species' *phenotype*, or what it looks like. Study of phenotypic traits usually occurs in one-dimensional (traditional morphometric descriptors) or multi-dimensional (geometric

morphometric descriptors) continuous trait space (see chapter ▶ "Morphometrics in Evolutionary Developmental Biology"). Discrete character descriptors, however, such as the presence or absence of a trait, can also be studied. Such morphological traits can then be plotted against time or onto a phylogeny to measure rates of change and to test competing models of evolution (*mode*; see chapter ▶ "Morphological Disparity").

A significant ongoing debate in macroevolution is whether rates of morphological change are predominately higher during speciation events compared to within the lifetime of a species. The theory of *punctuated equilibrium* by Eldredge and Gould (1972) demonstrated that species in the fossil record tended to exhibit long periods of morphological stasis punctuated by temporally short bursts of substantial morphological change (Fig. 1). "Dynamic stasis," as this has since been dubbed, describes the hypothesis that natural selection continuously produces morphological change within populations of a species. However, the net sum of that change across the lifetime of the species is essentially zero (see Eldredge et al. (2005) for a detailed summary). For example, Rosemary and Peter Grant have spent decades studying morphological and ecological changes in Galapagos Finches (e.g., Grant and Grant 2011) – their work is an excellent example of a long-term, high resolution microevolutionary analysis. These birds show measurable morphological change in beak

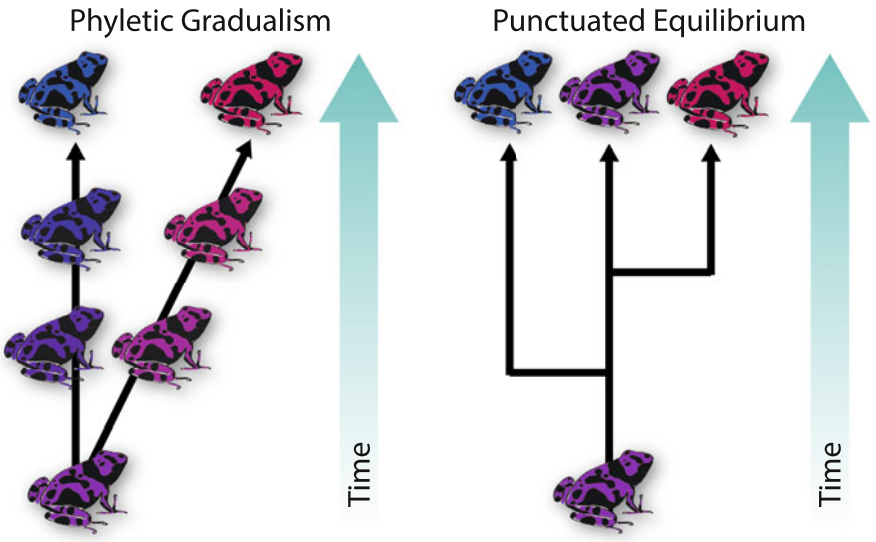


Fig. 1 In phyletic gradualism, morphological change occurs continuously over time, leading to divergence among populations and eventually speciation. In punctuated equilibrium, species exhibit relative net stability in morphological traits during their lifetime, with morphological change occurring rapidly at splitting (speciation) events. Both branching diagrams illustrate hypothesized relationships among species and are referred to as *phylogenies*. Compounding evidence from fossil and genetic data over the past 50 years supports dynamic stasis within lineages, punctuated by rapid morphological change at speciation

width through time that is linked to differences in food availability during wet and dry climate cycles. During wet periods, a larger variety of softer seeds are available and beaks tend to thin. In contrast, during dry periods, only hard, robust seeds are available to eat, and beaks tend to thicken. These data demonstrate a rollercoaster of morphological change: thin beaks → thick beaks → thin beaks → thick beaks, which summed across the lifetime of the study (40+ years) do not support net morphological change in a single direction (thin or thick).

The observation that species undergo no directional morphological change during their lifetime contrasts with the theory of *phyletic gradualism* (originally described, but not named, by Charles Darwin in *The Origin of Species*). Phyletic gradualism posits that morphological change is a slow, uniform, and gradual process occurring continuously during the lifetime of a species. Notably, examples of both punctuated equilibrium and phyletic gradualism have been identified. Thus, the important question is not whether rapid or gradual morphological change is true, but what is the relative frequency of each *tempo* throughout biological history. Compounding evidence from both fossil and genetic data over the past 50 years provides more support for the punctuational model (Fig. 1) (Gould 2002; Eldredge et al. 2005). These data lend directly to hypotheses of speciation *mode*, addressed below.

Morphological stasis observed across a suite of species in the same community is called *coordinated stasis*. Coordinated stasis is a pattern wherein groups of coexisting lineages display concurrent stability over extended intervals of geologic time separated by episodes of relatively abrupt *turnover*, defined as the coordinated extinction or local extirpation of several species or clades that are then replaced by newly originating or immigrating groups (Brett et al. 1996).

Rates of morphological change are likely tied intimately to rates of speciation and extinction. Just as with speciation and extinction, the rate at which morphological changes accrue is likely variable through time and across species, clades, and habitat. An important question in macroevolutionary studies of morphological change is the frequency of trait change to explain the diversity we observe in modern groups. For example, crown birds are an exceptionally diverse group that evolved <75 Myrs ago. The clade includes species that are very large and small, herbivores and carnivores, polar and tropical, volant and nonvolant. Did birds evolve their range of traits early and rapidly in their evolutionary history, or did they accrue these traits slowly as they diversified in the Cenozoic? This question explores the link between the accumulation of morphological diversity, referred to as *disparity,* and taxonomic diversity, which can be decoupled (Fig. 2) (see chapter ▶ "Morphological Disparity").

Additional questions pertaining to the *tempo* of morphological change include whether traits evolve faster when a species or clade is younger or older? Do the same traits evolve at different rates within different evolutionary lineages? Some groups, for example, seem to be characterized by particularly slow rates of evolutionary change, sometimes referred to as "living fossils." Living fossils were first recognized by Darwin and can refer to clades with either low rates of morphological change and/or low rates of speciation for much of their evolutionary history (Stanley 1979): classic examples include the coelacanth, horseshoe crab, and ginkgo tree. Why some

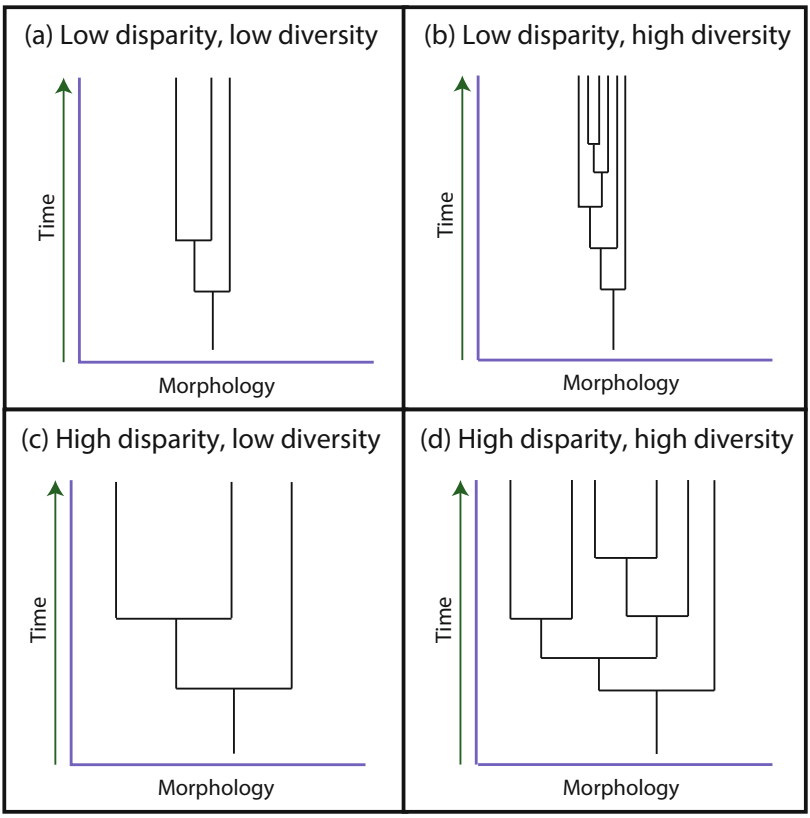


Fig. 2 The relationship between *disparity* (morphological diversity) and taxonomic diversity described using phylogenetic trees with simulated evolutionary histories. These phylogenies show morphological change occurring at splitting (speciation) events (for morphological change occurring within lineages, see Fig. 1). The concept of a living fossil is best represented in (**a**), whereas the concept of adaptive radiations is best represented in (**d**). In reality, many more tree shapes (topologies) exist that differ in number of splitting events and the incorporation of extinct taxa

clades appear more or less *evolvable* – that is, capable of morphological and ecological change – is another important area of macroevolutionary study (see chapter ▶ "Evolvability"). Quantifying the *tempo* at which traits evolve within a lineage and throughout life history can inform on underlying evolutionary processes of *mode*.

The Modes of Macroevolution

The modes of macroevolution are those mechanisms that spur speciation, extinction, and morphological and genetic change. Researchers focus on the spatial and temporal scales across which these changes occur and try to disentangle biological or

environmental factors that act to initiate, prohibit, or modify the magnitude and rate of these patterns.

Two overarching categories of factors have been proposed to drive evolutionary change. *Extrinsic* factors include abiotic variables such as changes in temperature or precipitation, mountain building, tectonic plate movement, formation and migration of rivers, etc. *Intrinsic* factors pertain to biological phenomena such as the appearance of evolutionary novelties governed by changes in evolutionary development (see chapter ▶ "Developmental Innovation and Phenotypic Novelty"), response to predation or competitive pressures, and modification in food location, quantity or type, etc. Most often, intrinsic (or biotic) factors affect the genetic makeup of individuals, organisms, and populations. These processes tend to occur on geologically short timescales (yrs–kyrs), which places them in the realm of microevolutionary change (Myers and Saupe 2013). There are some important exceptions; for example, the evolution of predation in the Proterozoic fundamentally altered the evolution of eukaryotes, and the ecological dominance of predation in Cambrian ecosystems likely contributed to the radiation of shelly invertebrates (e.g., arthropods and mollusks; Erwin et al. (2011)). However, while these examples are notable, they are also infrequent; most intrinsic evolutionary change seems rooted in microevolutionary processes, such as natural selection, evolutionary development, or possibly gene selection.

Extrinsic factors, however, have been demonstrated to affect macroevolution substantially, and may include cyclical or continuous drivers such as the movement of tectonic plates, climate changes, and sea level fluctuations. These types of drivers have been related to background levels of speciation and extinction. They have also been shown to produce synchronous macroevolutionary change across lineages, for example, in the climate-related turnover of fossil African bovids that formed the basis of Elizabeth Vrba's Turnover Pulse Theory (1993). However, some extrinsic factors may result in major environmental perturbations that dramatically change the Earth system for thousands to millions of years. Factors such as large asteroid impacts or large igneous provinces (LIPs) tend to be infrequent and difficult to predict, but the macroevolutionary consequences of these types of events are profound (Table 1). Unanswered questions in macroevolutionary *mode* include: whether extinction triggers yield predictable kill mechanisms that can be extrapolated through time, what mechanisms act to couple or decouple speciation and extinction rates, what causes long periods of relative stability in Phanerozoic biodiversity, what factors initiated the explosion of diversity and disparity at the start of the Cambrian period, and what factors instigate large-scale morphological changes and/or evolutionary novelties.

Modes of Speciation

Historical thinking on *modes* of speciation can be traced back to pre-evolutionary scholars such as Carl Linneaus, Louis Agassiz, and William Paley's Natural Theology. Secular views of speciation and the definition of evolution as *descent with*

modification did not arise until Charles Darwin and Alfred Russel Wallace's theories of evolution by natural selection. *Natural selection* is a microevolutionary process – or mode – that operates on individual organisms to remove unfavorable traits from a population (and thereby species). Notably, neither Darwin nor Wallace was explicit regarding the mode of producing new species under their theory of evolution. Implicit in their theory was that gradual and continuous change in organism-level traits within populations by natural selection at some time reached a tipping point and produced a definable new species. Speciation in this *mode* reflects a steady transformation of one species into a new one through a process called *anagenesis*. This is the process by which *phyletic gradualism* transpires. Anagenesis contrasts with the mode of speciation discussed above under the model of *punctuated equilibrium* (Eldredge and Gould 1972; Gould 2002). Under this model, speciation is discrete and rapid and better described as the "splitting" of lineages versus gradual transformation. This process is called *cladogenesis* (Fig. 1).

The difference between cladogenetic and anagenetic evolutionary change is linked in part to the processes (*modes*) hypothesized to produce new species. These were codified in the mid-twentieth century through the research of Modern Synthesis scientists. In particular, ornithologist Ernst Mayr defined two primary types of speciation: sympatry and allopatry. *Sympatric speciation* occurs when a lineage diverges within a single geographic area. That is, an ancestral species splits into one or more daughter species without the aid of significant geographic isolation. In contrast, *allopatric speciation* occurs when a population becomes geographically separated from the rest of its lineage and divergence ensues in isolation (likely facilitated by different microevolutionary processes, including selection and/or genetic drift). The differences between these two *modes* of speciation are important because each provides specific predictions regarding the factors that might contribute to lineage divergence. For example, in sympatric speciation all populations within the species experience similar environmental conditions, and thus speciation is likely driven by intrinsic factors such as changes in feeding, mating, or other behaviors in response to competitive pressure, predator avoidance, or resource limitation.

In contrast, during allopatric speciation the isolated population may experience very different extrinsic environmental conditions (e.g., if blown and isolated at a new location) in addition to potential pressures from intrinsic factors. Studying the process of speciation itself bridges microevolution and macroevolution, since populations (microscale) eventually form new species (macroscale). However, as noted previously, the timescale over which speciation occurs (5–40 kyrs) makes studying the *modes* of speciation particularly difficult from either a micro- or macroevolutionary perspective.

Whether speciation occurs in sympatry or allopatry is only one important piece of the evolutionary puzzle. Another important piece is how the "modification" in *descent with modification* is produced. The traditional microevolutionary view aligns with Darwin's hypothesis of natural selection. However, evolutionary change has been proposed to also occur at the macroevolutionary level, implying the existence of unique macroevolutionary *modes*. Selection, for example, may not

only work on organisms and their genes, but may also operate on species-level traits (Stanley 1979; Gould 2002; Jablonski 2008b). Under this contentious hypothesis, selection acts directly on species-level traits to produce new species or preferentially remove existing species. As with natural selection, this process would have reverberating effects on populations, individuals, and their genes. Similarly, selection at the species level would have an upward influence on the types of species comprising a clade. The effects of selection on one level will "sort" the types of traits preserved at both higher and lower levels. Sorting differs from selection in that the *process* responsible for evolutionary change at the focal level transpired at a lower or higher level. An example of sorting at the genetic level would be observed changes in gene frequencies caused by the process of natural selection on organisms.

These ideas, termed the Effect Hypothesis by Elizabeth Vrba (1980), imbue a strict definition of how selection might operate. The Effect Hypothesis limits selection on species, or any other level, to "emergent" traits – that is, those traits that arise from the combined organization and structure of lower levels (i.e., populations, individuals, and their genes). Traits that represent "aggregates" of these lower levels could not represent species selection because any selection on these traits would reflect natural selection operating on individuals or populations, not selection at the species level (Gould 2002). An example of an aggregate trait would be plumage color or number of digits on a limb. All Scarlet Macaws have red plumage, but one would not say that red plumage is caused by the organization of all populations and individuals defined as Scarlet Macaws. True emergent species-level traits are difficult to define; some suggested examples include species mate recognition systems, geographic range size, population structure, or evolvability (e.g., Jablonski 2008b). For this reason, some researchers have argued that emergent traits are not necessary to delineate species selection, but effects on emergent fitness are required. Others, however, have suggested that selection does not occur at levels higher than the population and there are no unique *modes* of macroevolution distinct from those of microevolution (Futuyma 2015).

Modes of Extinction

Extinction has a dramatic effect on macroevolution via the process of sorting genetic, morphological, and behavioral traits in individuals, populations, species, and clades. Surprisingly, unlike the swirl of ideas surrounding evolutionary change, it was not until the research of Georges Cuvier, a French naturalist and the "Father of Paleontology," that extinction was established as a legitimate concept in science. As mentioned above, extinction may transpire as *background extinction* or *mass extinction*, and these occur on different timescales with different intensities and causes (Table 1) (Bond and Grasby 2017; Jablonski 2008a; Harnik et al. 2012).

Extinction is rarely random across geological timescales, and certain traits may confer a higher or lower chance of species' survival. For example, species may be at higher risk for extinction during background times based on organismal traits such as poor dispersal ability or large body size, population level traits such as low

population density, or potential species level traits such as small geographic range size or narrow environmental niche. These observations demonstrate *ecological* or *extinction selectivity*, but they are not (by themselves) indications of species- or clade-level selection (i.e., a macroevolutionary vs. microevolutionary mode). Notably, studies comparing background and mass extinctions have demonstrated that patterns of ecological selectivity may differ in each regime (Jablonski 2008a).

Extinction *modes* associated with background and mass extinctions likely relate to both extrinsic environmental and intrinsic biological factors. Examples of extrinsic factors linked to species' extinction include rapid climate modification, tectonic changes, volcanism, and other natural disasters that affect habitat size, food supply, and physiological functioning (Myers and Saupe 2013). Milankovitch cycles (changes in the Earth's tilt, wobble, and orbit around the Sun) are one example of periodic (kyrs) climate change that is responsible for background extinction.

In addition to extrinsic factors, intrinsic biotic factors may also contribute to background extinctions. Developmental constraints may limit species' evolvability and thus their chance of survival. Disease, predation, and competition may also affect habitat, food, and physiology, leading to the deaths of individuals, populations, and potentially species. A classic example is when macro-predation evolved in the Cambrian (541 Ma). During this period, large-bodied predators appeared in the fossil record coincident with macro-invertebrates developing hard shells (mollusks) and exoskeletons (arthropods). One hypothesis explaining these congruent patterns is that predators selectively extinguished prey that did not have predator-defense mechanisms in the form of hard body coverings (Erwin et al. 2011). This is observed again nearly 400 Myrs later, termed the Mesozoic Marine Revolution (Vermeij 1977), when marine predators developed enhanced morphological tools for crushing or dissolving shells. This development was matched by prey developing thicker shells with deterring ornaments (e.g., large spikes), suggesting that prey without these traits were selectively eaten.

These examples are infrequent in the history of life but do demonstrate that predation pressure can be a significant biotic factor shaping patterns of extinction and evolutionary change. Other biotic factors that may influence background extinction are more nebulous in their macroevolutionary effect. These include factors such as competitive exclusion and disease. The former is still debated as a macroevolutionary versus microevolutionary process (e.g., see Sepkoski (1996) in the affirmative and Benton (1996) in the negative). Unfortunately, disease does not typically leave a fossil record and thus is mostly untenable for study across the large-scale history of life.

Mass extinction *modes* are debated, but the major hypotheses that match geological and fossil record observations relate almost exclusively to extrinsic environmental perturbations. In particular, mass extinctions seem to have substantial and irreversible effects on macroevolution when they are selective. For example, the mass extinction at the Triassic–Jurassic boundary (~201 Ma) wiped out reef dwellers in higher proportions than other marine species (Kiessling et al. 2007). This fundamentally altered shallow ocean ecosystems for tens of Myrs, and survivors of this crisis determined the trajectory of reef evolution from that point forward.

The degree and specifics of mass extinction selectivity is determined by the events *triggering* the mass extinction, which set off a cascade of environmental and ecosystem changes – *kill mechanisms* – that directly affected the survival of individual organisms, populations, species, and clades (Table 1). For example, the end-Permian mass extinction is thought be *triggered* by massive volcanic eruptions in Siberia. However, volcanism directly extinguished only those species that lived in Siberia. Global extinctions were instead caused by the *kill mechanisms* of climate change (warming), ocean acidification, and ocean anoxia caused by injection of volcanic gases (mainly carbon-based) into the atmosphere and oceans.

Thus, mass extinction *triggers* can be defined as things that initiate the conditions leading to elevated global extinction. Common triggers in the Phanerozoic are events such as large asteroid impacts or the emplacement of Large Igneous Provinces (LIPs; Bond and Grasby 2017). In contrast, *kill mechanisms* are defined as factors directly causing the deaths of organisms. Using the Phanerozoic fossil record as a case study, four major kill mechanisms have been identified: (1) climate change (especially warming), (2) ocean acidification, (3) ocean anoxia, and (4) habitat change (including loss, fragmentation, and degradation of habitat at local and regional scales, and continental configuration at global scales). Importantly, different triggers may initiate the same or multiple kill mechanisms. For example, LIPs often cause climate change, ocean acidification, and ocean anoxia, as observed during the end-Permian mass extinction.

Modes of Morphological Change

Patterns of morphological change can be observed on macroevolutionary timescales and the *tempo* quantified. However, discerning the *mode* of morphological change is difficult for macroevolutionary studies. This is because morphological change ultimately derives from changes in the genetic makeup and developmental program of individuals, the purview of microevolution. That said, advances in evolutionary development are elucidating the scales at which processes responsible for morphological change are observed in animals and plants. Moreover, macroevolutionary study of the fossil record may reveal large-scale drivers of morphological change and innovation, and how morphology has been constrained (or not) over time.

Concluding Remarks

In this chapter, we defined macroevolution as the study of patterns and processes at and above the species level. Macroevolutionary investigations have revealed that microevolutionary processes do not sufficiently explain patterns observed at the species level (and higher) on long time spans and broad spatial scales. For example, the observation that dynamic morphological stasis is observed commonly in fossil

species is incongruent with the many morphological changes observed among individuals in populations on microevolutionary timescales. The identification of dynamic stasis requires observations at the species level over long timescales and suggests that morphological change accrues at speciation events rapidly. This pattern of *punctuated equilibrium* was one of the first contributions of macroevolution to evolutionary theory.

This chapter focused on punctuated equilibrium and other unique macroevolutionary *patterns* that have been identified and contribute to a unified evolutionary theory. The example of punctuated equilibrium also illustrates that the *tempo* of evolutionary change is non-constant. Macroevolutionary studies on long timescales show that rates of speciation, extinction, and morphological change vary across clades, among habitats, and through time. This observation is a fundamental contribution of macroevolution to our understanding of evolutionary theory.

In contrast to macroevolutionary patterns, many proposed macroevolutionary *processes* are still debated. This is a common phenomenon given that patterns are based on empirical observations, whereas processes are hypothesized explanations for what generated those patterns. Science is based on a method of rejecting hypotheses unsupported by data, thus identifying the "right" answer (vs. rejecting the "wrong" answer) is difficult and often contentious. However, by studying variations in evolutionary *tempo*, we can begin to slowly untangle evolutionary *mode* to glean the full picture of how life evolves on Earth.

There are many remaining puzzles in macroevolution, which makes the field an exciting one to study. Some of the biggest questions pertain to the relative role of intrinsic versus extrinsic factors in directing evolutionary trajectories, including production of evolutionary novelties; the degree to which speciation and extinction rates are coupled; whether there are limits or carrying capacities to biodiversity and how they have changed through time; whether evolutionary and ecological rules have transformed throughout Earth history; and the degree to which disparity and diversity are coupled.

Cross-References

- ▶ A Macroevolutionary Perspective on Developmental Constraints in Animals
- ▶ Coevolution and Macroevolution
- ▶ Developmental Exaptation
- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Evo-Devo of the Fin-to-Limb Transition
- ▶ Evolution of Complexity
- ▶ Evolvability
- ▶ Levels of Organization in Evo-Devo
- ▶ Micro-Evo-Devo
- ▶ Morphological Disparity

► Morphometrics in Evolutionary Developmental Biology
► Stephen Jay Gould (1941–2002)
► The Origin of Angiosperms

Acknowledgments We were limited in the references we could cite and encourage the interested reader to follow citation trails in the contributions listed.

References

Alroy J (2008) Dynamics of origination and extinction in the marine fossil record. Proc Natl Acad Sci 105:11536–11542

Alroy J (2015) A more precise speciation and extinction rate estimator. Paleobiology 41:633–639

Balseiro D, Powell MG (2020) Carbonate collapse and the late Paleozoic ice age marine biodiversity crisis. Geology 20:118–122

Bambach RK (2006) Phanerozoic biodiversity mass extinctions. Annu Rev Earth Planet Sci 34:127–155

Barnosky AD (2001) Distinguishing the effects of the Red Queen and Court Jester on Miocene mammal evolution in the northern Rocky Mountains. J Vertebr Paleontol 21:172–185

Benton MJ (1996) Testing the roles of competition and expansion in tetrapod evolution. Proc R Soc B 263:641–646

Bond D, Grasby SE (2017) On the causes of mass extinctions. Palaeogeogr Palaeoclimatol Palaeoecol 478:3–29

Brett CE, Ivany LC, Schopf KM (1996) Coordinated stasis: an overview. Palaeogeography, Palaeoclimatology, Palaeoecology 127:1–20

Condamine FL, Rolland J, Morlon H (2019) Assessing the causes of diversification slowdowns: temperature-dependent and diversity-dependent models receive equivalent support. Ecology Letters 22:1900–1912

Daane JM, Dornburg A, Smits P, MacGuigan DJ, Hawkins MB, Near TJ, Detrich HW III, Harris MP (2019) Historical contingency shapes adaptive radiation in Antarctic fishes. Nat Ecol Evol 3:1102–1109

Eldredge N, Gould SJ (1972) Punctuated equilibria: an alternative to phyletic gradualism. In: Schopf TJM (ed) Models in paleobiology. Freeman Cooper, San Francisco, pp 82–115

Eldredge N, Thompson JN, Brakefield PM, Gavrilets S, Jablonski D, Jackson JBC, Lenski RE, Lieberman BS, McPeek MA, Miller W III (2005) The dynamics of evolutionary stasis. Paleobiology 31:133–145

Erwin DH (2001) Lessons from the past: biotic recoveries from mass extinctions. Proc Natl Acad Sci 98:5399–5403

Erwin DH, Laflamme M, Tweedt SM, Sperling EA, Pisani D, Peterson KJ (2011) The Cambrian conundrum: early divergence and later ecological success in the early history of animals. Science 334:1091–1097

Fan JX, Shen SZ, Erwin DH, Sadler PM, MacLeod N, Cheng QM, Zhang H (2020) A high-resolution summary of Cambrian to Early Triassic marine invertebrate biodiversity. Science 367 (6475):272–277

Futuyma DJ (2015) Can modern evolutionary theory explain macroevolution? In: Serelli E, Gontier N (eds) Macroevolution. Springer, Cham, pp 29–85

Gould SJ (2002) The structure of evolutionary theory. Harvard University Press, Cambridge, MA

Grant PR, Grant BR (2011) How and why species multiply: the radiation of Darwin's finches. Princeton University Press, Princeston

Harnik PG, Lotze HK, Anderson SC, Finkel ZV, Finnegan S, Lindberg DR, Liow LH, Lockwood R, McClain CR, McGuire JL (2012) Extinctions in ancient and modern seas. Trends Ecol Evol 27(11):608–617

Hunt G (2012) Measuring rates of phenotypic evolution and the inseparability of tempo and mode. Paleobiology 38:351–373
Jablonski D (2008a) Extinction and the spatial dynamics of biodiversity. Proc Natl Acad Sci 105:11528–11535
Jablonski D (2008b) Species selection: theory and data. Annu Rev Ecol Evol Syst 39:501–524
Jablonski D (2017) Approaches to macroevolution: 1. General concepts and origin of variation. Evol Biol 14:427–450
Kaiser SI, Aretz M, Becker RT (2016) The global Hangenberg Crisis (Devonian–Carboniferous transition): review of a first-order mass extinction. Geol Soc Lond Spec Publ 423:387–437
Kiessling W, Aberhan M, Brenneis B, Wagner PJ (2007) Extinction trajectories of benthic organisms across the Triassic–Jurassic boundary. Palaeogeogr Palaeoclimatol Palaeoecol 244:201–222
Kuussaari M, Bommarco R, Heikkinen RK, Helm A, Krauss J, Lindborg R, Öckinger E, Pärtel M, Pino J, Rodà F, Stefanescu C, Teder T, Zobel M, Steffan-Dewenter I (2009) Extinction debt: a challenge for biodiversity conservation. Trends Ecol Evol 24:564–571
Lieberman BS (2012) Adaptive radiations in the context of macroevolutionary theory: a paleontological perspective. Evol Biol 39:181–191
McGhee GR Jr, Clapham ME, Sheehan PM, Bottjer DJ, Droser ML (2013) A new ecological-severity ranking of major Phanerozoic biodiversity crises. Palaeogeogr Palaeoclimatol Palaeoecol 370:260–270
Myers CE, Saupe EE (2013) A macroevolutionary expansion of the modern synthesis and the importance of extrinsic abiotic factors. Palaeontology 56:1179–1198
Pagel M (2020) Can't see the wood for the trees. Nature 580:461–462
Rabosky DL (2013) Diversity-dependence, ecological speciation, and the role of competition in macroevolution. Annu Rev Ecol Evol Syst 44:481–502
Raup DM, Sepkoski JJ (1982) Mass extinctions in the marine fossil record. Science 215:1501–1503
Saupe EE, Myers CE, Peterson AT, Soberón J, Singarayer J, Valdes P, Qiao H (2019) Spatio-temporal climate change contributes to latitudinal diversity gradients. Nat Ecol Evol 3:1419–1429
Sepkoski JJ (1986) Phanerozoic overview of mass extinction. In: Raup DM, Jablonski D (eds) Patterns and processes in the history of life. Springer, Berlin/Heidelberg, pp 277–295
Sepkoski JJ Jr (1996) Competition in macroevolution: the double wedge revisited. In: Jablonski D, Erwin DH, Lipps JH (eds) Evolutionary paleobiology. University of Chicago Press, Chicago, pp 211–255
Silvestro D, Warnock RC, Gavryushkina A, Stadler T (2018) Closing the gap between palaeontological and neontological speciation and extinction rate estimates. Nat Commun 9:1–14
Stanley SM (1979) Macroevolution: pattern and process. W. H. Freeman, San Francisco
Van Valen L (1973) A new evolutionary law. Evol Theory 1:1–33
Vermeij GJ (1977) The Mesozoic marine revolution: evidence from snails, predators and grazers. Paleobiology 3:245–258
Vrba ES (1980) Evolution, species and fossils: how does life evolve? S Afr J Sci 76:61–84
Vrba ES (1993) Turnover-pulses, the Red Queen, and related topics. Am J Sci 293:418–452
Wiley EO, Lieberman BS (2011) The theory and practice of phylogenetic systematics, 2nd edn. Wiley-Blackwell, Hoboken

Evolution of Complexity

Daniel W. McShea

Contents

Abstract

To study the evolution of complexity in organisms, we need an understanding of complexity that enables us to measure it. In biology today, organismal complexity has two main operational senses: (1) a horizontal sense: the number of different part types at a given hierarchical level (e.g., the number of cell types in a multicellular individual) and (2) a vertical sense: the number of levels of nestedness of parts within wholes (e.g., a eukaryotic multicellular individual is one level of nestedness above a free-living protist). How do horizontal and vertical complexity behave in evolution? For horizontal complexity, an increasing trend is predicted by current theory, that is, by the zero-force evolutionary law (ZFEL), but at most hierarchical levels, evidence is lacking and the existence of a trend is uncertain. For vertical complexity, there is unambiguous evidence for a trend in the maximum, a rise in the maximum hierarchical level achieved by organisms over the history of life. However, the underlying mechanism of change and the forces driving the trend are unknown. Interestingly, there is some evidence that

D. W. McShea (✉)
Biology Department, Duke University, Durham, NC, USA
e-mail: dmcshea@duke.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_123

the rise in vertical complexity, the addition of new levels, is – when it occurs – accompanied by systematic losses in horizontal complexity at lower levels.

Keywords

Horizontal complexity · Vertical complexity · ZFEL · Complexity drain · Evo-devo

Introduction

The study of the evolution of organismal complexity is not about progress, performance, or perfection in evolution. It is not about genes or about the genetic basis of sophisticated adaptations like the eye or the brain. It is not an application of nonlinear equations, information theory, or any subfield of mathematical biology. At least, it is not these things to start with. At some point, when the basic patterns of change in complexity in evolution have been discovered, we will certainly want to investigate the relationship between complexity and adaptedness, to study the genetic basis of eyes and brains and such, and to consider what mathematical gadgetry might be deployed to capture and predict these relationships. But until we know just what it is that is changing, how it changes, and under what circumstances it changes, until then, we do not even know what we are trying to investigate.

So the first priority must be to devise a working understanding of complexity, one that will enable us to measure it in real organisms, in practice, not just in principle. The second is to actually measure it in organisms over the history of life and to discover the pattern of change of complexity in evolution. We will be interested in the whole pattern of change, not just the increases – the emergences of new spectacularly complex organisms – but also the decreases, the frequent retreats into simplicity.

The assumption, the hope, is that complexity will turn out to be more than a word that we use to capture the wonder, awe, and mystery of biology and that it will prove to be an important causal factor, one that can be – like temperature – measured and its effects quantified.

Horizontal and Vertical, Objects and Processes

We begin with a simple and intuitive view of complexity as number of different part types. A fish with 130 cell types has a complexity of 130. An arthropod with 50 cell types is less complex, more precisely it is 50/130 or 0.38 times as complex. This is complexity in a horizontal sense, where horizontal refers to the fact that we are counting part types at a single level of organization, in this case the cell level. And therefore, technically, when we count part types, we also need to specify the level of organization. The complexity of a fish *at the level of its cells* is 130. But its complexity at a higher level, the level of tissues and organs, is about 90. There is no contradiction here. Complexity is simply a level-relative concept, with different values at different levels.

Thus, it is not meaningful to speak about the "real" or "true" complexity of an organism in a way that implies there is some level-independent sense of the word. And contrary to conventional intuitions, an organism has no "true" complexity that is some function of its genes. A pufferfish has 20,000–25,000 genes (about the same as a human), but that number is just the complexity of the fish *at the molecular level*, and it no more represents a "true" complexity of the organism than does the number of cell types. (Actually, properly speaking, the complexity of the fish at the molecular level would be a count of all of the types of molecule that it contains, a number far greater than the number of genes.) Organisms – indeed all objects – simply have different complexity values at different levels.

Complexity as part types is a measure of difference, the number of parts that are significantly different from each other. It is a discrete measure in that it treats the differences among parts as discontinuous. In many cases however, especially in biology, differentiation is continuous, and in those, the appropriate measure is a more general one, complexity as degree of differentiation among parts. The vertebrae of a fish are all very similar from one end of the column to the other. In a mammal, the vertebrae are more differentiated – cervicals are different from thoracics, which in turn are different from sacrals, and so on. So a mammal column is more complex than a fish column (at the level of vertebrae). Any of the usual measures of continuous differentiation – such as standard deviation or variance – can be used as measures of complexity in such cases.

The notion of "parts" is not well established in biology, but it turns out not to be very troublesome. Organisms are not as cleanly separable into parts as machines are, but many parts are – like organs and cells – reasonably discrete objects, sufficiently so that they can be counted. Philosophical issues associated with the use of the parts concept have been addressed (McShea and Venit 2001), and solutions to some of the practical problems involved in counting have been devised (McShea 2002).

We are treating organisms here as objects, ignoring initially the intricacies of their development and their marvelous adaptations. Those will all become important as we explore further. In particular, we will want to ask about the relationship between complexity and development and between complexity and adaptation. But in order to ask those questions, we need an independent way to measure complexity. That is, in order to ask, say, whether more complex organisms are better adapted, we need a way to measure complexity that is independent of adaptation, just as asking about the relationship between health and happiness requires us to devise a measure of happiness that is independent of health.

Notice that the independence required here is conceptual. That is, it may turn out that as an empirical matter, complexity and adaptation are correlated with each other, and if the two variables are conceptually independent, that is an interesting finding. But if we adopt a measure of complexity that has the concept of adaptation built into it, definitionally, the discovered empirical correlation would be at least in part an autocorrelation, between adaptation and itself, and that would tell us nothing.

Horizontal complexity is just one variety of complexity. There is also vertical complexity, or the number of levels of nestedness of parts within wholes (see section on "Vertical Complexity" below). Both have to do with physical structure. Both treat organisms as objects. But there is also complexity of processes, the number of

different kinds of interaction among the parts of an organism, either in its physiology or its development. And there is the irregularity of the arrangement of parts (or processes) within an organism, in time or space. Even more types can be imagined (McShea 1996). What they all share is a view of complexity as a physical property, a property purely of an organism's structure and independent of function, one that at least in principle can be operationalized and measured in a straightforward way. As it happens, only the horizontal and vertical senses have been operationalized and applied to organisms so far in biology, so those are the senses I focus on here.

There is nothing necessary or inevitable about this way of thinking about complexity. Other ways can be imagined. But this view has become standard in biology in the last two decades (Valentine et al. 1994; Doolittle 2012). Also, not long ago, our best assessments of organismal complexity were entirely impressionistic, or based surreptitiously on the Great Chain of Being, with the consequence that it was impossible to give answers to questions about trends in complexity that were both serious and scientific. But as methods in evolutionary developmental biology have advanced, and as our knowledge of organismal structure at multiple levels of organization has grown, the data necessary to investigate trends are for the first time becoming available. The viewpoint described here offers methods and measures to transform those data into objective answers.

Horizontal Complexity and the Zero-Force Law

For evolution and horizontal complexity, theory has for the time being outstripped empirical investigation. We know what horizontal complexity is expected to do in evolution, under certain ideal conditions – namely, to increase, on average – but we do not yet know much about whether in fact it has.

The theory that predicts increase is called the zero-force evolutionary law (ZFEL, McShea and Brandon 2010), which says that in the absence of natural selection favoring increase or decrease in complexity (and of any constraints favoring increase or decrease), the number of part types or degree of differentiation among types is expected to increase (McShea and Brandon 2010). The reason is simply that in evolution, everything varies, so that parts that are initially identical will tend to become different from each other and parts that are already somewhat different will tend to become even more different. Concretely, in the absence of constraint or contrary selection, the segments in segmented animals will tend to become more different from each other, from one generation to the next. More generally, in any system with both heritability and variation, chance variation in parts accumulates, leading to divergence of parts. This is true only when such divergence is not blocked by forces (in biology, natural selection) or constraints of various kinds. (For more on constraint.

The ZFEL is consistent with standard evolutionary theory, which in the absence of selection predicts divergence due to drift. But the ZFEL goes further, predicting increasing complexity even when variation is under tight control by natural selection

acting at lower levels. That is, if one segment of a segmented animal – say a segment near the mouth – is selected for ability to manipulate food and another more distal segment is selected for ability to walk or swim, the two segments will tend to become more different from each other. And that is the ZFEL. Calling this the ZFEL would seem to violate the no-selection requirement, but it does not, because the law predicts increasing complexity whenever no selection acts directly on the complexity of the structure in question. In the case here, there is no selection acting on the degree of differentiation of the segment series as a whole, no selection acting to keep the segments similar to each other, and none favoring their becoming different. There is only selection on each one, separately, for a special purpose. The segments are not drifting, but they are changing randomly in a special sense: randomly with respect to each other, in other words independently of each other. Thus, the ZFEL predicts increasing complexity under a wide range of circumstances.

Finally, consider what happens when selection does act on complexity, not just on individual parts independently but on the degree of differentiation among them. Now the prediction changes. Selection acting against complexity could overwhelm the ZFEL tendency, producing morphological stasis or even simplification. On the other hand, selection favoring complexity is expected to reinforce the ZFEL, producing complexification at a rate greater than expectation due to passive divergence of part types. Detecting selection of this sort would require a quantitative version of the ZFEL (not yet developed), in order parse any given increase into its ZFEL-driven and selection-driven components.

Horizontal Complexity and the Structure of Development

Besides selection acting on complexity, internal constraints of various kinds could deflect the increase predicted by the ZFEL. In particular, if organismal development were structured in such a way as to make a loss of parts more likely than a gain of parts, the complexity increase predicted by the ZFEL might be thwarted. In the conceptual scheme of the ZFEL, the understanding in such a case would be that the accumulation of differences among parts proceeds nevertheless, but that it is simply overwhelmed by the bias in favor of part loss imposed by the structure of development (McShea and Brandon 2010). Is development in fact structured in this way? There is some reason in theory to think it might be. It has been suggested that part loss might be easier than part gain in evolution simply because mutations are more likely to destroy developmental pathways leading to existing parts than to originate new pathways leading to new ones (discussed in McShea 1996). Destruction is easier than creation, the argument goes.

This logic of loss is powerful, but it is worth pointing out that, unlike machine parts, biological materials are what have been called “excitable media” (Goodwin 1996), with the result that removal of a part can lead to a novel contact between tissues in development, sometimes inducing a new part (Müller and Streicher 1989). What is more, development is known to be to some degree hierarchically structured, to consist of processes organized in cascades of developmental dependency that

make losses extremely difficult. Many metazoans, for example, pass in early development through a phylotypic stage in which the basic body plan is laid down (Raff 1996). Later developmental structures are built on top of the phylotypic stage, so to speak, and therefore depend on it, as a house depends on its foundation. When development is structured this way, losses of parts affecting the phylotypic stage are likely to lead to an inviable embryo and thus are expected to be strongly opposed by selection, while buffering mechanisms which prevent such losses will be highly favored (i.e., canalization). A similar principle has been documented at a smaller scale, the molecular level, in recent years. Finnigan et al. (2012) discovered a kind of complexity ratchet in an oligomeric ring that forms part of an ATPase in fungi. In the abstract version of this ratchet described by Doolittle (2012), an initially homo-oligomeric ring, initially consisting of six molecules of type A bound together in a ring, becomes hetero-oligomeric by neutral drift, coming to consist of As and Bs. In a second step, A loses by drift the ability to bind to itself, with the result that B is required for a functional ring to form. In other words, the complexity of the ring increases by drift (essentially, by the ZFEL), but cannot decrease, because B cannot be lost without destroying ring functionality.

The hierarchical structure of early development, recognized in Von Baer's law, is widely acknowledged, but it has so many exceptions that it would be hazardous to claim that it results in a general bias toward gain over loss in development. The same goes for the ratchet described by Doolittle and Finnigan et al. Thus, at present, despite the huge increase in research on development in particular biological systems in the past two decades, there is nothing that can be said about the on-average structure of development, about whether organizations favorable to accumulation are more prevalent than those favorable to loss. In other words, there is no data on whether development in general is structured in a way that promotes either part loss or part gain, or indeed whether it biases complexity generation at all, on average.

A Trend in Horizontal Complexity?

Empirically we know very little about any overall directionality in horizontal complexity in evolution. Valentine et al. (1994) document an increase in the maximum number of cell types over the Phanerozoic in metazoans. Many increasing trends have been documented within certain metazoan groups at the tissue/organ level, such as the increase in degree of differentiation among arthropod limb types (discussed in McShea 1996). But decreasing trends have been demonstrated as well, such as the decrease in number of types of skull bones in vertebrates (Sidor 2001). At a lower level of organization, within cells, a loss of part types – organelles and other structures – has been documented in the transitions to multicellularity in plants and animals (see below, under "An Evolutionary Syndrome"). And the general tendency for complexity to decrease in organisms living in constant environments, especially parasites, has long been acknowledged. In fact, losses of complexity have been recognized in recent years to be much more pervasive than previously thought (O'Malley et al. 2016). Still, the overall pattern for horizontal complexity in the

history of life is unknown. The ZFEL predicts increase as the default expectation, but we do not know whether the default conditions are met, on average, or whether selection or constraints routinely overcome the ZFEL.

It might seem that the literature on novelty or innovation in evolution is relevant here (see chapter ▶ "Developmental Innovation and Phenotypic Novelty"). And in fact it could be relevant, if these terms are understood in a purely structural sense, as number of part types. But novelty and innovation are often used in a functional sense or in some hybrid sense combining both structure and function, and in that case, its relationship to complexity is uncertain. A novel function could arise with the gain of a part, as occurred in the gain of mitochondria in the lineage leading to eukaryotes. But it could also arise as a loss of parts, as in the loss of limbs in whales, leading to a fully aquatic life, an innovative functional mode for that lineage.

Vertical Complexity

Vertical complexity is number of levels, in other words, the depth of nestedness, or number of tiers of parts within wholes in an organism. A multicellular eukaryotic individual has greater vertical complexity than a solitary eukaryotic protist, because a multicellular is – historically speaking – an aggregation of single cells and therefore one level of nestedness deeper than a solitary protist.

The trend in vertical complexity over the history of life is well known and well documented (Heim et al. in press; McShea and Changizi 2003). It has four unambiguous data points. By convention, the basement level for organisms is set at the level of the prokaryotic cell. The second level is occupied by the eukaryotic cell, which arose as an aggregation of prokaryotic cells (initially, presumably, a eubacterial endosymbiont living within an archaebacterial host). The third level is occupied by the multicellular eukaryotes and the fourth by societies or colonies of multicellular eukaryotes. Figure 1 shows the trajectory of the trend in first occurrences, in other words, the trend in the maximum.

A scale based on nestedness, parts within wholes, raises some conceptual issues: (1) The starting point is somewhat arbitrary. If non-organismal systems are included, the scale could presumably have to extend downward to include entities at the molecular scale, such as chemical cycles, that is, entities that are the components of prokaryotes, either presently or historically. (2) Occupation of a level of nestedness can be understood to be continuous, rather than discrete. A green alga like *Gonium* that is a simple aggregation of eukaryotic cells occupies the multicellular level, but an oak tree with more cells and greater internal organization can be said to occupy that level to a greater degree. In other words, it is more individuated at that level. McShea and Changizi (2003, and references therein) devised an expanded vertical complexity scale that interpolates two sublevels, marking degrees of individuation, between the major levels. (3) Vertical complexity is usually understood to be a property of the structure of organisms, but there is no reason the concept could not be extended to include ecological associations of many sorts. Multispecies bacterial associations, including some biofilms, might be considered a level above a

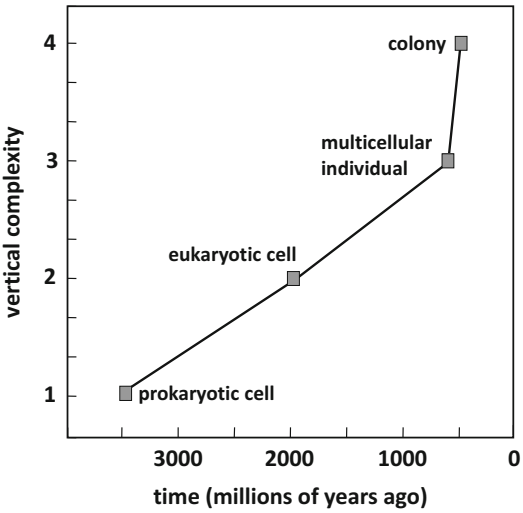


Fig. 1. The trend in vertical complexity over the history of life, showing first occurrences of organisms at each hierarchical level

solitary bacterium. And certain trophic webs might reach to levels higher than level 4, societies and colonies. Interestingly, the standard four-level scale for organisms already implicitly includes ecological associations, since the first eukaryotic cell arose as a multispecies association. (See Eldredge and Salthe 1984 for a treatment of hierarchy scales in biology generally.)

The trajectory of the trend in Fig. 1 raises a number of puzzles. One of the most salient has to do with the cause of the apparent acceleration. The transitions from prokaryotic cell to eukaryotic cell and from eukaryotic cell to multicellular eukaryote took about 1.4 billion years each. But the next transition, from multicellular to well-individuated societies/colonies, took only a few 100 million years. Increasing vertical complexity is a route to larger body size, so the acceleration could be connected with the general rise in body size among organisms at that time, perhaps triggered by the rise in atmospheric oxygen (Payne et al. 2011). A second puzzle is the apparent slowing, or even truncation, of the trend at the level of society/colony. It has been about 500 million years since the first well-individuated colonies (bryozoans) appeared in the fossil record, but the next level – colonies of colonies – has not yet arisen, so far as we know (McShea and Changizi 2003). It is possible that human societies are on their way to that level, but the degree of individuation of our larger social units has not been assessed in any rigorous way, and indeed a case could be made that the human commitment to sociality does not even rise to the bryozoan level. If that is right, then the slowing of the vertical complexity trend in the past 500 million years is an unsolved puzzle.

A third puzzle has to do with the dynamics of the trend, the pattern of change in vertical complexity within lineages that accounts for the overall trend over the history of life. One possibility is that the trend is the result of passive diffusion away from a lower limit or wall. The first organisms may have been as vertically simple as possible, so that as diversity increased, vertical complexity could only have increased as well.

With a wall on the left, there is nowhere to go but up. And in fact, a study of changes in degree of individuation within and among levels revealed no upward tendency (Marcot and McShea 2007), consistent with a diffusive dynamic. But another possibility, advanced by Knoll and Bambach (2000), is that whenever a new vertical level is achieved (e.g., the eukaryotic cell), change occurs by diffusion away from a left wall (arising from the improbability of eukaryotic cells returning a prokaryotic condition), but this diffusion is limited by a wall on the right (e.g., the biomechanical difficulties presented by multicellularity). Heim et al. (in press) offer evidence, based on body size distributions, that the history of life can be understood as a series of expansions away from left walls and repeated scaling of right walls.

Some excitement about the vertical aspect of complexity has been generated in biology in the past two decades by Maynard Smith and Szathmáry's (1995) book on the "major transitions" in the history of life. They understand major transitions as the origin of new levels of replication or of significant changes in the way that information is transmitted. Their new levels of replication correspond closely with the levels of complexity addressed here (prokaryotic cell, eukaryotic cell, multicellular eukaryote, society/colony), but the other major transitions (including the origin of sexual reproduction and of human language) do not. The relationship between levels and information, as well the meaning of the term "major transition," is currently under scrutiny in biology and the philosophy of biology (Calcott and Sterelny 2011), and in the meantime, the relationship of that literature to vertical complexity remains uncertain.

There is also a fair-sized literature on mechanisms underlying change in vertical complexity, seeking to understand the forces at work in the transition from solitary individual to simple aggregate to highly individuated whole. In particular, the question has been how selection on the whole is able to suppress the tendency of lower-level individuals to behave selfishly, undermining the interests of the whole (Maynard Smith and Szathmáry 1995; see also discussion in Simpson 2012). There has also been some interest in the prior question of how aggregates arise in the first place, necessarily prior because selection on the whole cannot act until the whole has arisen (Brandon and Fleming 2015). A related theme in that literature has to do with the structural features of new higher levels, with the engineering aspects of hierarchical structure, as opposed to fitness issues (Calcott 2008). For example, Venit (2007) investigated the relationship between degree of division of labor among lower-level entities and the degree of connectedness among them. And he found that in marine invertebrate species that form highly individuated colonies, lower-level individuals did not share body cavities (complete connectedness) nor were they fully walled off from each other (complete isolation), but instead showed intermediate levels of connectedness.

An Evolutionary Syndrome

It is worth reiterating that horizontal and vertical complexity are conceptually independent, meaning that nothing in the definition of either term necessarily implies anything about the other. A system can be vertically complex (having many levels) and horizontally simple (having few part types at some level), vertically complex

and horizontally complex, and the reverse of both of these. In other words, all four combinations are possible in principle. Still, there is some data to suggest that there is a connection in fact, more specifically, that as a new higher level emerges, parts are lost at the next level down, what has been called a complexity drain (McShea 2002; O'Malley et al. 2016). In the emergence of the eukaryotic cell, the eubacteria that evolved into mitochondria became simpler, losing molecular components. More generally, it is thought that in multispecies bacterial associations, one level up from solitary bacteria, certain species may tend to lose the molecular machinery associated with certain metabolic functions as other species in the association take over those functions (also known as the "black queen" effect). Also, in multicellular organisms, the cells have fewer part types on average than free-living protists (McShea 2002). To see this, consider an extreme case, human blood cells, which have essentially no parts at all, compared to free-living protists, which have many. Finally, there is some reason to think that the same pattern of loss occurred in the origin of intensely social/colonial organisms: as the individuals in clonal associations differentiated into multiple types (castes in insects, polymorphs in marine invertebrates), they lose parts, becoming simpler as they specialized.

These observations suggest a possible pattern at the largest scale, a recurring set of changes at the scale of life as a whole, in other words a kind of evolutionary "syndrome" (McShea 2015) with three signature "symptoms." The first is the trend in vertical complexity itself, the episodic addition of ever-higher levels of nestedness in organisms. The second is the increase in horizontal complexity within each level, that is, the increase in number of part types. And the third is a decrease in horizontal complexity within those parts, the complexity drain. More research is needed to see if these patterns are robust and then to investigate possible causes.

Cross-References

- ▶ A Macroevolutionary Perspective on Developmental Constraints in Animals
- ▶ Complexity in Evo-Devo
- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Levels of Organization in Evo-Devo
- ▶ Macroevolution
- ▶ Morphological Disparity

References

Brandon R, Fleming L (2015) Why flying dogs are rare: a general theory of luck in evolutionary transitions. Stud Hist Phil Biol Biomed Sci 49:24–31

Calcott B (2008) The other cooperation problem: generating benefit. Biol Philos 23:179–203

Calcott B, Sterelny K (eds) (2011) The major transitions in evolution revisited. The MIT Press, Cambridge

Doolittle WF (2012) A ratchet for protein complexity. Nature 481:270–271

Eldredge N, Salthe SN (1984) Hierarchy and evolution. Oxf Surv Evol Biol 1:184–208

Finnigan GC, Hanson-Smith V, Stevens TH, Thornton JW (2012) Evolution of increased complexity in a molecular machine. Nature 481:360–364
Goodwin BC (1996) How the leopard changed its spots. Simon and Schuster, New York
Heim NA, Payne JL, Finnegan S, Knope ML, Kowalewski M, Lyons SK, McShea DW, Novack-Gottshall PM, Smith FA, Wang SC (in press) Hierarchical complexity and the size limits of life. Proc R S Lond B Biol Sci
Knoll AH, Bambach RK (2000) Directionality in the history of life: diffusion from the left wall or repeated scaling of the right? Paleobiology 26:1–14
Marcot J, McShea DW (2007) Increasing hierarchical complexity throughout the history of life: phylogenetic tests of trend mechanisms. Paleobiology 33:182–200
Maynard Smith J, Szathmáry E (1995) The major transitions in evolution. Oxford University Press, New York
McShea DW (1996) Metazoan complexity and evolution: is there a trend? Evolution 50:477–492
McShea DW (2002) A complexity drain on cells in the evolution of multicellularity. Evolution 56:441–452
McShea DW (2015) Three trends in the history of life: an evolutionary syndrome. Evol Biol 43:531–542
McShea DW, Brandon RN (2010) Biology's first law. University of Chicago Press, Chicago
McShea DW, Changizi MA (2003) Three puzzles in hierarchical evolution. Integr Comp Biol 43:74–81
McShea DW, Venit EP (2001) What is a part? In: Wagner GP (ed) The character concept in evolutionary biology. Academic Press, San Diego, pp 259–284
Müller GB, Streicher J (1989) Ontogeny of the syndesmosis tibiofibularis and the evolution of the bird hindlimb: a caenogenetic feature triggers phenotypic novelty. Anat Embryol 179:327–339
O'Malley MA, Wideman JG, Ruiz-Trillo I (2016) Losing complexity: the role of simplification in macroevolution. Trends Ecol Evol 31:608–621
Payne JL, McClain CR, Boyer AG, Brown JH, Finnegan S, Kowalewski M, Krause RA Jr, Lyons SK, McShea DW, Novack-Gottshall PM, Smith FA, Spaeth P, Stempien JA, Wang SC (2011) The evolutionary consequences of oxygenic photosynthesis: a body size perspective. Photosynth Res 107:37–57
Raff RA (1996) The shape of life: genes, development, and the evolution of animal form. University of Chicago Press, Chicago
Sidor CA (2001) Simplification as a trend in synapsid cranial evolution. Evolution 55:1419–1442
Simpson C (2012) The evolutionary history of division of labour. Proc R Soc B Biol Sci 279:116–121
Valentine JW, Collins AG, Meyer CP (1994) Morphological complexity increase in metazoans. Paleobiology 20:131–142
Venit EP (2007) Evolutionary Trends in the individuation and polymorphism of colonial marine invertebrates. PhD thesis, Department of Biology, Duke University

Convergence

George R. McGhee

Contents

Abstract

Convergence is the evolution of the same or very similar traits independently in different lineages of organisms. There exist three different pathways by which evolution may produce convergent forms: allo-convergence, iso-convergence, and retro-convergence. Allo-convergent evolution is the independent evolution of the same or very similar new trait from *different precursor traits* in different lineages; iso-convergent evolution is the independent evolution of the same or very similar new trait from the *same precursor trait* in different lineages; and retro-convergent evolution is the independent *re-evolution* of the same or very similar trait to an *ancestral trait* in different lineages. In addition to convergent phenotypic and molecular evolution, ecological niche convergence is the evolutionary occupation of the same ecological niche, the same ecological role in life, independently by different lineages of organisms. Ecological niche convergence may not result in morphological convergence at all, in that ecologically convergent organisms may vary widely in their morphologies but their ecological niches, their modes of life, are the same.

G. R. McGhee (✉)
Department of Earth and Planetary Sciences, Rutgers University, New Brunswick, NJ, USA
e-mail: mcghee@eps.rutgers.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_124

Analyzing the phenomenon of convergence in evolution is now becoming as active a field of evolutionary research as the analysis of the phenomenon of divergence. The results of future convergence research should provide definitive answers to current questions concerning the degree to which evolutionary processes are predictable or unpredictable, limited or unbounded, directed or directionless, and the degree to which those processes are extrinsically (selectively) limited, intrinsically (developmentally) limited, or unlimited (random).

Keywords

Convergent evolution · Evolutionary repeatability · Evolutionary limits · Functional constraint · Developmental constraint

Introduction

Convergence is the evolution of the same or very similar traits independently in different lineages of organisms. This evolutionary phenomenon was clearly known to Charles Darwin even in the infancy of evolutionary analysis in biology, as he wrote in the first edition of *On the Origin of Species* that "In all cases of two very distinct species furnished with apparently the same anomalous organ, it should be observed that, although the general appearance and function of the organ may be the same, yet some fundamental difference can generally be detected. I am inclined to believe that in nearly the same way as two men have sometimes independently hit on the very same invention, so natural selection, working for the good of each being and taking advantage of analogous variations, has sometimes modified in very nearly the same manner two parts in two organic beings, which owe but little of their structure in common to inheritance from the same ancestor" (Darwin 1859, pp. 193–194). And before Darwin, the great systematist Linnaeus finally moved the whales from the fish and placed them with the mammals in the tenth edition of *Systema Naturae* in 1758, realizing that although whales looked like fish they simply possessed too many mammalian traits to be fish.

Convergence is currently at the center of multiple debates – some highly contentious – concerning the process, pattern, and essential nature of evolution in Earth history (Losos 2011; McGhee 2011). Convergent evolution was once thought to be a rare curiosity in the history of life, but now, it is being argued to be ubiquitous at all levels of life and the dominant evolutionary process – the evolutionary expectation rather than the exception (Conway Morris 2003; McGhee 2011; Martin and Orgogozo 2013). In the twentieth-century modern synthesis of evolutionary biology, convergence was argued to be a classic adaptive phenomenon and a prime example of natural selection at work, whereas in the twenty-first century extended evolutionary synthesis much of evolutionary convergence is being argued to be produced by developmental constraints and developmental bias, not by natural selection (Laland et al. 2015). In the twentieth century, the evolutionary process was argued to be unpredictable due historical contingency, whereas in the twentieth-first century

historically contingent evolution is being argued to be constrained to occur within a finite number of limited convergent pathways, and thus to be probabilistically predictable (McGhee 2011, 2015; Hordijk 2016; see chapter ▶ "Inherency").

Analyzing Convergent Evolution

A phylogenetic-systematics analysis is essential to the recognition of convergent evolution in any given group of organisms (Lecointre and Le Guyader 2006). Only in a phylogenetic analysis can it be revealed whether a similar trait found in different species is shared by those species simply because they inherited it from a common ancestor with that derived trait – that is, that the trait is a synapomorphy – or whether the similar trait has arisen independently in each different and separate species lineage – that the trait is convergent.

Given an initial phylogenetic analysis (Fig. 1), convergence can be shown to arise in three different ways (Figs. 2, 3, and 4). In Fig. 1, the phylogenetic relationships of six hypothetical species are shown. Species 1, 2, and 3 all possess synapomorphy S and belong to the monophyletic clade S. Likewise, species 4, 5, and 6 all belong to clade T as all possess synapomorphy T. Although clade S and clade T have diverged in their evolution, they nevertheless evolved from a common ancestor that evolved the derived trait R, a trait that all six species still possess by inheritance. Thus trait R is a synapomorphy for the larger monophyletic clade R, which contains both clade S and clade T.

The three different pathways by which evolution may produce convergence are allo-convergent, iso-convergent, and retro-convergent (Figs. 2, 3, and 4). Allo-convergent evolution is the independent evolution of the same or very similar new trait from *different precursor traits* in different lineages (Pontarotti and Hue 2016). The phenomenon of allo-convergence is illustrated in Fig. 2, in which a new trait Z independently evolves in species 3 and species 6 from different preexisting traits, trait A in the case of species 3 and trait B in the case of species 6. We can prove that trait Z

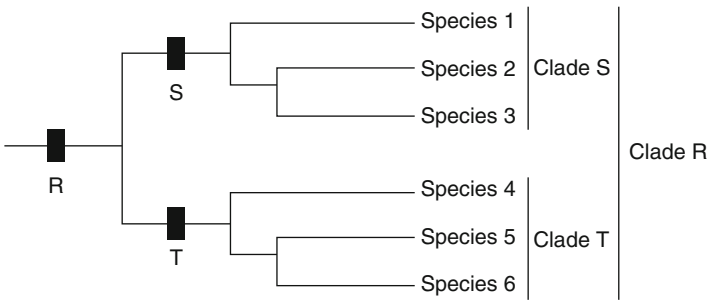


Fig. 1 Cladogram of evolutionary relationships between six hypothetical species (Modified from McGhee 2011)

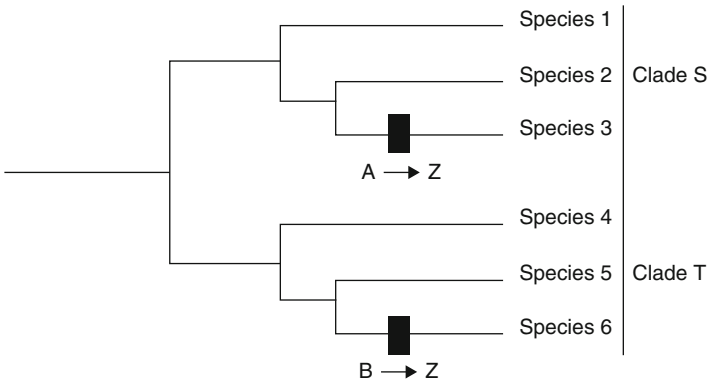


Fig. 2 Allo-convergent evolution of trait Z in species 3 and 6 (Modified from McGhee 2011)

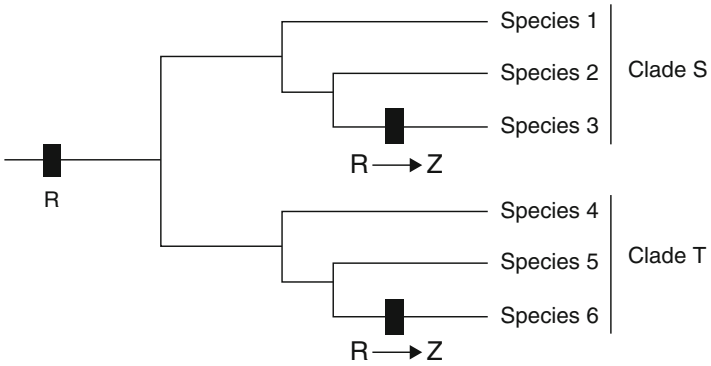


Fig. 3 Iso-convergent evolution of trait Z in species 3 and 6 (Modified from McGhee 2011)

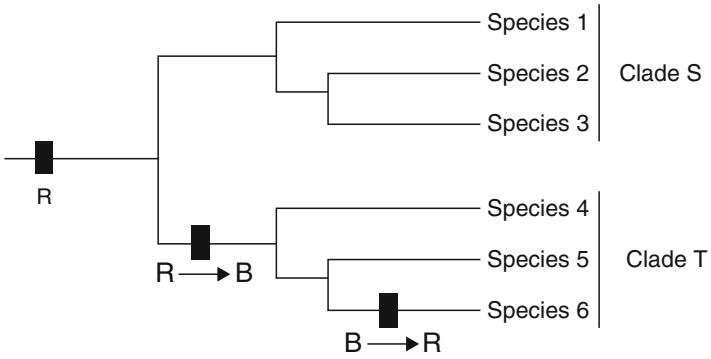


Fig. 4 Retro-convergent evolution of trait R in species 6 within clade T, whereas all species of clade S simply inherit trait R from a common ancestor (Modified from McGhee 2011)

evolved independently in these two species lineages and that trait Z is not a synapomorphy that species 3 and species 6 inherited from a common ancestor, because we have previously conducted a phylogenetic analysis for the entire group of species (Fig. 1) and know that species 3 and species 6 belong to two entirely different clades, clades S and clade T.

A classic example of allo-convergence is the independent evolution of wings in bats and dragonflies. The evolutionary lineage of a bat (Bilateria: Deuterostomia: Chordata: Vertebrata) is very different and divergent from that of a dragonfly (Bilateria: Protostomia: Ecdysozoa: Arthropoda), yet both have evolved wings – but from radically different precursor traits. The bat's wings are modified vertebrate forelimbs, whereas the dragonfly's wings are modified larval-stage gill branches.

Iso-convergent evolution is the independent evolution of the same or very similar new trait from the *same precursor trait* in different lineages (Pontarotti and Hue 2016). The phenomenon of iso-convergence is illustrated in Fig. 3, in which a new trait Z independently evolves in species 3 and species 6 from the same preexisting trait, trait R, in both species 3 and species 6. A classic example of iso-convergence is the independent evolution of wings in bats, birds, and the extinct pterodactyls of the Mesozoic. A bat is a member of the clade of the mammals, a bird is a member of the clade of the dinosaurs, and a pterodactyl is a member of the clade of the pterosaurs – yet all three animals are members of the larger inclusive clade of the vertebrates. And the same precursor trait – the vertebrate forelimb – was independently modified to form wing structures iso-convergently in bats, birds, and pterodactyls.

Retro-convergent evolution is the independent *re-evolution* of the same or very similar trait to an *ancestral trait* in different lineages (this definition is not the same as that for the proposed retroconvergent or cyclic evolutionary process of Krassilov 1995). The phenomenon of retro-convergence is illustrated in Fig. 4, in which trait R has been modified into trait B, a new trait possessed by species 4 and species 5 in clade T. In species 6, however, trait B has been modified back into trait R – an evolutionary reversion or "reverse convergent evolution." Species 6 did not inherit trait R directly from its ancestor (which possessed trait B), whereas species 1, 2, and 3 all do possess trait R by inheritance from a common ancestor (Fig. 4), thus we would create an erroneous polyphyletic clade if we were to mistakenly include species 6 in clade S along with species 1, 2, and 3 on the basis that species 6 also possesses trait R. Species 6 independently acquired trait R by the process of retro-convergent evolution; we know this because species 6 possesses synapomorphy T (Fig. 1) and thus belongs in clade T along with species 4 and 5, even though species 6 does not possess trait B like species 4 and species 5 do (Fig. 4). A classic example of retro-convergence is the independent re-evolution of ancestral fish-like traits in dolphins and the extinct ichthyosaurs. A dolphin is a mammal and an ichthyosaur is a reptile yet they both retro-convergently re-evolved fish-like ventral fins, a tail fin, and a dorsal fin when they made the evolutionary habitat shift from the land back into the oceans.

In the older terminology of evolutionary convergence, allo-convergent evolution is equivalent to convergent evolution sensu stricto, iso-convergent evolution replaces the term parallel evolution, and retro-convergent evolution replaces the

term reverse evolution. The problem with the terms "parallel" and "reverse" evolution is that they generated confusion in that these two types of convergent evolution were often misunderstood not only to be different from convergent evolution but in some cases the actual disproof of convergent evolution (see discussion in McGhee 2011). Phylogenetic systematists consider that the phenomenon of "parallelism is a special case of convergence" in which the same trait has independently evolved "from the same ancestral character in different taxa" (Lecointre and Le Guyader 2006, p. 541). Likewise, the phenomenon of reverse evolution is a special case of convergence in which the same ancestral trait has independently re-evolved in different taxa.

The term "parallel" evolution has created even greater confusion in that this same term has been used to describe different evolutionary phenomena. For example, in some molecular analyses Pontarotti and Hue (2016, p. 4) point out that the term parallel evolution has been used to describe molecular convergence from the same ancestral amino acid in different taxa and convergent evolution only used to describe molecular convergence from different ancestral amino acids in different taxa, whereas in other molecular analyses "the distinction between parallel and convergent evolution is not based on the evolutionary history of the characters, but on the similarity of the genetic mechanisms that are involved in the repeated phenotype" such that "if the molecular mechanisms are the same, the evolution is said to be parallel; if the genetic mechanisms are different, the evolution is said to be convergent." Even more confusing, in phenotypic analyses the concept of "phylogenetic proximity" (however defined) has been used to differentiate parallel and convergent evolution, where the independent evolution of the same trait in phylogenetically close species is described as parallel evolution, whereas the independent evolution of the same trait in phylogenetically distant species is described as convergent evolution (Pontarotti and Hue 2016).

Pontarotti and Hue (2016, p. 4) thus proposed that "in order to help in the conceptual understanding of evolutionary convergence, we propose two neologisms that can be applied to all biological levels: iso-convergent and allo-convergent evolution (iso from the same ancestral state and allo from a different ancestral state)." The advantage of the new terminology is that the terms themselves make it clear that the fundamental phenomenon is convergent evolution (the suffix of the terms), and that the difference between the two phenomena lies in the evolutionary pathway (the prefixes of the terms) producing that convergence (Figs. 2 and 3). The term retro-convergent extends this same logic to the case of "reverse" (retro-) convergent evolution, where the evolutionary pathway producing that convergence involves the re-evolution of an ancestral trait (Fig. 4). The general term homoplasy (similarity not inherited from a common ancestor) covers all three phenomena; allo-, iso-, and retro-convergence.

Last, there remains one final convergence phenomenon – ecological niche convergence. Ecological niche convergence may be defined as "the evolutionary occupation of the same ecological niche, the same ecological role in life, independently by different lineages of organisms" (a phenomenon also known to Darwin; for a discussion of Darwin's research into the convergent evolution of carnivory in plants

see McGhee 2011). In stark contrast to the conventional concept of convergent evolution – the independent evolution of the same morphological traits in different lineages – ecological niche convergence may not result in morphological convergence at all. Ecologically convergent organisms may vary widely in their morphologies but their ecological niches, their modes of life, are the same.

The concept behind ecological niche convergence is that there exist a limited number of ways to make a living in nature, a finite number of ecological niches in nature. If the number of ecological niches were infinite in the universe, every separate species would have its own unique way of living, different from all other species. When we examine the ecological structure of living organisms on Earth, we can clearly see that we do not inhabit such a universe (McGhee 2011).

Ecological niche convergence is best recognized by considering truly bizarre ways of making a living – ecological roles that are so strange that, at first glance, it would seem probable that only one species would have evolved such a restricted ecological pathway. And then to observe, astonishingly, that the ecological evolution of life on Earth is so constrained that multiple species have ecologically converged on that same odd pathway in their evolution. Prime examples of ecological niche convergence are the evolution of carnivorous plants, which has independently occurred in 12 different plant lineages, the evolution of parasitic plants, which has independently occurred in seven different plant lineages, and the evolution of a highly-restricted form of insectivory – eating insects that bore into tree bark – which has independently evolved in seven different lineages of animals; from avian-dinosaurian woodpeckers, finches, and honeycreepers, to marsupial mammals (striped possums), and to primate mammals (the Aye-aye; see discussion in McGhee 2011). All of these animals have strikingly different morphologies but their way of making a living in nature, their ecological niche, is the same (see chapter ► "Evo-Devo and Niche Construction").

Causes of Convergent Evolution

Why does convergent biological evolution occur? Convergence arises because the evolutionary pathways available to life are not endless but instead are *limited*. If the number of possible evolutionary pathways was infinite, then each species on Earth would be morphologically different from every other species, and each species would have its own unique ecological role or niche. Such an Earth does not exist. Instead, repeated evolutionary convergence on similar morphologies, niches, molecules, or even mental states is the norm for life on Earth (McGhee 2011).

Evolutionary limits are the product of functional constraints, developmental constraints, and the two processes acting in concert. Functional constraints are imposed by the laws of physics, chemistry, and geometry and are extrinsic to the organisms affected by those constraints (McGhee 2007). Convergence results from the fact that there are limited number of ways to solve a functional problem within the constraints imposed by the laws of physics, chemistry, and geometry. A fast-swimming organism moving in the dense medium of water (1,027 kg/m^3 at sea level;

over a tonne per cubic meter) must have a streamlined, fusiform body to minimize drag hence fast-swimming sharks, bony fishes, reptilian ichthyosaurs, and mammalian dolphins were all functionally constrained to evolve this body form – nature had no other choice. Organisms flying in the thin atmosphere of the Earth (density of 1.23 kg/m^3 at sea level and 15 °C) must have wings to generate enough lift to overcome the gravity of the Earth hence arthropod insects, reptilian pterodactyls, dinosaurian birds, and mammalian bats were all functionally constrained to evolve wing forms – and humans were functionally constrained to construct heavier-than-air flying machines with wings.

Functional constraint may underlie much of the allo-convergent evolution observed in nature. And, as the laws of physics, chemistry, and geometry are believed to be the same throughout the universe, the same functional constraints that we observe in action in the convergent evolution of life on Earth should apply equally to any alien life forms evolving under the same physical conditions in other regions of the universe. That is, we should expect fast-swimming organisms in alien seas on an Earth-like alien planet to have evolved streamlined, fusiform bodies, and flying alien organisms in the skies of that alien planet to have evolved wings.

In contrast to functional constraints, developmental constraints are intrinsic and are imposed by the biology and phylogeny of specific organisms (McGhee 2007). First, the developmental pathways that are available to specific organisms are limited by what has been variously called phylogenetic legacy, phylogenetic inertia, or phylogenetic constraint (see discussion in McGhee 2007). For example, the vertebrate pterodactyls, birds, and mammals all were developmentally constrained to iso-convergently evolve only two wings because their vertebrate phylogenetic legacy provided them with only two forelimbs that could be modified to form wings. However, that vertebrate developmental constraint does not apply to the arthropod insects and the dragonfly therefore is able to develop four wings, not just two, because dragonfly wings are allo-convergently evolved from entirely different precursor traits – larval-stage gill branches.

Second, even given a certain phylogenetically available repertoire of traits, developmental bias may make the generation of some of those traits more probable than others, including processes such as the "repeated, differential re-use of developmental modules, which enables novel phenotypes to arise by developmental rearrangements of ancestral elements, as in the parallel evolution of animal eyes" (Laland et al. 2015, p. 3). Third, "phenotypic variation can be channeled and directed towards functional types by the process of development," a developmental phenomenon known as facilitated variation that can "sometimes elicit substantial, non-random, well-integrated and apparently adaptive innovations in the phenotype" (Laland et al. 2015, p. 3).

The interaction between developmental constraint and functional constraint may underlie much of the iso-convergent and retro-convergent evolution seen in nature (Stern 2013; Rosenblum et al. 2014). However, developmental constraints are intrinsic and imposed by the laws of biology of specific organisms, in this case the biology of Earth organisms. Unlike functional constraints, we cannot expect the same developmental constraints to apply to alien life forms.

Last, Stayton (2015) has argued that some convergent evolution hypothetically may be produced by chance alone and not by evolutionary limits. This analytic phenomenon can be produced in phenotypic data sets of low dimensionality and leads us to the next topic – can convergent evolutionary data be quantified and mathematically analyzed?

Quantifying Convergent Evolution

Any developing science usually passes through an initial, natural history phase that primarily describes a phenomenon and seeks causal explanations for the phenomenon, and then moves into a quantification phase with mathematical analyses and modeling of the phenomenon. The science of convergence is now in the process of moving into that quantification phase.

An excellent review of the current state of quantifying the phenomenon of convergent evolution has been given by Speed and Arbuckle (2017), and a practical guide to useful computer software and mathematical tools in the quantification of convergent evolution by Arbuckle and Speed (2016). Two aspects of convergence that Speed and Arbuckle (2017) are particularly interested in quantifying are the frequency of convergent evolution and the strength of convergent evolution.

Just how frequent is convergence in evolution; is it rare, is it ubiquitous, or is it somewhere in between? Can we actually measure, put a number on, the frequency of convergences in nature? Is evolution more frequently convergent in some phylogenetic lineages rather than others, in some environmental situations rather than others, in certain types of selection rather than others? How do we standardize convergence data to remove biases? For example, in clades with a large number of species, the frequency of convergences could be greater than in clades with a small number of species simply as a function of sample size, "so we would expect more instances of convergence in larger groups just by chance" (Speed and Arbuckle 2017, p. 817). Likewise, older clades with a longer duration in geologic time may have more instances of convergence than younger or shorter-duration clades simply as a function of sample size.

A quick, easily understandable method to detect and quantify the frequency of convergences in a given clade is to conduct both a phylogenetic analysis of the clade's species (producing a cladogram of sequenced synapomorphies) and a numerical taxonomic analysis of the clade's species (producing a phenogram of shared traits), and then to compare the cladogram results with the phenogram results. "If convergent evolution is common then the phenogram will tend to cluster species that are not grouped into clades in the phylogenetic tree," and the differences in the topologies of the two types of tree can be quantified (Speed and Arbuckle 2017, p. 819). In addition to this example, Speed and Arbuckle (2017) proceed to discuss numerous quantification metrics and indices that may be applied to measuring the differential frequency of convergences in nature.

What are the strengths of the observed cases of convergent evolution? As Speed and Arbuckle (2017) note, this question is at the heart of potential evolutionary

predictability – are some traits consistently, strongly convergent and hence predictable, whereas other traits are not? For example, traits that are "repeated across life in very many selective contexts (such as are likely present in different habitats) are almost certainly very highly constrained; traits that vary with local conditions are less so" (Speed and Arbuckle 2017, p. 817). Mahler et al. (2017) have pointed out specific biogeographic and ecological predictions that may be made, given the particular type of convergence mechanism in operation, from phylogenetic comparative studies of convergence. Last, Speed and Arbuckle (2017) also discussed the question of the level of convergence in life and its relationship to functional and developmental constraints (Stern 2013; Rosenblum et al. 2014), and the usage of spatial techniques in convergence quantification, which will be considered next.

Theoretical morphospace analyses allow spatial approaches to the quantification and analysis of convergent evolution, where theoretical morphospaces are *n*-dimensional geometric hyperspaces produced by systematically varying the parameter values of a geometric model of form (McGhee 2007). Different species with different phenotypes will plot in different regions of such a mathematical space, and their allo-convergent evolution will plot as different trajectories within that space to a common region in the space – the region of the convergent phenotypic form. Iso-convergent evolution will produce parallel trajectories within the space, and actual cases of both allo-convergent evolution and iso-convergent evolution have been analyzed by the usage of theoretical morphospaces (McGhee 2007).

Empirical morphospaces have also been used to quantify convergent evolution, where empirical morphospaces are multidimensional phenotypic spaces produced by the mathematical analysis of phenotypic measurement data using the techniques of principal components analysis, factor analysis, Fourier analysis, or other polynomial series approximations of natural morphology (McGhee 2007). Stayton (2015) pointed out that convergent evolution produces descendant species that are more similar to each other than they are to their own nonconvergent ancestors and has used both distance-based measures within empirical morphospaces to quantify the magnitude of convergent trajectories and degree of similarity of convergent species in such a space, and frequency-based measures to quantify how often certain regions of the space are occupied by convergently evolving species. Stayton (2015) also discussed and illustrated the quantification of convergent evolution to the next mathematical step – the computer simulation of convergence, where modeled evolutionary lineages are forced to converge on a target region in space. Last, Oke et al. (2017) have used both empirical morphospaces and phenotypic-trait spaces to quantitatively analyze their rhetorical question "how parallel is parallel evolution?" and discuss the usage of phenotypic-change vector analyses and phenotypic-trajectory vector analyses in the examination of actual iso-convergent evolution in fishes.

One final quantification of the phenomenon of convergent evolution is the active experimentation with the evolutionary process to determine how often convergence occurs, and how that convergence occurs. Such experiments must necessarily involve organisms with very short generation times if evolution is to be observed, organisms like the bacterium *Escherichia coli* (e.g., Tenaillon et al. 2012). In his review of

experimental-evolutionary and other studies of convergent genetic evolution, Stern (2013, p. 762) concludes that genetic convergences "provide evidence that genetic evolution is historically predictable."

Conclusion

Analyzing the phenomenon of convergence in evolution is now becoming as active a field of evolutionary research as the analysis of the phenomenon of divergence. The repeated evolution of the same or very similar forms in independent, disparate lineages of life has profound implications concerning the relative roles of adaptation via natural selection and developmental processes in constraining and directing the process of evolution.

Ongoing and future research into the phenomenon of convergence has the goal of producing precise quantification of the frequency and magnitude of convergence in the total suite of the phylogenetic lineages of life, and at each hierarchical level within those lineages – from the molecular (nucleotides, genes, genetic and epigenetic regulatory network structures, developmental processes) to the phenotypic (trait forms, form complexes, and functions) to populations and ecosystems (niche and community structures). The results of future convergence research should provide conclusive answers to current questions concerning the degree to which evolutionary processes are predictable or unpredictable, limited or unbounded, directed or directionless, and the degree to which those processes are extrinsically (selectively) limited, intrinsically (developmentally) limited, or unlimited (random).

Cross-References

- ▶ A Macroevolutionary Perspective on Developmental Constraints in Animals
- ▶ Canalization: A Central but Controversial Concept in Evo-Devo
- ▶ Developmental Homology
- ▶ Evo-Devo and Niche Construction
- ▶ Evo-Devo's Contributions to the Extended Evolutionary Synthesis
- ▶ Form and Function in Evo-Devo
- ▶ Inherency

References

Arbuckle K, Speed MP (2016) Analysing convergent evolution: a practical guide to methods. In: Pontarotti P (ed) Evolutionary biology: convergent evolution, evolution of complex traits, concepts and methods. Springer, Berlin, pp 23–36

Conway Morris S (2003) Life's solution: inevitable humans in a lonely universe. Cambridge University Press, Cambridge

Darwin C (1859) On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. John Murray, London

Hordijk W (2016) Evolution: limited and predictable or unbounded and lawless? Biol Theory 11(4):187–191. https://doi.org/10.1007/s13752-016-0251-5
Krassilov VA (1995) *Scytophyllum* and the origin of angiosperm leaf characters. Paleontol J 29(1A):63–73
Laland KN, Uller T, Feldman MW, Sterelny K, Müller GB, Moczek A, Jablonka E, Odling-Smee J (2015) The extended evolutionary synthesis: its structure, assumptions and predictions. Proc R Soc B 282:20151019. https://doi.org/10.1098/rspb.2015.1019
Lecointre G, Le Guyader H (2006) The tree of life: a phylogenetic classification. Belknap Press of Harvard University Press, Cambridge, MA
Losos JB (2011) Convergence, adaptation, and constraint. Evolution 65:1827–1840
Mahler DL, Weber MG, Wagner CE, Ingram T (2017) Pattern and process in the comparative study of convergent evolution. Am Nat 190(S1):S13–S28. https://doi.org/10.1086/692648
Martin A, Orgogozo V (2013) The loci of repeated evolution: a catalog of genetic hotspots of phenotypic variation. Evolution 67:1235–1250
McGhee GR (2007) The geometry of evolution: adaptive landscapes and theoretical morphospaces. Cambridge University Press, Cambridge
McGhee GR (2011) Convergent evolution: limited forms most beautiful. Massachusetts Institute of Technology Press, Cambridge, MA
McGhee GR (2015) Limits in the evolution of biological form: a theoretical morphologic perspective. Interface Focus 5:1–6; 20150034. https://doi.org/10.1098/rsfs.2015.0034
Oke KB, Rolshausen G, LeBlond C, Hendry AP (2017) How parallel is parallel evolution? Am Nat 190:1–16. https://doi.org/10.1086/691989
Pontarotti P, Hue I (2016) Road map to study convergent evolution: a proposition for evolutionary systems biology approaches. In: Pontarotti P (ed) Evolutionary biology: convergent evolution, evolution of complex traits, concepts and methods. Springer, Berlin, pp 3–21
Rosenblum EB, Parent CE, Brandt EE (2014) The molecular basis of phenotypic convergence. Annu Rev Ecol Evol Syst 45:203–226
Speed MP, Arbuckle K (2017) Quantification provides a conceptual basis for convergent evolution. Biol Rev 92:815–829
Stayton CT (2015) The definition, recognition, and interpretation of convergent evolution, and two new measures for quantifying and assessing the significance of convergence. Evolution 69:2140–2153
Stern DL (2013) The genetic causes of convergent evolution. Nat Rev Genet 14:751–764
Tenaillon O, Rodríguez-Verdugo A, Gaut RL, McDonald P, Bennett AF, Long AD, Gaut BS (2012) The molecular diversity of adaptive convergence. Science 355:457–461

Coevolution and Macroevolution

John N. Thompson, Kari A. Segraves, and David M. Althoff

Contents

Abstract

Coevolution is reciprocal evolution of interacting species driven by natural selection. Selection imposed by interactions between or among species can cause trait changes that alter ecological outcomes, patterns of local adaptation, and diversification of lineages. For example, selection can reduce the effect of the interaction when one species suffers a loss in fitness (antagonistic interactions) or increase the effect when species benefit from the association (mutualistic interactions). The selected traits may either change the cost of the interaction or the probability that the interaction occurs at all. These evolutionary changes can lead to local coadaptation as interacting species adapt and counteradapt to one another over time. In some cases, one or more of the locally coadapted species may

J. N. Thompson (✉)
Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, Santa Cruz, CA, USA
e-mail: jnthomp@ucsc.edu

K. A. Segraves · D. M. Althoff
Department of Biology, Syracuse University, Syracuse, NY, USA

Archbold Biological Station, Venus, FL, USA
e-mail: ksegrave@syr.edu; dmalthof@syr.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_125

become reproductively isolated from other populations as local coevolution decreases the chance of mating among populations. This cessation of gene flow, coupled with further evolutionary change, could lead to the formation of nascent species. There is, then, a direct potential connection between local coadaptation of populations, speciation, and macroevolutionary diversification. Some of the most challenging questions in coevolutionary biology center on understanding how coevolving traits change as they are expressed in a diversity of genetic and environmental backgrounds, how such traits can directly or indirectly lead to reproductive isolation, and whether these traits are likely to cause recurrent patterns of speciation that produce macroevolutionary patterns. This article considers what is currently known about the steps of this hierarchical process of evolutionary, and sometimes coevolutionary, diversification of interactions among species and how shifts in development may play an instrumental role in diversification.

Keywords

Coadaptation · Coevolution · Diversification · Geographic mosaic theory · Macroevolution · Evo-devo · Reciprocal selection · Speciation

Introduction

Throughout evolutionary history, organisms have evolved in response to their physical environment and to the other species with which they interact. Together, selection imposed by abiotic and biotic environments continually reshapes the phenotypes of organisms. Selection imposed by biotic environments (i.e., other species), though, can be especially strong in shaping trait evolution, because it can produce an ongoing feedback in the traits of interacting species. That is, changes in one species can precipitate changes in the second species, and this in turn can cause further change in the first species. The same process can occur within larger webs of interaction through a combination of direct and indirect feedbacks. This ongoing process of reciprocal evolutionary change among interacting species, termed coevolution, is an important mechanism that drives changes in organismal phenotypes.

From the beginning of life on Earth, coevolution was likely an instrumental force that shaped not only the communities of single-celled microbes, but also perhaps gave rise to multicellular organisms and the evolution of eukaryotes (Margulis and Fester 1991). Coevolution of proto-mitochondria, proto-chloroplasts, and heterotrophic single-celled organisms spurred the integration of these separate organisms into a single functioning unit that eventually led to the diversity of life forms we see today. Given the fact that every organism interacts with a multitude of other species during its lifetime and the large potential for coevolution to shape these species interactions, there is no question that coevolution is one of the processes molding the diversity of life. Indeed, coevolutionary interactions are diverse and have been found among predators and prey, parasites and hosts, competing species, and mutualistic

species (Thompson 1994). Hence, coevolutionary selection will produce traits that are advantageous for interacting with different species. Many times, the differences we observe among closely related species are a direct result of their adaptations for interacting with different suites of species.

Changes in development are crucial for these adaptations and can be responsible for fine-tuning traits or initiating larger phenotypic changes. Even so, there has been surprisingly little integration of coevolutionary theory into developmental biology and vice versa. The goal of this chapter is to explore how coevolution and development could be integral in driving adaptations that eventually lead to speciation and large-scale macroevolutionary diversification. We start by first describing the coevolutionary process within and among populations and then discuss how this process could drive speciation and fuel macroevolutionary diversification. Along the way, we highlight how development has been critical for promoting coevolution and creating traits that may spur speciation. We end by outlining several major unanswered questions about the linkage among coevolution, development, and macroevolution.

Adaptive Coevolutionary Divergence and Geographic Mosaics

Populations, rather than species, are the unit of evolutionary and coevolutionary change. The evolution of a species is the composite of all its populations that have adapted to varying degrees to their local physical environments and to their interactions with other species. The exchange of migrants among these populations is the connection that maintains the cohesiveness of a species across its geographic range. During the process of local adaptation and coadaptation with other species, some populations diverge strongly from one another in ecologically and evolutionarily important traits. Such strong divergence occasionally provides the basis for ecological speciation by reducing gene flow among populations, and even more rarely, leading to adaptive radiations of new lineages. The microevolutionary processes at the level of populations can scale up to drive macroevolutionary patterns. During this process, minor or major changes in the developmental program are sometimes necessary to modify or produce new traits that are important for interactions. If changes to the developmental program are large enough, there may be shifts in life histories or correlated changes in traits important for reproduction that begin the process of reproductive isolation among populations that are diverging.

For interactions among species, local coadaptation may result in a geographic mosaic of coevolution. The geographic mosaic theory of coevolution stipulates that the coevolutionary process is fueled by three sources of variation in interactions among species (Thompson 1994, 2013). Selection mosaics arise across ecosystems as natural selection favors different traits or trait combinations in different environments. Coevolutionary hotspots and coldspots arise as natural selection favors reciprocal evolutionary change among interacting species in some environments but not in others. Trait remixing occurs as gene flow, genomic processes, and metapopulational processes continue to reshuffle the combinations of genes that

are locally available for selection to act upon. Hence, the structure of selection, the strength of reciprocal selection, and the traits available for selection all vary among environments.

The formal population genetic way of envisioning these sources of coevolutionary variation is as a genotype by genotype by environment interaction (GxGxE). If a genotype of one species interacts with a genotype of a second species in two different environments, the effect of the interaction on the two species would often differ between the two environments. An example would be two identical twins living in different places. Both are infected with the same virus clone, but the virus is expressed in a more virulent way in one environment than in the other environment. If now, instead of one genotype, a population of individuals with many different genotypes interacts with another genetically variable population, the distribution of outcomes from their interactions is almost bound to vary among environments. Genotype by genotype by environment variation in outcome is the basis for coevolutionary divergence. The distribution of outcomes can change not just through selection on mutations with new structural properties but also through the developmental timing of expression of genes important in species interactions.

The combination of selection mosaics, coevolutionary hotspots and coldspots, and trait remixing therefore produces the first steps in macroevolutionary divergence driven by coevolution. It does so, though, not just by acting differently on the same genes or their expression in different environments, but also by acting on different genes in different environments. Selection may act mostly on morphological traits in some environments, but on physiological or behavioral traits in other environments. An interaction between the same two or more species may even evolve to be antagonistic in some environments, commensalistic (one species benefits with no effect on the other) in other environments, and mutualistic in yet other environments. This variation in interaction outcomes and traits provides the raw material for differences to evolve among populations and, eventually, species.

Examples of geographic mosaics of coevolution are now known for many kinds of interactions. Plants and fungal pathogens show geographic differences in defense and counterdefense genes (Laine et al. 2014). Coevolving *Taricha* newts and garter snakes differ geographically in the levels of tetrodotoxin in the skin of the newts and the ability of garter snakes to resist high levels of the toxin after ingesting newts (Hague et al. 2016). Similarly, wild parsnip plants differ among populations in the combination of defensive furanocoumarins they employ against specialist parsnip webworms, and the webworms match those differences with detoxifying P450 enzymes in their gut (Li et al. 2014). These geographic mosaics can also include differences in the ecological outcomes of interactions. The outcome of interactions between woodland star (*Lithophragma*) plants and *Greya* moths range from antagonistic to mutualistic among ecosystems (Thompson and Fernandez 2006). As interactions change in structure, strength, and outcome across environments, evolutionary changes in developmental pathways, either early on or late in developmental cascades, can help provide trait variation to fuel adaptation.

The genotype by genotype by environment variation that forms the basis of the geographic mosaic of coevolution can therefore be driven by geographic differences

in the developmental expression of traits or by differences in the effectiveness of an expressed trait in different environments. Thus, developmental plasticity may be a major component of trait evolution. Natural selection can even restrict the expression of coevolved traits to some environments but not others. For example, some species of *Daphnia* water fleas plastically develop hardened "helmets" only in environments in which they detect the chemical signatures of potential predators in the surrounding water (Petrusek et al. 2009). Escape from predation has also been suggested as the basis of developmental differences between wet season and dry season in the wing colors and eyespots on *Bicyclus* butterflies in Africa (Brakefield 2010).

From Coevolutionary Geographic Mosaics to Ecological Speciation

For the geographic mosaic to shape speciation, gene flow among diverging populations must weaken relative to the strength of divergent selection. Ecological speciation, in which strong divergent selection directly promotes reproductive isolation among populations, can be a key component of this process. For example, populations of threespine sticklebacks have diverged repeatedly into locally adapted populations during and following the Pleistocene as marine populations became trapped in coastal freshwater lakes and ponds during rising and falling sea levels (Schluter 2016). Those freshwater populations then diverged repeatedly into benthic forms (bottom dwelling) and limnetic (open water) forms, driven in part by competitive interactions among individuals and strong selection for specialization in prey use that favored different traits (e.g., body armor and shape) in different aquatic microhabitats. These forms also differ in their propensity to mate with one another based on body size differences that exist between the benthic and limnetic forms (Conte and Schluter 2013).

Similarly, species of *Anolis* lizards have diverged in repeatable ways among Caribbean islands, depending on which *Anolis* species are present on an island (Losos 2009). Through competition, lizards have radiated into different morphological forms that exploit different microhabitats (e.g., tree trunks vs. small limbs), and each island has predictable combinations of *Anolis* species with specific body types or ecomorphs. These morphological forms differ in a number of traits that correlate with shifts in body size and shape as well as the substrates on which they forage (Harmon et al. 2003). Importantly, the ecomorphs do not have a single origin that then dispersed to different islands. Instead, the same forms have evolved repeatedly in predictable ways as lizard populations have colonized different islands (Mahler et al. 2013). These morphological changes have occurred through differences in the early development of the lizards (Sanger et al. 2012). The adaptive radiation of *Anolis* species has therefore been driven at least to some extent by ongoing coevolution of competing species.

Coevolutionary divergence can also be driven by interactions between predators and prey. Red crossbill birds in North America have diverged into at least nine ecotypes that specialize on feeding on the seeds of different conifer species. These

ecotypes use different calls, vary in the extent to which they are nomadic or sedentary, and show some genomic divergence from one another (Parchman et al. 2016). More extreme divergence, however, is found in one ecotype called the South Hills crossbill that specializes on lodgepole pines and shows substantial divergence from other crossbills at a small number of genetic loci. This ecotype has bills that are adapted to extracting seeds from closed cones of lodgepole pines, and the pines have adaptations that make it difficult for the birds to extract the seeds. Both the birds and the pines differ from other populations in ways that suggest local coadaptation (Benkman et al. 2009). The traits involved in this divergence involve allometric changes in size and shape that could readily evolve through small developmental changes. Unlike many other crossbill populations, these local birds have become sedentary rather than nomadic and, in the process, have become increasingly reproductively isolated from other crossbill populations over the past 6000 years.

The most famous example of ecological speciation that has led to an adaptive radiation is Darwin's finches (Grant and Grant 2014). Within the Galapagos Island chain are approximately 14 species of *Geospiza* finches that differ primarily in body size and bill morphology. These species evolved from a common finch ancestor into specialist and generalist species that differ in the type (seeds vs. insects) and size of food they consume. The changes in bill morphology necessary to specialize on different food types are driven by changes in allometry as well as developmental changes in individual traits (Foster et al. 2008). In this case, the extent to which coevolution has directly fueled diversification of the birds is not known, but competitive interactions among these species combined with specialization in food types suggest that interspecific interactions rather than adaptations to different physical environments have been the major driver of this adaptive radiation (Grant and Grant 2014). The radiation of Darwin finches highlights the difficulty in disentangling the relative role of coevolution versus other factors in creating and maintaining divergence among species.

Determining the Role of Coevolution in Patterns of Macroevolutionary Divergence

Coevolution of two or more lineages has the potential to generate a wide range of macroevolutionary patterns because the relationships between adaptive divergence among populations, phylogeographic divergence, and speciation are probably rarely the same across interacting lineages. Moreover, adaptive divergence of populations may lead to speciation in both (or all) partners; however, it is also possible that speciation only occurs on one side of an interaction even if populations on both sides are coevolving. Despite the lack of general expectations for macroevolutionary patterns associated with coevolution, a common starting point is to examine phylogenetic patterns of speciation between interacting lineages because this may give an indication when coevolution may have had a strong role in speciation. One caveat of this approach, however, is that because there are many patterns of speciation that can be generated between coevolving lineages, from strong congruence of speciation

events to more disconnected ones, macroevolutionary patterns cannot be attributed to the coevolutionary process without additional forms of evidence (Althoff et al. 2014).

One of the most common popular expectations of coevolutionary divergence is to observe parallel speciation in two coevolving lineages, but that is probably a very rare outcome. Part of that expectation arose from a deep misinterpretation of Paul Ehrlich and Peter Raven's 1964 paper on macroevolutionary patterns, which is commonly cited as predicting parallel cladogenesis. The process they describe, however, cannot, by definition, result in parallel speciation. Ehrlich and Raven (1964) suggested that, during a coevolving interaction between two lineages, a mutation in a species in a host lineage would free it from interactions with a parasite lineage. That would allow the mutant host lineage to undergo speciation and potentially adaptively radiate in the absence of interactions with the parasites. Eventually, a mutant in the parasite lineage would be able to colonize the mutant host lineage and, in turn, radiate across the host lineage. This process does not require the mutant parasite lineage to colonize the mutant host lineage in ancestral-descendent order. As a result, any parallelism in speciation in the two coevolving lineages would occur only at higher taxonomic levels, and not at the species level. Instead, we would predict to observe starbursts of speciation followed by lag phases that alternate in timing between the interacting lineages.

The predictions of "escape-and-radiate" coevolution, coupled with what is now known about the geographic mosaic of coevolution, suggest that parallel speciation of coevolving species is highly unlikely except under certain circumstances. These include interactions between hosts and vertically transmitted parasites and interactions between hosts and species that control the movement of gametes among host individuals. The best examples of the latter situation are interactions between plants and pollinating floral parasites, such as yuccas and yucca moths, woodland stars and *Greya* moths, leafflower plants and leafflower moths, and figs and fig wasps. These interactions involve insect pollinators that eat the developing seeds in the same flowers that they pollinate.

Because diversification fueled by coevolution produces no single pattern, inferring the role of coevolution requires a multifaceted approach that combines (1) phylogenetic and, when possible, phylogeographic investigation of species relationships, (2) analysis of how natural selection shapes trait evolution among the interacting species, and (3) analysis of how selection on traits involved in interactions may directly or indirectly lead to reproductive isolation. The overall evaluation therefore requires analyses at both microevolutionary and macroevolutionary levels. Microevolutionary and macroevolutionary analyses of extant species allow analyses of selection on potentially coevolving traits and the causes of geographic and interspecific differences in current maintenance of those traits and ecological outcomes. The fossil record can greatly deepen this understanding by providing important information on the potential origin of coevolving traits, their diversification over longer periods of time, and the potential limits on what appears to be directional selection on traits in short-term studies of extant species. For this reason, there are still few clear examples in which coevolution has been shown to

lead directly to large-scale patterns of speciation within interacting lineages (Althoff et al. 2014; Hembry et al. 2014).

We also do not understand whether antagonistic or mutualistic interactions are more likely to influence speciation. And, at a deeper level, we do not know if different forms of antagonism (e.g., competition, predation) or mutualism (e.g., mutualisms between symbionts and hosts, mutualisms between free-living species) differ in how they shape speciation and macroevolutionary change. For example, natural selection often acts on competing species to mitigate or dissolve the interaction, whereas in interactions between parasites and hosts, selection acts on the parasite to increase the interaction and on the host to decrease the interaction or its negative effects. Whether any form of antagonism or mutualism is more likely to lead to trait divergence that could drive reproductive isolation is currently unclear. That said, parasites or mutualists that control host reproduction seem to be the clearest candidates for a major role of coevolution in shaping diversification.

Interactions between plants and pollinating floral parasites provide some of the best examples of how control of host reproduction by symbionts may lead to an extraordinarily high diversification of species. Hundreds of fig species worldwide are each pollinated by their own one or two fig wasps (Cruaud et al. 2012), and potentially hundreds of leafflower plants (*Glochidion*) throughout the tropical Pacific region are similarly each pollinated by their own one or a few *Epicephala* moths (Kawakita 2010). Diversification has also occurred in other insect lineages that act as pollinating floral parasites and in the plants they pollinate, but the resulting diversification occurs sometimes not only at the species level but also at higher taxonomic levels through host shifts. For example, prodoxid moths have colonized a diverse array of plant families. Many of the interactions between these moths and the host plants are antagonistic, with moth larvae feeding on seeds, floral, scape, or other plant tissues. But one lineage of the moths, called yucca moths, has become the sole pollinators of a lineage of monocots, and another lineage of the moths, called *Greya* moths, has become the major pollinators of a group of eudicots. The yucca moths actively pollinate yuccas as they oviposit into the flowers, whereas the *Greya* moths passively pollinate their host flowers during oviposition or nectaring using different mechanisms (Pellmyr and Leebens-Mack 1999, Thompson et al. 2013).

Developmental shifts have been crucial in shaping the traits important to these interactions. For example, changes in development played a central role in the evolution of the specialized tentacular mouthparts used by yucca moths to actively collect and deposit pollen on yucca flowers (Fig. 1). Similarly, changes in the relative and absolute lengths of the abdominal segments of *Greya* moths among species and populations have affected how they pollinate different host species during oviposition with pollen adhering to the abdomen (Thompson et al. 2013). The tentacles of yucca moths are particularly impressive, because they evolved de novo and are not present in any other insect group. Morphological work suggests that the movement of the tentacles is linked to the proboscis and likely stem from the same developmental template as the proboscis (Pellmyr and Krenn 2002). The evolution of the tentacles occurred once at the base of the radiation of yucca moths and was a key trait for increasing the pollination efficiency of the moths (Pellmyr and Leebens-Mack

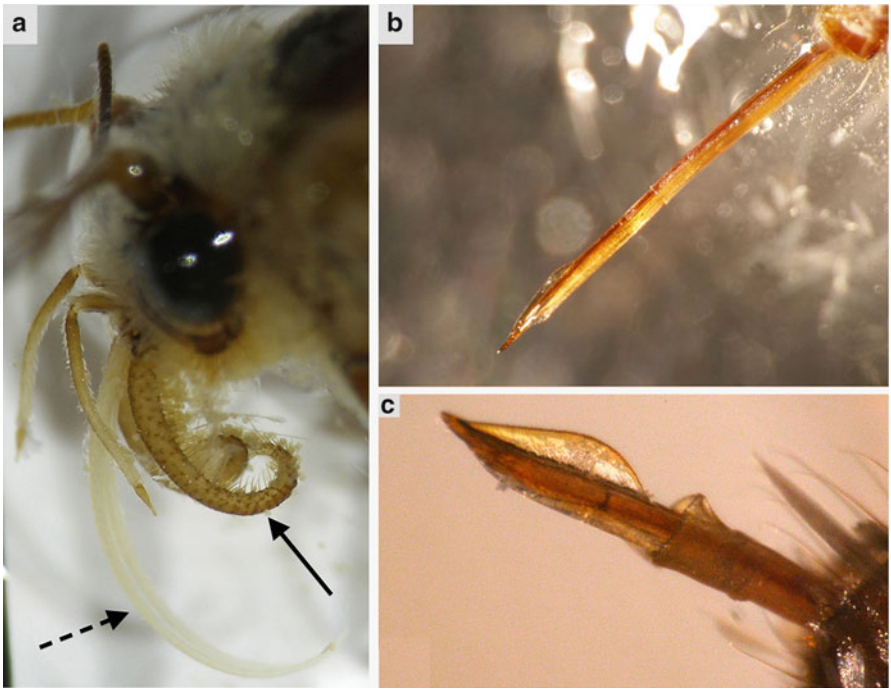


Fig. 1 Mutualistic and antagonistic traits in yucca moths (*Tegeticula* spp.; Prodoxidae). (**a**) Close-up of female *T. altiplanella* mouthparts showing "tentacles" used for pollination (*solid arrow*) and proboscis (*dotted arrow*). (**b**) Thin, needle-like ovipositor of female *T. yuccasella* that deposits eggs deep within yucca pistil. (**c**) Short, stout ovipositor of *T. cassandra* female that deposits eggs just below pistil surface

1999). The tentacles are truly a mutualistic trait, because cheater moth species that evolved from pollinator species deposit eggs into yucca fruits rather than flowers and lost functional tentacles (Pellmyr 1999).

There is no question that the evolution of the specialized mouthparts was instrumental in the relationship among yuccas and yucca moths. What is less clear, however, is whether this key trait is directly responsible for the burst of speciation in the pollinator moths and yuccas or if it sets the stage for other aspects of the interaction to drive speciation. Among pollinator species, there are two radiations of moths that differ in the how they place their eggs in yucca flowers. The shift in egg placement from deep within the flower next to ovules to just on the pistil surface corresponded with changes in the morphology of the egg-laying structure of the moths (Pellmyr 1999). Deep ovipositing species have a long, thin, needle-like ovipositor that damages plant ovules in contrast to shallow ovipositing species that have short, stout ovipositors (Fig. 1). This shift in oviposition strategy has resulted in correlated evolution of the male intromittent organ as well, suggesting a means of mechanical reproductive isolation among moth species that differ in oviposition strategy (Althoff 2014). The current results from yucca moths suggest that the

antagonism might be more directly responsible for the diversification of moths rather than the mutualism (Althoff 2016). Even so, it is clear that development has played a major role in creating key traits and modifying mutualistic and antagonistic traits important in the overall relationship between plant and moth lineages.

Unresolved Questions on the Evolutionary Developmental Biology of Coevolution

As species continually interact and coevolve, the traits important in interactions will be under constant selective pressure either to diverge or remain fixed at optimal trait values. Development influences every aspect of an organism's biology, and small changes in developmental pathways can produce extraordinary changes in the phenotypic expression of traits. In many ways, coevolution has the potential to act strongly on development to help shape the traits of interacting species. Because coevolutionary biology has only begun to grapple with how shifts in development can affect coevolution (and vice versa) among species and shape the diversification of interacting lineages, there are major research questions that are ripe for exploration including these:

1. *How does coevolution shape interactions throughout development?* Individuals at different ages and sizes are susceptible to attack by different parasites and predators, and they may depend on different mutualists. For example, some mutualistic interactions between plants and mycorrhizal fungi may be very important during plant seedling establishment, but have little or no impact on adult performance. Many fish species need to avoid gape-limited predators as juveniles but then escape predation once they reach a size threshold. There is, therefore, strong potential for developmental changes to fundamentally affect how individuals within a population interact with other species and how those interactions affect fitness. In particular, species with morphologically and physiologically distinct developmental stages, such as holometabolous insects, amphibians, and marine species with pelagic larvae have vastly different interactions between development stages. We know relatively little about how coevolution with species at one stage of life may affect interactions with other species at later stages of life.
2. *How does the interplay between coevolution and development coordinate coevolved defenses and counterdefenses across developmental stages?* Some interactions span several life history stages and may require different responses at different stages. For example, parasitoid wasps and flies use other insects as hosts for development. Adults must locate hosts, overcome host defenses, and deposit eggs in or on host insects. In turn, the parasitoid larvae have to overcome the host's defenses, especially internal physiological/immunological defenses. Selection at different parts of the life stages will favor different traits and those traits must be coordinated to be expressed at the right time during the development of the parasitoid. Similarly, the host insects must be able to respond to all the different stages of attack by the parasitoid. If we add in the other interactions that

both parasitoids and host insects have with other species, we can begin to see the complexity of traits that need to appear during the right stages of development. We have little understanding of how the genes responsible for the development of different traits important for different interactions may act synergistically or as constraints on evolution.

3. *How does developmental plasticity contribute to coevolution and, ultimately, speciation into new adaptive zones?* Development has a dual role in the production of trait variation. For many traits, environmental inputs over the course of development can have large impacts on phenotypic expression. This plasticity along with underlying genetic variation provides the wealth of phenotypic variation that is available for natural selection. Development, however, also constrains the range of trait variation within certain values of trait space. For example, within *Bicyclus* butterfly species, the number and size of eyespots vary between the wet and dry seasons. Across species, eyespots vary more, but this variation is constrained to a subset of possible eyespot positions, colors, and sizes (Brakefield 2010), and eyespot shape is due to major genes and hormonal gradients that control eyespot development (Oostra et al. 2014). It is also unclear how often major or minor shifts in development can open up a new adaptive zone that fuels speciation in interacting lineages. For example, changes in development can produce de novo phenotypes in mutualistic interactions, such as in the evolution of yucca moth mouthparts (Pellmyr and Krenn 2002), or in antagonistic interactions, such as the allometric changes in body size in *Anolis* lizards that have led to repeated speciation among competing species (Losos 2009). Each of these changes has been instrumental in opening up new avenues of speciation through coevolution with other species.

Conclusions

We are only just beginning to understand how developmental mechanisms shape the ecological diversification of coevolving species and the macroevolutionary patterns that result. Changes in development can provide the phenotypic variation required for natural selection, potentially creating major changes in phenotype from simple changes in developmental pathways. These changes could cause direct modification of traits important for mating or produce a cascade of changes via correlated evolution with other traits that secondarily influence reproductive isolation. The synergy between development and continued coevolution of interacting species has the potential to move species to new adaptive zones, increase reproductive isolation, and spur diversification.

Cross-References

► Convergence
► Developmental Plasticity and Evolution

- ▶ Eco-Evo-Devo
- ▶ Macroevolution
- ▶ Morphological Disparity

References

Althoff DM (2014) Shift in egg-laying strategy to avoid plant defense leads to reproductive isolation in mutualistic and cheating yucca moths. Evolution 68:301–307

Althoff DM (2016) Specialization in the yucca-yucca moth obligate pollination mutualism: a role for antagonism? Am J Bot 103:1803–1809.

Althoff DM, Segraves KA, Johnson MTJ (2014) Testing for coevolutionary diversification: linking pattern with process. Trends Ecol Evol 29:82–89

Benkman CW, Smith JW, Keenan PC, Parchman TL, Santisteban L (2009) A new species of the red crossbill (Fringillidae: *Loxia*) from Idaho. Condor 111:169–176

Brakefield PM (2010) Radiations of mycalesine butterflies and opening up their exploration of morphospace. Am Nat 176(Suppl 1):S77–S87

Conte GL, Schluter D (2013) Experimental confirmation that body size determines date preference via phenotype patching in a stickleback species pair. Evolution 67:1477–1484

Cruaud A, Ronsted N, Chantarasuwan B, Chou LS, Clement WL, Couloux A, Cousins B, Genson G, Harrison RD, Hanson PE, Hossaert-Mckey M, Jabbour-Zahab R, Jousselin E, Kerdelhue C, Kjellberg F, Lopez-Vaamonde C, Peebles J, Peng YQ, Pereira RAS, Schramm T, Ubaidillah R, van Noort S, Weiblen GD, Yang DR, Yodpinyanee A, Libeskind-Hadas R, Cook JM, Rasplus JY, Savolainen V (2012) An extreme case of plant-insect codiversification: figs and fig-pollinating wasps. Syst Biol 61:1029–1047

Ehrlich P, Raven P (1964) Butterflies and plants: a study in coevolution. Evolution:586–608

Foster DJ, Podos J, Hendry AP (2008) A geometric morphometric appraisal of beak shape in Darwin's finches. J Evol Biol 21(1):263–275

Grant PR, Grant R (2014) Forty years of evolution. Princeton University Press Princeton, Princeton

Hague MT, Avila JLA, Hanifin CT, Snedden WA, Stokes AN, Brodie ED (2016) Toxicity and population structure of the rough-skinned newt (*Taricha granulosa*) outside the range of an arms race with resistant predators. Ecol Evol 6:2714–2724

Harmon LJ, Schulte JA II, Larson A, Losos JB (2003) Tempo and mode of evolutionary radiation in iguanian lizards. Science 301:961–964

Hembry DH, Yoder JB, Goodman KR (2014) Coevolution and the diversification of life. Am Nat 184:425–438

Kawakita A (2010) Evolution of obligate pollination mutualism in the tribe Phyllantheae (*Phyllanthaceae*). Plant Species Biol 25:3–19

Laine AL, Burdon JJ, Nemri A, Thrall PH (2014) Host ecotype generates evolutionary and epidemiological divergence across a pathogen metapopulation. Proc R Soc B Biol Sci 281:20140522

Li W, Zangerl AR, Schuler MA, Berenbaum MR (2014) Characterization and evolution of furanocoumarin-inducible cytochrome P450s in the parsnip webworm, *Depressaria pastinacella*. Insect Mol Biol 13:603–613

Losos JB (2009) Lizards in an evolutionary tree. University of California Press, Berkeley

Mahler DL, Ingram T, Revell LJ, Losos JB (2013) Exceptional convergence on the macroevolutionary landscape in island lizard radiations. Science 341:292–295

Margulis L, Fester R (1991) Symbiosis as a source of evolutionary innovation. MIT Press, Cambridge, MA

Oostra VA, Mateus RA, van der Burg KRL, Piessens T, van Eijk M, Brakefield PM, Beldade P, Zwaan BJ (2014) Ecdysteroid hormones link the juvenile environment to alternative adult life histories in a seasonal insect. Am Nat 184:E79–E92

Parchman TL, Buerkle CA, Soria-Corrasco V, Benkman CW (2016) Genomic divergence and diversification within a geographic mosaic of coevolution. Mol Ecol 22:5705–5718

Pellmyr O (1999) Systematic revision of the yucca moths in the *Tegeticula yuccasella* complex (Lepidoptera: Prodoxidae) north of Mexico. Syst Entomol 24:243–271

Pellmyr O, Krenn HW (2002) Origin of a complex key innovation in an obligate insect-plant mutualism. Proc Natl Acad Sci U S A 99:5498–5502

Pellmyr O, Leebens-Mack J (1999) Forty million years of mutualism: evidence for eocene origin of the yucca-yucca moth association. Proc Natl Acad Sci U S A 96:9178–9183

Petrusek A, Tollrian R, Schwenk K, Haas A, Laforsch C (2009) A "crown of thorns" is an inducible defense that protects *Daphnia* against an ancient predator. Proc Natl Acad Sci U S A 106:2248–2252

Sanger TJ, Revell LJ, Gibson-Brown JJ, Losos JB (2012) Repeated modification of early limb morphogenesis programmes underlies the convergence of relative limb length in *Anolis* lizards. Proc R Soc Lond Ser B Biol Sci 279:739–748

Schluter D (2016) Speciation, ecological opportunity and latitude. Am Nat 187:1–18

Thompson JN (1994) The coevolutionary process. University of Chicago Press, Chicago

Thompson JN (2013) Relentless evolution. University of Chicago Press, Chicago

Thompson JN, Fernandez CC (2006) Temporal dynamics of antagonism and mutualism in a geographically variable plant-insect interaction. Ecology 87:103–112

Thompson JN, Schwind C, Guimarães PR Jr, Friberg M (2013) Divergence through multitrait evolution in coevolving interactions. Proc Natl Acad Sci U S A 110:11487–11492

Part III

History of Evo-Devo

Theories of Inheritance: The Evolution and Development of Form

David Haig

Contents

Abstract

Questions of how form is inherited have long been entangled with questions of how form develops. In the late nineteenth century, cytologists converged on an understanding in which the hereditary substance resided in the nuclei of cells and did not itself undergo processes of development in its transmission from parents to offspring. The modern understanding of nuclear DNA as the hereditary material is a direct descendant of these ideas.

Keywords

Lamarck · Weismann · Germ cells · Adaptation · Epigenetics

D. Haig (✉)
Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, USA
e-mail: dhaig@oeb.harvard.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_62

Introduction

From ancient times, it has been observed that like begets like and that adults develop from embryos in which adult parts are not readily distinguishable. For many investigators, the question of how form develops was perceived as related to, perhaps the same as, the question of how form is inherited. The nineteenth century saw the overthrow of ideas of fixity of type, in which variation within species was tightly constrained, and the triumph of evolutionary theories in which changes of form could accumulate indefinitely. The nineteenth century also saw the replacement of vague notions of the direct inheritance of form (what one might call morphogenetic memory) with models in which form was indirectly inherited via the transmission of material determinants of form. In the latter models, processes of development evolved via changes to the determinants of form rather than via changes of form that were directly inherited. Such models conceptually distinguished mechanisms of transmission from mechanisms of development.

Descent with Modification in Lamarck and Darwin

Jean-Baptiste Lamarck (1809/1984) proposed that life was subject to an essentially linear drive toward increasing complexity. Although simple living forms were continuously transforming into more complex forms, simple forms were unceasingly replenished by spontaneous generation from inanimate matter. The motive force for morphological change was the molding of substance by fluid movements. The motions that generated simple animals from nonliving gelatinous substances, and simple plants from nonliving mucilaginous substances, were external, but the movements of fluids that shaped the organs and tissues of more complex forms were internal. Such internal flows became progressively accelerated as organismal complexity increased. As a subsidiary cause, the use and disuse of organs in response to changes in the environment resulted in adaptation of organisms to their position in life. Such responses to the environment, arising from immediate organismal needs, obscured the overall upward trend by causing forms to branch from the main path of increasing complexity. Lamarck believed that changes of form, whether sculpted by flow of internal fluids or by use and disuse of parts, were inherited by offspring, but he offered no mechanistic account of inheritance.

Charles Darwin (1859), like Lamarck, accepted that the effects of use and disuse could be inherited and could thereby contribute to evolutionary change but emphasized the importance of spontaneous undirected variation. Slight deviations that by chance increased the fit of an organism to its environment were retained by natural selection, whereas those that decreased adaptation were discarded by natural rejection. He later published his "provisional hypothesis of pangenesis" to account for hereditary phenomena, including the inheritance of the effects of use and disuse. In this hypothesis, all parts of an organism continually released minute gemmules that congregated in the reproductive organs from where the gemmules were inherited by offspring (Darwin 1868).

For Lamarck, adaptation to the environment caused forms to deviate from the main path of progress, but, for Darwin, there was no main path: adaptation created diverging branches pruned by extinction. Lamarck's and Darwin's respective visions can be exemplified in two extended quotations:

> By these wise precautions, everything is thus preserved in the established order; the continual changes and renewals which are observed in that order are kept within limits that they cannot pass; all the races of living bodies continue to exist in spite of their variations; none of the progress made towards perfection of organisation is lost; what appears to be disorder, confusion, anomaly, incessantly passes again into the general order, and even contributes to it; everywhere and always the will of the Sublime Author of nature and of everything that exists is invariably carried out. (Lamarck 1809/1984, p. 55)

> Thus, from the war of nature, from famine and death, the most exalted object which we are capable of conceiving, namely, the production of the higher animals, directly follows. There is grandeur in this view of life, with its several powers, having been originally breathed into a few forms or into one; and that, whilst this planet has gone circling on according to the fixed law of gravity, from so simple a beginning endless forms most beautiful and most wonderful have been, and are being evolved. (Darwin 1859, p. 489)

Nuclear Inheritance and Continuity of the Germ Plasm

In the years 1883–1885, multiple German cytologists proposed theories in which a hereditary substance of complex structure was located in cell nuclei and exhibited continuity between generations. These theories involved a conceptual separation of mechanisms of transmission from mechanisms of development (Churchill 1987). August Weismann's (1834–1914) advocacy of continuity of the germ plasm is best known today.

In Weismann's theory, germ plasm (*Keimplasma*) resided in the *nuclei* of a germ track (*Keimbahn*) that was the cellular path by which germ plasm was passed unchanged from parents to offspring (Weismann 1892/1902; Haig 2016). The germ plasm did not undergo cyclical processes of development and had a hierarchical structure of biophors (at the lowest level) organized into determinants, determinants organized into ids, and ids organized into idants (chromosomes). Each id contained a complete set of all determinants necessary to produce an organism. In Weismann's model of development, the nuclei of the germ track contained multiple ids, but subsets of determinants were parceled out to daughter nuclei as they diverged from the germ track. "Id" survives today hidden in the words *haploid* (simple id) and *diploid* (double id).

Weismann categorically rejected the inheritance of acquired characters because somatic changes (in the body or cytoplasm) were of a different nature from the change in the nuclear germ plasm that would be necessary for a somatic change to be inherited by offspring (Weismann 1892/1902; Haig 2007). In his theory, the germ track could include somatic cells if these retained intact germ plasm. Therefore, his rejection of "Lamarckian" inheritance was *not* based on early segregation of germ cells from somatic cells but on something much closer to the modern distinction

between genotype and phenotype. Weismann also believed that changes in the germ plasm had physical causes that could include environmental causes. Such changes were the necessary source of variation on which natural selection could act. However, this heritable variation originated in direct changes of the germ plasm itself, not changes in soma or cytoplasm that were then communicated to the germ plasm.

Although Weismann believed that many organisms derived their germ cells from somatic cells, "Weismannism" is commonly presented as the theory that germ cells are set aside early in development and do not contribute to the somatic body (Griesemer and Wimsatt 1989). The latter theory was proposed by Nussbaum (1880, p. 112) among others. One source of confusion has been that "germ plasm" was used by different researchers to refer to different substances. For Weismann, germ plasm was a *nuclear* substance residing in chromatin that contained a complete set of "determinants" for all the cells of the body. For others, "germ plasm" referred to *cytoplasmic* materials present in the egg that were inherited by germ cells, but not somatic cells, and were considered the "determinants" of the germ line.

Mendelian Genetics and Its Critics

Gregor Mendel (1822–1884)'s experiments with peas revealed that some characters that differed between parents segregated in simple ratios in the progeny of their progeny and that the character of one parent could be dominated by the character of the other parent in their joint progeny but could reappear in subsequent generations (Mendel 1865). These discoveries attracted little attention until their "rediscovery" in 1900 by de Vries and Correns who presented Mendel's results as prefiguring their own work on heritable factors (Brannigan 1979).

The key methodological innovation of the new Mendelian genetics was counting the number of occurrences of discrete, alternative character states among progeny of controlled crosses. A key conceptual innovation was the establishment of a clear distinction between character states (*phenotype*) and the heritable factors responsible for character states (*genotype*). Some early Mendelians resisted proposals that the heritable factors (*genes*) were material entities located on chromosomes, but the chromosomal theory soon prevailed (Carlson 1966). The idea that the bearers of heredity were located on chromosomes was not new, but "gene mapping" added the understanding that genes were linearly arranged on chromosomes, with fixed locations, and that different chromosomes possessed different sets of genes. Mendelian genetics also enabled the detection of changes to genes (*mutations*), and methods were developed for inducing such changes (Muller 1927). Genes were thus identified as chemical structures on which experiments could be performed.

Critics of the new genetics believed that its emphasis on transmission neglected questions of development. For some, genetics explained relatively superficial differences among organisms but did not explain major features of the development of organisms considered as integrated and purposeful wholes (Russell 1930). For others, a role for genes in all aspects in development was conceded, but genes were recognized as only one factor in complex processes of embryonic development

in which the cytoplasm and environment also had roles. Conrad Hal Waddington (1905–1975) ceded that which was *preformed* in the fertilized egg to genetics but coined the term *epigenetics* for the study of the causal mechanisms of development (Waddington 1942; see Haig 2004).

Material Basis of Heredity

Experiments in bacterial transformation strongly indicated that the hereditary substance was a nucleic acid of the desoxyribose type (Avery et al. 1944). The subsequent elucidation of the structure of DNA as a double helix of two complementary antiparallel strands immediately suggested how the specificities of the hereditary material could be faithfully copied (Watson and Crick 1953). Biologists, for the first time, gained a clear insight into how the trick of inheritance could be achieved. The question of how hereditary information determined cellular functions remained.

Early molecular biologists believed that information contained in the sequence of nucleic acid bases in some manner coded for the sequence of amino acids in proteins and it was the latter that performed most of the work within cells. Crick (1958) articulated the emerging consensus in his "central dogma of molecular biology": information about sequence could be transferred from nucleic acid to nucleic acid or from nucleic acid to protein but not from protein to protein or protein to nucleic acid. The discovery of messenger RNA and the decipherment of the genetic code by which triplets of nucleic acid bases specified the sequential addition of amino acids to growing polypeptides were achievements of the 1960s (Judson 1979). A major development of subsequent years has been the growing appreciation of the role of various noncoding RNAs (rRNAs, tRNAs, snRNAs, snoRNAs, miRNAs, lncRNAs, etc.) in cellular functions and of forms of "epigenetic" inheritance that do not involve changes to DNA sequence.

Epigenetics and Epigenetic Inheritance

Cells of the same genotype manifest distinct phenotypes, and the alternative phenotypes sometimes persist through multiple cell divisions. Nanney (1958) proposed that systems of *epigenetic* control determined which genetic specificities were expressed in particular cells and that these specificities could be stably maintained through cell division in the absence of inducing stimuli. In his view, mechanisms of epigenetic inheritance must exist at the cellular level in addition to mechanisms of genetic inheritance. Nanney's concept of epigenetic inheritance has morphed into a label for heritable modifications to chromatin, including to DNA or histone proteins, that do not involve changes to the sequence of DNA bases (Probst et al. 2009).

Epigenetic "memories" can sometimes persist through meiotic divisions and be transmitted to offspring (Jablonka and Raz 2009). It has been repeatedly suggested that transgenerational epigenetic inheritance makes possible a form of Lamarckian inheritance of acquired characteristics and that this will require a radical change in

our understanding of the evolutionary process (Jablonka and Lamb 1995). A couple of general observations can be made about the evolutionary and physiological implications of epigenetic inheritance. First, heritable genetic and epigenetic variation are both subject to natural selection. In this respect, a stable epimutation is no different from a stable genetic mutation. If there are indeed epimutations that, once having occurred, are impervious to subsequent epigenetic change on a timescale similar to the rate of change in genetic sequences, then such epimutations are subject to the same Darwinian processes as genetic mutations and are, from an adaptationist perspective, no different from genetic mutations (Haig 2007).

Second, much interest in epigenetic inheritance has been generated by the possibility of metastable epialleles that switch back and forth among a limited set of states, with changes of state cued to environmental inputs. The paradigmatic example is provided by imprinted genes that switch their expression depending on the sex of the body (somatic environment) from which they were inherited (Reik and Walter 2001). If the epigenetic state of a typical gene in this generation does not reflect its epigenetic state several generations in the past nor predict its epigenetic state several generations into the future, then whether a gene exists in one epigenetic state or the other does not contribute to long-term evolution. Instead, what is subject to natural selection is alternative switching behaviors of genetic sequences. Genetically determined switching behaviors that enhance fitness are retained by differential survival and reproduction, and those that impair fitness are eliminated (Haig 2007).

Consider skin color of Australians of different descent. People of predominantly Aboriginal ancestry are dark-skinned, whereas people of predominantly British ancestry are fair-skinned. The darker skins of the former are an *evolutionary adaptation* to high levels of solar radiation in Australia, whereas the lighter skins of the latter are an evolutionary adaptation to lesser sun exposure in northern Europe. The maladaptiveness of fair skin in the Australian environment is manifest in a high rate of melanoma in light-skinned Australians. Most Australians also exhibit some degree of *developmental adaptation* to sun exposure, expressed as a variable genetic propensity to change the skin color of exposed body surfaces – darker in summer, fairer in winter. This process of physiological adaptation is itself a genetically determined evolutionary adaptation (Haig 2007).

The significance of epigenetic inheritance depends on the timescale of epigenetic changes of state and how this relates to the timescale of a particular research question. If one's focus is understanding the reasons why offspring inherit a disease present in a parent or why offspring develop as the same morphotype as a parent, then epigenetic inheritance of unstable states may be of key importance. However, if one's focus is long-term evolutionary adaptation, then repertoires of epigenetic states, and their correlations with variable environments, may be characters under genetic selection.

Evolution and Development

Evolution and development can both be characterized as processes of morphological trans*form*ation, and the two processes have been historically entangled. Indeed, for the first two-thirds of the nineteenth century, "evolution" was commonly used as a

synonym for ontogenetic development (Bowler 1975). This entanglement can be seen in the use of the same words to describe the two processes. Thus "adaptation" is used to refer to the products of evolution by natural selection and phenotypic changes during an individual life in response to environmental variables. "Epigenetics" has been used to refer both to processes of development in an individual life and to transgenerational inheritance of information. Relations between evolutionary biologists and developmental biologists have not always been harmonious with each group believing the other is ignoring their insights. For more than a century, evolutionary theory has been characterized by a tension between investigators who have found conceptual clarity in a separation of evolutionary from developmental questions and critics of this separation who emphasize "reciprocal causation" between evolutionary and developmental processes (Laland et al. 2015).

Cross-References

- ▶ Conrad Hal Waddington (1905–1975)
- ▶ Evo-Devo's Contributions to the Extended Evolutionary Synthesis

References

Avery OT, MacLeod CM, McCarty M (1944) Studies on the chemical nature of the substance inducing transformation of pneumococcal types. J Exp Med 79:137–158

Bowler PJ (1975) The changing meaning of "evolution". J Hist Ideas 36:95–114

Brannigan A (1979) The reification of Mendel. Soc Stud Sci 9:423–454

Carlson EA (1966) The gene: a critical history. Saunders, Philadelphia

Churchill FB (1987) From heredity theory to Vererbung. The transmission problem, 1850–1915. Isis 78:337–364

Crick FHC (1958) On protein synthesis. Symp Soc Exp Biol 12:138–163

Darwin C (1859) On the origin of species. John Murray, London

Darwin C (1868) The variation of animals and plants under domestication. John Murray, London

Haig D (2004) The (dual) origin of epigenetics. Cold Spring Harb Symp Quant Biol 69:67–70

Haig D (2007) Weismann rules! OK? Epigenetics and the Lamarckian temptation. Biol Phil 22:415–428

Haig D (2016) Transposable elements: self-seekers of the germline, team players of the soma. BioEssays 38:1158–1166

Griesemer JR, Wimsatt WC (1989) Picturing Weismannism: a case study of conceptual evolution. In: Ruse M (ed) What the philosophy of biology is. Kluwer, Dordrecht, pp 75–137

Jablonka E, Lamb MJ (1995) Epigenetic inheritance and evolution. The Lamarckian dimension. Oxford University Press, Oxford

Jablonka E, Raz G (2009) Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. Q Rev Biol 84:131–176

Judson HF (1979) The eighth day of creation. Simon and Schuster, New York

Laland K, Uller T, Feldman M, Sterelny K, Müller GB, Moczek A, Jablonka E, Odling-Smee J (2015) The extended evolutionary synthesis: its structure, assumptions and predictions. Proc R Soc B 282:20151019

Lamarck JB (1809/1984) Zoological philosophy. University of Chicago, Chicago

Mendel G (1865) Experiments in plant hybridisation. (trans: Bateson W (1909) Mendel's principles of heredity. Cambridge University, Cambridge)

Muller HJ (1927) Artificial transmutation of the gene. Science 66:84–87
Nanney DL (1958) Epigenetic control systems. Proc Natl Acad Sci U S A 44:712–717
Nussbaum M (1880) Zur Differenzirung des Geschlechts im Thierreich. Archiv für mikroskopische Anatomie 18:1–121
Reik W, Walter J (2001) Genomic imprinting: parental influence on the genome. Nature Rev Genet 2:21–32
Russell ES (1930) The interpretation of development and heredity. Oxford University Press, Oxford
Probst AV, Dunleavy E, Almouzni G (2009) Epigenetic inheritance during the cell cycle. Nature Rev Mo Cell Biol 10:192–206
Waddington CH (1942) The epigenotype. Endeavour 1:18–20
Watson JD, Crick FHC (1953) A structure for deoxyribose nucleic acid. Nature 171:737–738
Weismann A (1892/1902) The germ-plasm. Scribner, New York

Alexander Onufrievich Kowalevsky (1840–1901)

Alexander V. Ereskovsky

Contents

Abstract

Alexander O. Kowalevsky (1840–1901), a founder of cellular and comparative evolutionary embryology, was one of the most prominent biologists of the nineteenth century. He worked at the intersection of zoology, embryology, and evolution. His studies on the lancelet, tunicates, insects, and germ layer homologies pioneered comparative embryology and confirmed the evolutionary continuity between invertebrates and vertebrates. In this chapter I present a short description of the life of A.O. Kowalevsky and his achievements and, with their help, illustrate the development of comparative evolutionary embryology in the last third of the nineteenth century. The chapter also presents the full bibliography of Kowalevsky.

A. V. Ereskovsky (✉)
Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), Aix Marseille University, CNRS, IRD, Avignon University, Station Marine d'Endoume, Marseille, France

Department Embryology, Faculty of Biology, Saint-Petersburg State University, Saint-Petersburg, Russia

Koltzov Institute of Developmental Biology of Russian Academy of Sciences, Moscow, Russia
e-mail: alexander.ereskovsky@imbe.fr

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_9

Keywords

Alexander Kowalevsky · Comparative evolutionary embryology · Evo-devo

Life

Alexander O. Kowalevsky was born on November 7, 1840, in the village of Vorkovo (Russia). His father, Onufriy I. Kowalevsky, owned a rural estate in Polessye (the Vitebsk Province, now Republic of Byelorussia). Another son, Vladimir (1842–1883), who was born two years later, would become a renowned evolutionary palaeontologist. Both brothers spent their childhood in the estate and were tutored at home until the age of 16. Their parents envisaged a practical career for the children, and their education was organized correspondingly (Dogiel 1945; Pilipchuk 2003; Fokin 2012).

In 1855 Alexander Kowalevsky entered the third year of the School of Railway Engineers in St. Petersburg. In 1858 he left it for the Department of Natural Sciences of the Physical-Mathematical Faculty of St. Petersburg University. A year later (1859) Kowalevsky left the university to continue his studies at Heidelberg University (Germany). Kowalevsky spent about two years in Heidelberg. At first he studied chemistry with Robert Wilhelm Bunsen (1811–1899) and Georg Ludwig Carius (1829–1875) and then anatomy, histology, and zoology under the supervision of Heinrich Georg Bronn (1800–1862) and Heinrich Alexander Pagenstecher (1825–1889). It was in Heidelberg that Kowalevsky began to work on the lancelet (*Branchiostoma lanceolatum*), which was to become his favorite research subject. It should be noted, however, that formally Kowalevsky was not a student at the university (Fokin 2012).

At the end of 1861 Kowalevsky moved to Tübingen University. His teachers were well-known scientists: Hubert von Luschka (1820–1875; anatomy), Friedrich Eduard Reusch (1812–1891; general physics), Hugo von Mohl (1805–1872; botany), Friedrich Quisted (1809–1889; geology), and Franz von Leydig (1821–1908; zoology and histology). In Tübingen Kowalevsky also improved his skills in microscopy (Fokin 2012).

In 1861 Kowalevsky briefly returned to St. Petersburg, where he became a Candidate of Natural Sciences for his research on the anatomy of the crustacean *Idothea entomon*. At the end of 1863 Kowalevsky returned to Tübingen and continued zoological studies under the supervision of F. von Leydig for several months. Then he moved to Naples and started his own scientific research. At that time Kowalevsky was already under a strong influence of Charles Darwin's (1809–1882) *The Origin of Species*, which he read in the early 1860s in the German translation (Dogiel 1945; Pilipchuk 2003).

Kowalevsky spent two years in Naples, completing his famous research, which became the basis for his Master dissertation "The Developmental History of the *Amphioxus lanceolatus* or *Branchiostoma lunbricum*" defended in St. Petersburg University in 1865 (Kowalevsky 1865). The main conclusions of the dissertation

were as follows: (1) the lancelet, previously considered a fish-like vertebrate, actually belongs to cephalochordates, and (2) its embryo and embryonic development resembles a vertebrate. Many of the conclusions still appear to be true (see for instance, Arthur 2002; Holland 2010). Karl Ernst von Baer (1792–1876), a famous embryologist who was 73 at that time, referred to the dissertation of Kowalevsky as a "first-class study."

Between 1866 and 1867 Kowalevsky worked as a conservator of the Zoological Cabinet at St. Petersburg University. He took a special interest in the development of the bilateral, worm-like animals, which we now call phoronids. The results of these investigations provided the basis for his doctoral thesis presented publicly in 1867 under the title *Anatomy and Developmental History of Phoronis* (Kowalevsky 1867c). At the same time Kowalevsky published numerous articles on the embryology of worms, bryozoans, sea cucumbers, and ascidians. In the same year, Kowalevsky was awarded, together with Ilya Ilyich Mechnikov (1845–1916), the first Baer Prize from the Russian Imperial Academy of Sciences.

In 1868 Kowalevsky was elected professor at the Imperial Kazan University. At the end of 1869 he transferred to St. Vladimir Imperial Kiev University and was awarded the Baer Prize for the second time. He spent the money on two trips, first to Italy and then to the Red Sea, where he discovered the famous crawling ctenophoran *Coeloplana metschnikowii*. In 1871 Kowalevsky participated in the foundation of the Sevastopol Biological Station in the Crimea.

From 1873 to 1890 Kowalevsky worked as a full professor in Novorossiysk University (now Odessa, Ukraine) (Fig. 1). For him it was a relatively stable period

Fig. 1 Alexander Onufrievitch Kowalevsky. St. Petersburg, 1885. (From archives of the Department of Embryology of St. Petersburg State University)

of intense pedagogical and scientific work and established family life. However, he spent most of the summers in France, Switzerland, and Italy doing scientific research. Almost everywhere, Kowalevsky studied the embryology of various marine invertebrates. In the early 1880s he also participated in the research on the *Phylloxera*, a harmful vine pest that had appeared in southern Russian at that time.

In 1883, Kowalevsky was elected corresponding member of the Russian Imperial Academy of Sciences, and in 1890 he became a full academician. His interests were shifting toward physiology, in particular to excretion in different invertebrates. Kowalevsky made an outstanding achievement in the field of comparative physiology. Having modified and improved the technique of intravital injections, used to differentiate physiologically different parts of the excretory apparatus, Kowalevsky studied the effect of numerous substances on the excretory system of many invertebrates. As a result of the use of this new technique he managed to reveal the existence of different and diverse excretory apparatuses, many of which were previously unknown. The use of different substances also allowed him to identify the physiological significance of the discovered organs.

In 1890 Kowalevsky moved with his family to St. Petersburg. From 1890 until 1894 he was a professor at St. Petersburg University, where he was the head of the Anatomical-Histological Cabinet. In 1894 he founded a Special Zoological Laboratory in the Academy of Sciences, the first Russian center of experimental zoology. He worked there with his students, among them the embryologist Constantin Davydoff (1878–1960), the immunologist Sergei Metal'nikoff (1870–1946), the protistologist Vladimir Schewiakoff (1859–1930), and others (see Fokin 2000). He also supervised the construction of a special building for the Sevastopol Biological Station (1894–1897), the center for faunistic and morphological studies of the animals of the Black Sea and the Mediterranean basin (now Kowalevsky Institute of Marine Biological Research).

During this period, Kowalevsky became a recognized authority in evolutionary embryology and zoology. He was a member of the Society of Naturalists of Modena and the Cambridge Philosophical Society, a foreign member of the Royal Society, and a corresponding member of the Academies of Sciences of Brussels and Turin.

Kowalevsky died of cerebral hemorrhage on November 9, 1901, at the age of 61. He was buried at the Novo-Devichie Orthodox cemetery in St. Petersburg.

Kowalevsky was a tireless traveler. In total, he spent 12 years in expeditions and scientific missions. He undertook numerous expeditions and trips aimed at the exploration of marine animals at the Adriatic Sea (Trieste), the Mediterranean Sea (Naples, Messina, Villafranca, Marseille, Alger), the Caspian Sea, the Red Sea, in the English Channel (Roscoff), and other marine regions.

Work

Evolutionary developmental biology (evo-devo) compares developmental processes of different organisms to infer their ancestral relationships and the evolution of developmental processes. In its present form, evo-devo emerged in the 1970s.

However, its origins date back to the evolutionary embryology of the second half of the nineteenth century, and Kowalevsky was one of its pioneers. He used new histological techniques to determine homologies that were no longer visible in the adult organism (Mikhailov and Gilbert 2002). His studies using cell lineage to show the homologies of the notochord in tunicates, the lancelet, and vertebrates became a major argument in support of the theory of evolution and contributed to the transformation of descriptive embryology into evolutionary embryology, one of the bases of modern evo-devo.

The State of Comparative Animal Embryology Before Alexander Kowalevsky

Between 1828 and 1837 von Baer suggested the doctrine of the two main embryonic layers: the upper layer and the lower layer (renamed ectoderm and endoderm, respectively, by George James Allman (1812–1898) in 1853), and Robert Remak (1815–1865) (in 1855) added a third, middle, germinal layer – the modern mesoderm (Hall 1998).

Although von Baer himself was only involved with the embryology of various classes of vertebrates, he tried to build the entire system of the animal kingdom based on not only comparative anatomy but the development of different animal groups. He recognized four types of development: radial (in coelenterates), spiral (in mollusks), symmetrical (in articulates), and bisymmetrical (in vertebrates). These four types approximately coincide with the four types of anatomical structure of animals according to Georges Cuvier (1769–1832), and von Baer, similarly to Cuvier, considered the types of development as independent and unrelated.

In the first edition of *The Origin of Species* Darwin emphasized the importance of embryology as one of the "three pillars" of evolutionary theory (morphology, embryology, and palaeontology). However, he lacked the substantial evidence confirming such a statement. It was based only on Fritz Müller's (1821–1897) research on the history of the development of various crustacean groups and on his own studies of development in barnacles. It is Alexander Kowalevsky who should be considered as one of the first scientists who empirically proved the importance of embryology as a pillar of evolutionary theory.

By the time when Kowalevsky began his research on marine zoological material, Darwin's general principles of the theory of evolution were widely confirmed by evidence from comparative anatomy in the practice of breeding domestic animals and cultivated plants. Phylogenetic relationships within such well-defined groups as vertebrates or arthropods were not in doubt. One of the major aims of the evolutionists of the second half of the nineteenth century was to detect the relationship between vertebrates and invertebrates and then to establish a taxonomic position, and thus a phylogenetic relationship, of certain artificial groups such as worms and other questionable groups such as acrania, tunicates, bryozoans, brachiopods, and chaetognaths with the rest of the animal world. These forms later attracted Kowalevsky's attention.

When Kowalevsky and his friend I. Mechnikov (who was to become a Nobel Prize winner and the founder of immunology) began their embryological research, evolutionary embryology as a distinct field of science did not seem to exist. They decided to assess relationships across the animal kingdom on the basis of the identification of developmental similarities between the embryos of different phyla. Under a strong influence of *The Origin of Species*, Kowalevsky and Metchnikoff made comparative embryological studies on a huge number of invertebrate and vertebrate species, pioneering evolutionary embryology (see Mikhailov 2012; Mikhailov and Gilbert 2002; Levit 2007).

The most frustrating aspect of marine invertebrate research at that time was its episodic character. Field studies could only be pursued during inter-semester breaks. This was exacerbated by the problem that the animals, once collected, had to be kept alive and taken to laboratories for developmental studies and histological analysis, often over long distances. However, the scientific activity of Kowalevsky coincided with a surge of organization of marine biological stations throughout Europe. A permanent experimental marine station with an aquarium, preferably near invertebrate-rich waters, made it possible for zoologists not only to study the life history of various marine animals in detail but also to work with model organisms. This switch to the study of model organisms was an extremely important factor in the development of a new research direction, experimental embryology, which later became a major component of evo-devo.

The Works of Alexander Kowalevsky and the Foundation of Evolutionary Comparative Embryology

The fact that Kowalevsky turned to the study of the *Amphioxus* does not mean that he just came across an interesting object among the rich Mediterranean fauna. He consciously sought to investigate it, setting it as his task even before his arrival in Naples. At that time, *Amphioxus* was attributed to vertebrates, and Kowalevsky himself called it “a wonderful fish.” He hoped to find in its embryology common developmental features of invertebrates and vertebrates, a foresight was brilliantly justified (Dogiel 1945).

Kowalevsky used *Amphioxus* as a model system in his studies in Naples in 1866 (Fig. 2). He identified in its embryos the principal features common to all chordates such as the notochord, the dorsal nerve cord, and metameric muscles. He also showed that embryonic development in *Amphioxus* was that of a typical vertebrate, with a notochord, multiple gill slits, and medullary folds fusing to form a neural canal (Fig. 2c, d) (1867b). A remarkable discovery made by Kowalevsky in his work on *Amphioxus* development was the description of a two-layered embryo arising through typical invagination type gastrulation, which later turned out to be common for many invertebrates. Kowalevsky established the presence of the two main germ layers, the upper and the lower one, in *Amphioxus* and later in tunicates and ctenophores. Until that time the presence of the two germ layers was considered an exclusive feature of vertebrates (1867b).

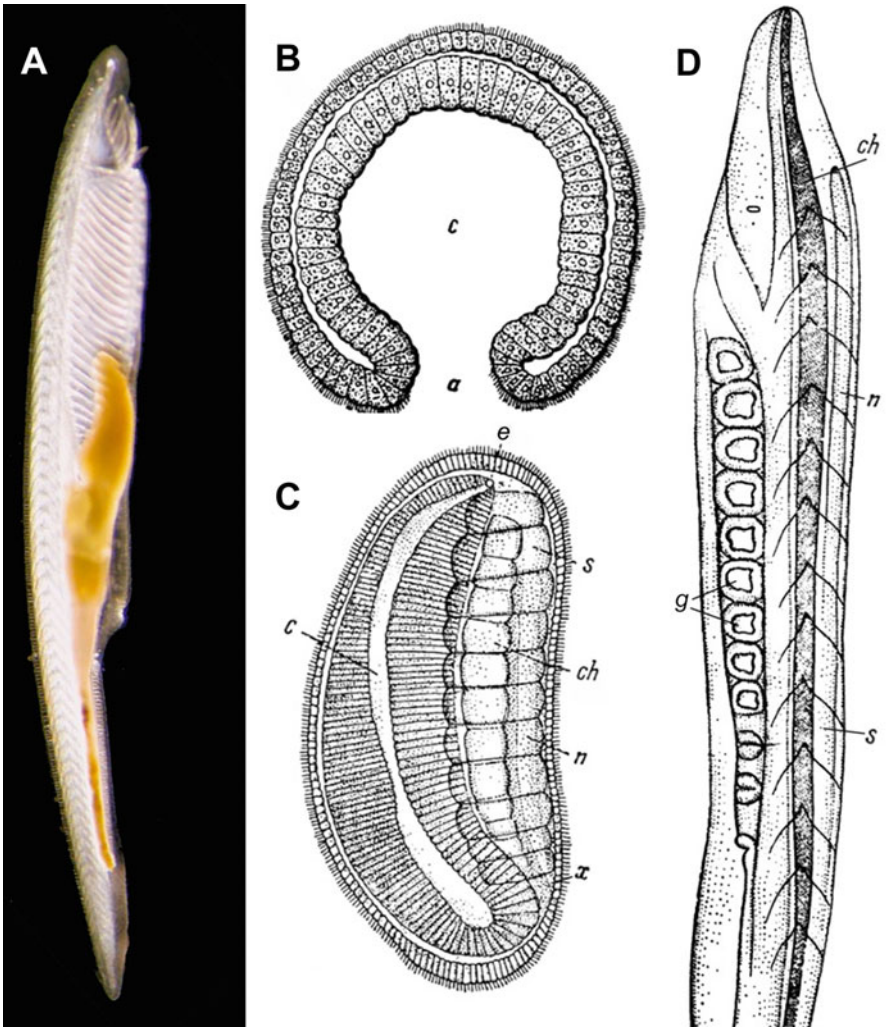


Fig. 2 (**a**) Adult lancelet *Branchiostoma lanceolatum.* B-D. Kowalevsky's drawing showing early stages of lancelet development (adapted from Kowalevsky 1865). (**b**) Mid-to-late gastrula; (**c**) newly hatched embryo with 11 somite pairs; (**d**) free larva with 11 gill slits. a – blastopore; c – archenteron; ch – notochord; e – ciliated ectoderm; g – gill slits; n – nerve cord; s – somites; x – neuropor

Concurrently with the study of *Amphioxus*, Kowalevsky studied the development of tunicates, ctenophores, and holothurians. Tunicates attracted him by the uncertainty of their taxonomic position. They are now recognized as the most basal group of chordates, but a century and a half ago, they were classified as mollusks related to shipworms, a group of wood-boring clams (Fig. 3a). The result of Kowalevsky's research was unexpected even for its author: according to the type of cleavage,

gastrulation, and other features of embryonic development, the tunicates turned out to be closely related to *Amphioxus* (Fig. 3b–g). He discovered an affinity between ascidian tadpoles and vertebrates and suggested that ascidians might have been vertebrate ancestors (Kowalevsky 1866d, 1868b, 1871b).

The tunicate larva, whose formation Kowalevsky traced in remarkable detail, resembles a vertebrate embryo. Its nervous system looks like a tube arising from the longitudinal depression of the upper germ layer, the chord passes along the tail of the larva, and there are gill slits in the anterior part of the alimentary canal (Fig. 3g). The discoveries of Kowalevsky concerning the development of *Amphioxus* and especially tunicates were so striking that at first they were met with suspicion. Our present understanding that vertebrates develop from a two-layered gastrula can be traced to this fundamental work, which revolutionized embryology and zoology (Hall 1998). Kowalevsky suggested that vertebrates, including the cephalochordate *Amphioxus*, might have evolved from tunicate-like ancestors. This idea was endorsed by Darwin (1874) and Ernst Haeckel (1834–1919) (1874), who considered tunicates as "connecting intermediate forms between the lower worms on the one hand and vertebrates on the other" (Holland and Gibson-Brown 2003).

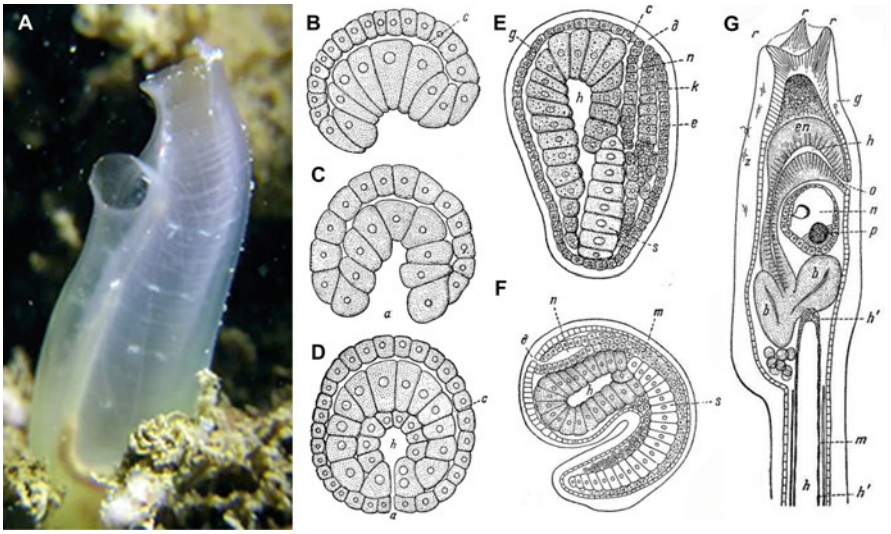


Fig. 3 (**a**) Adult ascidia *Ciona intestinalis*. (**b–g**) Kowalevsky's drawing showing embryonic development of ascidia *Ciona intestinalis.* (Aadapted from Kowalevsky 1866d). (**b**) Beginning of gastrulation by invagination; (**c**) advanced stage of invagination; (**d**) developing embryo at the stage of neurulation and notochord formation; (**e**, **f**) lateral view of later embryo, showing first appearance of neural tube and arrangement of notochord cells into a single column, and with short tail (**f**). a – blastopore; c – cleavage cavity; d – neuropor; e, g – ectoderm; h – archenteron; m – presumptive muscle cells; n – neural canal; s – notochord cells originated from the "lower" germ layer. (**g**) Anterior part of larva (tadpole). g – mantle; b – intestine; o – oral aperture; en – endostyle; n – cerebral vesicle; p – ocellus; h – notochord; m – muscle band; r – adhesive papillae

Kowalevsky's findings and conclusions were quickly accepted by other prominent evolutionists such as Karl Gegenbaur (1826–1903). The origin of vertebrates from an ascidian tadpole-like ancestor became a favored theory. In 1894, Arthur Willey (1867–1942) reviewed the information on the embryology of amphioxus and ascidians in an influential book "*Amphioxus* and the Ancestry of the Vertebrates" (Hall 1999).

The famous English zoologist Sir Edwin Ray Lankester (1847–1929) emphasized that although Albert Kölliker (1817–1905) and Remak had studied the development of some tissues from embryonic cells earlier, it was Kowalevsky who "in small transparent embryos (such as those of Ascidia, *Amphioxus*, *Sagitta* and *Argiope*) traced the history of adult organs cell by cell to the original egg-cell" (Lankester 1902).

Darwin delighted in the discovery that the tunicate was actually a chordate. Kowalevsky's research suggested to Darwin that the argument from embryonic recapitulation could be carried back beyond the vertebrates to non-vertebrates. The ascidians might then be considered in their earliest form the ancestor of the vertebrates (including man), while their present form showed a regression to a non-vertebrate character. Darwin believed that, should Kowalevsky's research be confirmed by other scientists, "the whole will form a discovery of the very greatest value" (Darwin 1874. Cited in Bljacher 1959).

Another important work of Kowalevsky in the 1860s was his research on the embryology of ctenophores (1866c, 1873). Kowalevsky described very accurately the peculiar cleavage of their eggs and for the first time discovered a cross-shaped cell anlage between ecto- and endoderm, giving rise to tentacle muscles. In other words, he found the mesoderm absent in the rest of the coelenterates, to which Ctenophora and Cnidaria then belonged.

Kowalevsky made the first embryological studies on several other invertebrate groups such as coral polyps, echiurids, phoronids, camptozoas, and acrania. In case of some other groups, his work was preceded only by superficial and incomplete studies (comb jelly, amphineura, scaphopods, chaetognaths, brachiopods, echinoderms, and tunicates). In the study of the development of annelids, insects, and arachnids, Kowalevsky had several predecessors, but his research provided so much fundamentally new data that Kowalevsky's works continue to be cited in modern works devoted to these subjects.

The technique of making histological sections used by Kowalevsky (1871a, 1886b, c, d, 1887) started a new era in insect embryology (Fig. 4). These studies were also of great significance for general embryology since they formed the basis of the classical germline theory. Kowalevsky (1885, 1887) made the first strictly scientific descriptions of internal processes during insect metamorphosis and organogenesis. These works were made under the influence of phagocytic theory, founded shortly before by a friend of Kowalevsky, I. Metchnikoff. Henri Viallanes (1856–1893) and August Weismann (1834–1914) noticed before Kowalevsky that the organogenesis of definitive organs during the metamorphosis of flies was accompanied by the destruction of most larval organs (Bljacher 1959) but did not describe this process in detail. Professor Mitrophan Ganin (1839–1894) in Warsaw

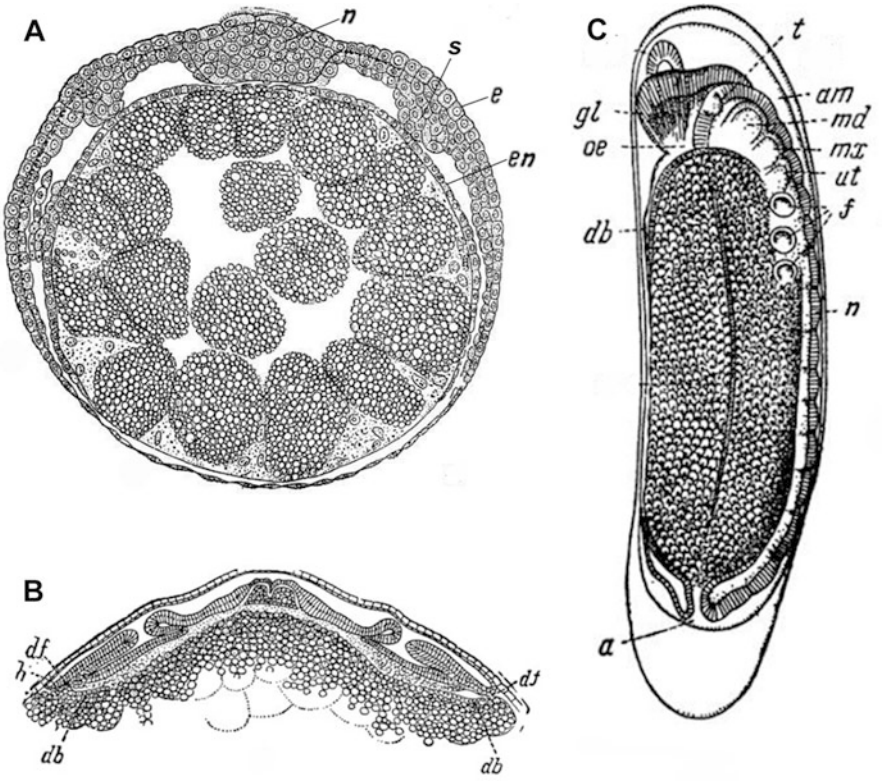


Fig. 4 Kowalevsky's drawing showing embryonic development of annelids and insects (adapted from Kowalevsky 1871a). (**a**) Development of *Euaxes* sp. (Annelida, Clitellata). Cross section of the anterior end of the embryo at the germ band stage. (**b**) The water beetle *Hydrophilus piceus* (Insecta, Coleoptera): Cross section of the ventral side of the embryo at the stage when germ band covers the entire ventral side. db – visceral mesoderm; df – somatic mesoderm. (**c**) Honey bee *Apis mellifica* (Insecta, Hymenoptera): longitudinal section of the embryo at the stage of dorsal closure of germ layers. a – anus; e – ectoderm, en – endoderm; f – anlage of legs; gl – brain lobe; md – mandibles; mx – maxilla; n – anlage of ventral nerve cord, t – antenna; s – anlage of somites

established the significance of the so-called germinal discs, from which the organs of an adult fly developed, but the process of destruction of the larval organs still remained obscure (Bljacher 1959). Kowalevsky was the one to solve the riddle by discovering the phenomenon of histolysis in the metamorphosis of flies, that is, the digestion of decaying tissues by phagocytes.

At the same time (in the late 1860s) Kowalevsky was much attracted by vertebrate development. He studied embryonic development of sharks and teleosts, amphibians, turtles, birds, and mammals (1869, 1870f, i). He identified the basic principles of fish development, in this way introducing "fish to the general theory of the development of vertebrates" (Poljanskij 1955, p. 40).

Homology of the Germ Layers

Homology of the germ layers as a universal principle that applies beyond separate individual "phyla" of animals was formulated by Kowalevsky in 1871. It was part of the empirical basis for the Darwinian monophyletic view of evolution. In the introduction and in the concluding remarks to his most extensive work "Embryological studies of worms and arthropods" (1871a), Kowalevsky states with utmost clarity the principles of the theory of germ layers as an anlage, the homology of which should be limited only to representatives within a phylum but also when comparing animals from different phyla. According to Kowalevsky, germ layer homology "speaks of the relationship of types, for which we find evidence in invertebrates at every step" (Kowalevsky 1871a, p. 19). Haeckel appreciated Kowalevsky's work, writing in his *Anthropogenie*: "The most significant embryo histories in the recent time were those of Kowalevsky" (Haeckel 1874, p. XX).

Kowalevsky did not doubt the true relationship between different animal phyla, based on the similarity of the early stages of their embryonic development. In this regard, he homologized the developmental stages, germ layers, and various organs. However, it is not quite clear what the homologization criteria were for Kowalevsky. Apparently, he relied on the similarity of the developmental processes and their corresponding stages, the topography of the germ layers and their further fate, that is, their derivatives, those parts of the body and organs that originate from different layers.

Legacy

In the literature devoted to the scientific legacy of Kowalevsky, it has often been discussed why there were no definitive phylogenetic conclusions and broad morphological generalizations in his works. The authors of the necrologies and memoirs, who knew Kowalevsky closely, answered this question in more or less the same manner, writing about Kowalevsky's adherence to well-established facts, his dislike for speculations, and his exceptional scientific sensitivity and caution (see Dogiel 1945; Pilipchuk 2003). His aim was not schematization but a detailed study of specific ontogenetic phenomena that undergo change in the course of the historical development of the animal world. From the beginning to the end of his scientific activities, Kowalevsky found explanations of these changes in Darwin's theory.

The largest contribution to science made by Alexander Kowalevsky was the proof of the unity of the laws governing the development of the entire animal world. In his famous "History of the development of the *Amphioxus*" (1865, 1867b, 1870h, 1876) and in a series of subsequent works on the development of ascidians, coelenterates, echinoderms, polychaetes and arthropods, Kowalevsky proved the universal prevalence of the same early larval stages (blastula, gastrula, and some others) in different animal phyla. He proved the homology of the germ layers throughout the animal world and the correspondence of the main types of cavities (gastral, primary and secondary) in different animals.

Fig. 5 The Alexander Kowalevsky bronze medal award of the Saint-Petersburg Society of Naturalists for extraordinary achievements in evolutionary developmental biology and comparative zoology. (**a**) – front face; (**b**) – reverse side

Many researchers wrote about accounting of how Kowalevsky fits into a history of evo-devo (see Mikhailov and Gilbert 2002; Raff and Love 2004; Mikhailov 2012). The main conclusion is that the widespread focus on the molecular biological techniques that have allowed the discovery of homologous regulatory genes, homologous developmental pathways, and changing patterns of homeotic gene expression over the past three decades confirmed many of the discoveries made by Kovalevsky in animal development (Satoh et al. 2012; Holland 2015; Stolfi and Brown 2015).

An international prize, the Alexander Kowalevsky Medal, has been established to honor Kowalevsky's discoveries in embryology (Fig. 5). It is awarded by the St. Petersburg Society of Naturalists in Russia for outstanding contributions to the understanding of evolutionary relationships among major groups in the animal kingdom, to evolutionary developmental biology, and to comparative zoology (see Mikhailov and Gilbert 2002; Ereskovsky 2012).

Cross-References

► Developmental Homology
► Evo-devo and Phylogenetics

► Evo-Devo's Contributions to the Extended Evolutionary Synthesis
► Macroevolution

Acknowledgments Author gratefully thanks Natalia Lentsman for English correction. This work was supported by the Russian Science Foundation grant no. 17-14-01089.

Bibliography

Kowalevsky AO (1865) Istoria razvitia lantsetnica (Amphioxus lanceolatus или Branchiostoma lumbricam). St. Petersburg (The developmental history of Amphioxus lanceolatus or Branchiostoma lumbricam). 47 pp
Kowalevsky AO (1866a) Anatomie des Balanoglossus délle Chiaje. Mém Acad Imp Sciences St. Petersburg 10(16):1–18
Kowalevsky AO (1866b) Beiträge zur Anatomie und Entwicklungsgeschichte des Loxosoma neapolitanum. Ibid 10(2):1–10
Kowalevsky AO (1866c) Entwicklungsgeschichte der Rippenquallen. Ibid 10:28
Kowalevsky AO (1866d) Entwicklungsgeschichte der einfachen Ascidien. Ibid 10(15):16
Kowalevsky AO (1867a) Beiträge zur Entwicklungsgeschichte der Holothurien. Ibid 10(6):16
Kowalevsky AO (1867b) Die Entwickelungsgeschichte des Amphioxus lanceolatus. Ibid 11(4):1–17
Kowalevsky AO (1867c) Anatomia i istoria razvitia Phoronis. ("Anatomy and Developmental History of Phoronis"). Doctoral disser, Zapiski Akademii nauk (St. Petersburg) 11(1):1–35
Kowalevsky AO (1867d) Über das Centralinervensystem und das Gehörorgan der Cephalopoden. Ibid 9(3):38. (With Ovsjannikov F.)
Kowalevsky AO (1868a) Untersuchungen über die Entwickelung der Coelenteraten. Nachr K Gesell Wiss Göttingen 7:154–159
Kowalevsky AO (1868b) Beitrag zur Entwickelungsgeschichte der Tunikaten. Ibid 19:401–415
Kowalevsky AO (1869) Die Entwickelungsgeschichte der Störe. Vorläufige Mitteilung. Mem Biol Bull Acad Sci St Petersburg 7(2):171–183. (With Ovsjannikov F., Vagner N)
Kowalevsky AO (1870a) Zametka o poezdke na Kaspijskoe more. (Notes to expedition on Kaspean Sea). Zapiski Kievskogo Obsh Estestvoispit 1:19–20
Kowalevsky AO (1870b) Predvaritelnoe soobshenie ob embriologii chervej i chlenistonogih. (Preliminary report on worms and arthropods embryology). Ibid 1:21–26
Kowalevsky AO (1870c) Pochkovanie Perophora Listeri Wiegm. (Budding in Perophora Listeri Wiegm). Ibid 1:70–100
Kowalevsky AO (1870d) J planarieobraznom samtse Bonellia. (About planariemorph mail of Bonellia). Ibid 1:101–107
Kowalevsky AO (1870e) Stroenie pishevaritelnogo kanala e planarii Dendrocoela. (The structure of the digestive canal y planarians in Dendrocoela). Ibid 1:109–110
Kowalevsky AO (1870f) K istorii razvitija akul po nabludenijam nad Mustelus laevis i Acanthias vulgaris. (On the history of the development of sharks on observations of Mustelus laevis and Acanthias vulgaris). Ibid 2:163–187
Kowalevsky AO (1870g) Razvitie jaits u Sternaspis thalassemoides Otto. (Eggs development in Sternaspis thalassemoides Otto). Ibid 2:287–290
Kowalevsky AO (1870h) K istorii razvitija Amphioxus lanceolatus. (On the history of the development of Amphioxus lanceolatus). Ibid 3:327–338
Kowalevsky AO (1870i) K istorii razvitija cherepahi (Emys europaea). (On the history of the development of turtles (Emys europaea)). Ibid 3:378–385
Kowalevsky AO (1871a) Embryologische Studien an Würmern und Arthropoden. Mem Acad Imp Sci St-Pétersbourg 16(12):70

Kowalevsky AO (1871b) Weitere Studien über die Entwickelung der einfachen Asciden. Arch Micr Anat 7:101–130
Kowalevsky AO (1871c) O razmnozhenii morskih zvezd deleniem i pochkovaniem. (On the reproduction of sea stars by dividing and budding). In: Proceedings of the 3rd congress Naturalists Kiev, pp 3–4
Kowalevsky AO (1871d) Ob anatomii i istorii razvitija Thalassema. (On anatomy and on the history of the development of Thalassema). Ibid 24–25
Kowalevsky AO (1871e) O bespolom razmnozhenii Amauroceum. (On asexual reproduction of Amauroceum). Ibid 25
Kowalevsky AO (1872) Sitzungsberichte der zoologischen Abtheilung der III. Versammlung russischer Naturforscher in Kiew. Zschr wiss Zool 22(3):283–304
Kowalevsky AO (1873) Nabludenija nad razvitiem Coelenterata. (Observations of development of Coelenterata). Izvestia Obshchestva liubitelei estestvoznanii antropologii etnografii Moscow 10(2):1–36
Kowalevsky AO (1874a) Nabludenija nad razvitiem Brachiopoda. (Observations of development of Brachiopoda). Ibid 14:1–40
Kowalevsky AO (1874b) Über die Knospung der Ascidien. Arch Microscop Anat 10:441–480
Kowalevsky AO (1875) Über die Entwickelungsgeschichte der Pyrosoma. Ibid 11:598–635
Kowalevsky AO (1876) Weitere Studien über die Entwickelungsgeschichte des Amphioxus lanceolatus nebst einem Beitrage zur Homologie des Nervensystems der Würmer und Wirbeltiere. Ibid 13:181–208
Kowalevsky AO (1879a) Über die Entwickelung der Chitonen. Zool Anz 2(37):469–473
Kowalevsky AO (1879b) Zur Entwickelungsgeschichte der Alcyoniden, Sympodium coralloides und Glavularia crassa. Ibid 2(38):491–493
Kowalevsky AO (1880a) Über Coeloplana Metschnikowii. Ibid 3(51):140
Kowalevsky AO (1880b) Bau und die Lebenserscheinungen von Neomenia gorgonophilus n. sp. Ibid 3(53):190–191
Kowalevsky AO (1881a) Neomenia gorgonophila. Izvestia Obshchestva liubitelei estestvoznanii antropologii etnografii Moscow 37:181–186
Kowalevsky AO (1881b) About Neomenia corallophila and Coeloplana Metschnikowii. Ibid 37:1–5
Kowalevsky AO (1882a) Sur le développement des Alcyonaires. Compt Rendus Inst Acad Sci XCL (13):562–565 (With Marion AF)
Kowalevsky AO (1882b) Études sur les Neomenia. Zool Anz 5(103):101–104. (With Marion AF)
Kowalevsky AO (1882c) Weitere Studien über die Entwickelung des Chitonen. Ibid 5(113):307–310
Kowalevsky AO (1883a) Études sur l'embryologie du Dentale. Ann Musée Hist Nat Marseille 1(4):7–43
Kowalevsky AO (1883b) Embryogénie du Chiton polii (Philipii) avec quelques remarques sur le développement des autres Chitons. Ibid 1(5):5–37
Kowalevsky AO (1883c) Documents pour l'histoire embryogénique des Alcyonaires. Ibid 1(4):7–43. (With Marion AF)
Kowalevsky AO (1883d) Matériaux pour servir à l'histoire de l'Anchinie. J Anat Physiol 19:1–22. (With Barrois J)
Kowalevsky AO (1884) Zur Entwicklungsgeschichte des Lucernaria. Zool Anz 7(184):712–717
Kowalevsky AO (1885) Beiträge zur nachembryonalen Entwicklung der Musciden. Ibid 8(186):98–103; (189):123–128; (190):153–157
Kowalevsky AO (1886a) K istorii razvitija kavkazskogo scorpiona (Androctonus ornatus). (To the history of the development of the Caucasian scorpion Androctonus ornatus). Zapiski Novorossiiskago obshchestva estestvoispytatelei 11:39–55. (With Shulgin M)
Kowalevsky AO (1886b) Zur Entwickelungsgeschichte des Scorpions (Androctonus ornatus). Biol Centrbl 6(17):525–532. (With Shulgin M)

Kowalevsky AO (1886c) Zum Verhalten des Rückengefasses und des girlanden förmigen Zellenstranges der Musciden während der Metamorphose. Ibid 6(3):74–79
Kowalevsky AO (1886d) Zur embryonalen Entwickelung der Musciden. Ibid 6(2):49–54
Kowalevsky AO (1886e) Organisation du Lepidomenia hystrix nouveau type de Solenogastre. Compt Rend Sean Acad Sci (Paris) 103:757–759. (With Marion AF)
Kowalevsky AO (1887) Beiträge zur Kenntnis der nachembryonalen Entwicklung der Musciden. Zeitschr Wiss Zool 45:542–588
Kowalevsky AO (1888) Sur les éspeces de proneomenia des cotes de Provence. Compt Rend Sean Acad Sci (Paris) 106(8):529–532. (With Marion AF)
Kowalevsky AO (1889a) O videlitelnih organah chlenistonogih. (On the excretory organs of arthropods). Zapiski Novorossiiskago obshchestva estestvoispytatelei 14:79–84
Kowalevsky AO (1889b) O videlitelnih organah nekotorih nasekomih, paukov i mnogonozhek. (On the excretory organs of certain insects, spiders and centipedes). Ibid 14(2):201–205
Kowalevsky AO (1889c) Ein Beitrag zur Kenntnisse der Excretionsorgane. Biol Centralbl 9(2):33–47; (3):65–76; (4):127–128
Kowalevsky AO (1889d) Contributions à l'Histoire des Solenogastes au Aplacophores. Ann Mus Hist Nat Marseille Zoologie 3(1):77. (With Marion AF)
Kowalevsky AO (1889e) O pishevarenii lichinok astsidij i ob obrazovanii mantii. (Ascidia larvae transformation and mantle formation). Vestnik Estestvoispit 1(9):378–390
Kowalevsky AO (1890) O selezenke u molluskov. (About the spleen in mollusks). Zapiski Novorossiiskago obshchestva estestvoispytatelei 15(2):175–186
Kowalevsky AO (1892a) Ein Beitrag zur Kenntniss der Excretionsorgane der Pantopoden. Mém Acad Imp Sci St Petersburg 38(12):1–9
Kowalevsky AO (1892b) Einige Beiträge zur Bildung des Mantels bei Ascidien. Ibid 38(1):1–20
Kowalevsky AO (1892c) Sur les organes excréteurs chez les Arthropodes terrestres. Trav Congres Internat. Zool Moscow in 1892. Moscou, 1893. 1–43
Kowalevsky AO (1892) O zhirovom tele u nasekomih, paukov i mnogonozhek. (About the fat body of insects, spiders and centipedes). Trudy St Petersburg Obsh Estestvoisp 23(1):53–57
Kowalevsky AO (1894a) Sur le coeur de quelques Orthoptères. Compt Rend Acad Sci (Paris) 119(7):409–411
Kowalevsky AO (1894b) Étude sur le coeur de quelques Orthoptères. Arch Zool Exper Gén 2:485–490
Kowalevsky AO (1894c) Études expérimentales sur les Glandes lymphatiques des Invertébrés. Mém Biol Bull Acad Imp Sci 13:437–459
Kowalevsky AO (1895a) Nabljudenia nad limphaticheskoy systemoy nasekomih i mnogonozhek. (Observations on the lymphatic system of insects and centipedes). Mém Acad Imp Sci St Petersburg (5) 2(1):1–8
Kowalevsky AO (1895b) Limphaticheskie zhelezi u Nereis cultrifera i Halla parthenopeia. (Lymphatic glands in Nereis cultrifera and Halla parthenopeia). Ibid 3(2):127–128
Kowalevsky AO (1895c) Une nouvelle glande lymphatique chez le Scorpion d'Europe. Bull Acad Sci St. Petersbourg 3(2):129–130
Kowalevsky AO (1895d) Une nouvelle glande lymphatique chez le Scorpion d'Europe. Compt Rend Sean Acad Sci (Paris) 121(2):106–108
Kowalevsky AO (1895e) Une nouvelle glande lymphatique chez le Scorpion d'Europe. Compt Rend Sean du 3-me Congr Internat Zool Leyde. 16–21 Sept 1895; Leyde; 1896, pp 501–530
Kowalevsky AO (1895f) Sur les glandes lymphatiques de quelques Nereides. Ibid 526–530
Kowalevsky AO (1896a) Études des glandes lymphatiques de quelques Myriapodes. Arch Zool Exp Gén 3(3):591–614
Kowalevsky AO (1896b) Étude sur l'anatomie de l'Acanthobdella peledina Grube et Archaeobdella Esmontii. Bull Acad Imp Sci St Petersburg 5(1):1–4
Kowalevsky AO (1896c) Étude sur l'anatomie de l'Acanthobdella peledina. Ibid 5(4):263–274
Kowalevsky AO (1896d) Étude sur l'anatomie de l'Archaeobdella Esmontii de O. Grimm. Ibid 5(5):331–335

Kowalevsky AO (1896e) Études biologiques de quelques Hirudinées. Compt Rend Sean Acad Sci (Paris) (4):165–168
Kowalevsky AO (1896f) Etude des glandes lymphatiques de quelques Myriapodes. Arch Zool Exper Gén 3:591–614
Kowalevsky AO (1897a) Études biologiques sur les Clepsines. Mém Acad Imp Sci St Petersburg 5:1–15
Kowalevsky AO (1897b) Une nouvelle glande lymphatique chez le Scorpion d'Europe. Ibid 5(10):1–18
Kowalevsky AO (1898a) Nabludenija nad razvitiem Archaeobdella i biologia Glepsine costata Müll. (Observations on the development of Archaeobdella and biology of Glepsine costata Müll.). Trudy St Petersburg Obsh Estestvoisp 285(1):23–30
Kowalevsky AO (1898b) Nekotorie dannie k istorii razvitia Archaeobdella Eismontii i biologii Clepsine costata. (Some data to the history of development of Archaeobdella Eismontii and biology of Clepsine costata). Ibid 28(1):1–9
Kowalevsky AO (1899a) K biologii *Haemtnteria* (Clepsine) costata Fil. (Miiller). Predvaritelnoe soobshenie. (To biology of *Haemtnteria* (Clepsine) costata Fil. (Miiller). Preliminary report). Ibid 30(1):23–30
Kowalevsky AO (1899b) Quelques mots sur l'Haementeria costata Müll. Compt Rend Sean Acad Sci (Paris) 128(19):1185–1188
Kowalevsky AO (1899c) Imprégnation hypodermique chez l'Haementeria costata de Müll. Ibid 129(5):261–264
Kowalevsky AO (1899d) Otchet o moih zoologicheskih issledovanijah na Sevastopolskoj stantsii letom 1899 g. (Report on my zoological studies at the Sevastopol station in the summer of 1899). Mém Acad Imp Sci St Petersburg 12:193–204
Kowalevsky AO (1900a) Étude biologique de l'Haementeria costata Müll. Ibid 11(1):66 pp
Kowalevsky AO (1900b) Phénomènes de la fécondation chez l'Helobdella algina Moq. Tandon. Mém Soc Zool France 13:66–88
Kowalevsky AO (1901a) Protsess oplodotvorenija u Haementeria costata Müll. (Processes of fecondation in Haementeria costata Müll.). Mém Acad Imp Sci St Petersburg 11 Ser. III(10):19
Kowalevsky AO (1901b) Les Hedilidées. Étude anatomique. Ibid 8 ser. XII(6):32
Kowalevsky AO (1901c) Sur le genre Ghaetoderma. Arch Zool Exp Gén 9:261–281
Kowalevsky AO (1901d) L'études anatomiques sur le genre Pseudovermis. Ibid XII(4):28

References

Allman GJ (1853) On the anatomy and physiology of *Cordylophora*: a contribution to our knowledge of *Tubularian zoophytes*. Philos Trans R Soc Lond 143:367–384
Arthur W (2002) The emerging conceptual framework of evolutionary developmental biology. Nature 415:757–764
Bljacher LJ (1959) Istorija Embriologii v Rossii: Bespozvonochnyje. Akademija Nauk, Moscow. (in Russian)
Darwin CH (1874) The descent of man, 2nd edn. J. Murray, London
Dogiel VA (1945) A. O. Kovalevskiy [A. O. Kowalevsky]. Academy of the sciences USSR Press, Moscow–Leningrad. (in Russian)
Ereskovsky AV (2012) Introduction to the Kowalevsky medal issue. Evol Dev 14:1–2
Fokin SI (2000) Professor W.T. Schewiakoff: life and science. Protist 151:182–189
Fokin SI (2012) Life of Alexander Onufrievich Kowalevsky (1840–1901). Evol Dev 14:3–8
Haeckel E (1874) Anthropogenie oder Entwickelungsgeschichte des Menschen. Gemeinverständliche wissenschaftliche Vorträge über die Grundzüge der menschlichen Keimes- und Stammesgeschichte. Wilhelm Engelmann, Leipzig

Hall BK (1998) Germ Layers and the germ-layer theory revisited. Primary and secondary germ layers, neural crest as a fourth germ layer, homology, and demise of the germ-layer theory. In: Hecht MK (ed) Evolutionary biology. Plenum Press, New York, pp 121–186
Hall BK (1999) Evolutionary developmental biology, 2nd edn. Kluwer, Dordrecht
Holland PWH (2010) From genomes to morphology: a view from Amphioxus. Acta Zool (Stockholm) 91:81–86
Holland LZ (2015) Cephalochordata. In: Wanninger A (ed) Evolutionary developmental biology of invertebrates 6: Deuterostomia. Springer, Wien, pp 91–133
Holland LZ, Gibson-Brown JJ (2003) The ciona intestinalis genome: when the constraints are off. Bioessays 25:529–532
Lankester ER (1902) Alexander Kowalevsky. Nature 1712(66):394–396
Levit GS (2007) The roots of evo-devo in Russia: is there a characteristic "Russian Tradition"? Theory Biosci 126:131–148
Mikhailov AT (2012) Russian comparative embryology takes form: a conceptual metamorphosis toward "evo-devo". Evol Dev 14:9–19
Mikhailov AT, Gilbert SF (2002) From development to evolution: the re-establishment of the "Alexander Kowalevsky Medal". Int J Dev Biol 46:693–698
Pilipchuk OJ (2003) Aleksandr Onufrievich Kowalevskiy 1840–1901. Nauka, Moscow. (in Russian)
Poljanskij J (1955) The letters of A.O. Kowalevsky to I.I. Metschnikoff (1866–1900). Akademia Nauk, Moscow, Leningrad. (in Russian)
Raff RA, Love AC (2004) Kowalevsky, comparative evolutionary embryology, and the intellectual lineage of evo-devo. J Exp Zool (Mol Dev Evol) 302B:19–34
Remak R (1855) Untersuchungen über die Entwickelung der Wirbelthiere. G. Reimer, Berlin
Satoh N, Tagawa K, Takahashi H (2012) How was the notochord born? Evol Dev 14:56–75
Stolfi A, Brown FD (2015) Tunicata. In: Wanninger A (ed) Evolutionary developmental biology of invertebrates 6: Deuterostomia. Springer, Wien, pp 134–204

Valentin Haecker (1864–1927)

Uwe Hoßfeld, Georgy S. Levit, and Elizabeth Watts

Contents

Abstract

The German zoologist Valentin Haecker (1864–1927) is one of the forerunners of experimental biology, genetics, and developmental physiology. In his "Entwicklungsgeschichtliche Eigenschaftsanalyse," published in 1918, Haecker tried to describe the earliest stages in the development of the phenotype (*Phenogenetics*). His major objective was to embrace two central concepts of Mendelian genetics, phenotype and genotype, within a well-articulated theory. Haecker realized that a proper analysis of how the genotype gives rise to the

U. Hoßfeld (✉) · E. Watts
Arbeitsgruppe Biologiedidaktik, Institut für Zoologie und Evolutionsforschung, Fakultät für Biowissenschaften, Friedrich-Schiller-Universität Jena, Jena, Germany
e-mail: uwe.hossfeld@uni-jena.de; elizabeth.watts@uni-jena.de

G. S. Levit
Institute for Biology, Kassel University, Kassel, Germany

Biology Education Research Group ("Bienenhaus"), Friedrich-Schiller University Jena, Jena, Germany

Department of Social Sciences and Humanities, ITMO National Research University, St. Petersburg, Russia
e-mail: uk059961@uni-kassel.de; georg.levit@uni-jena.de; gslevit@corp.ifmo.ru

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_23

phenotype requires joint efforts of morphology, physiology, and experimental embryology. In this sense, Haecker's theory of phenogenetics became not only indispensable for the future acquisition of knowledge in epigenetics but also in the field of evo-devo.

Keywords

Phenogenetics · Developmental genetics · Valentin Haecker · Pluripotency · Neo-Lamarckism

Life

Ferdinand Carl Valentin Haecker was born on September 15, 1864, in Ungarisch-Altenburg (today Mosonmagyaróvár, Hungary) as the son of the Professor of Agriculture Christian Ludwig Haecker and his wife Julie Charlotte Schübler. During his childhood, Haecker concentrated on the exact and natural sciences. Since 1870 he went to the elementary school in Altenburg. In 1873 his father died unexpectedly from a stroke. A year later, the family moved to Stuttgart, where Haecker continued his education at a secondary school. In 1879 he joined a Convent School in Maulbronn (Neckarkreis). After two years his class moved to Blaubeuren (Swabian Alb), where he passed through a difficult examination and got his matriculation examination in 1883. After the school, Haecker served one mandatory year in the German army as a lieutenant. In the fall 1884, together with his brother Walter, he enrolled first at the Convent of Tübingen (Stift), and later at the Tübingen University to study mathematics and natural sciences. At the University of Tübingen, Valentin attended lectures of Theodor Eimer (1843–1898), a founder of the orthogenesis theory. Being moved for a while to Straßburg, he graduated in the spring of 1889. After receiving his PhD with Eimer with a dissertation "About the Colours of Bird's Feathers" in 1889, he spent a decade in Freiburg with the co-founder of neo-Darwinism August Weismann (1834–1914), first as an assistant, and later as a Privatdozent, after defending his habilitation thesis. In 1895 he became Professor of Zoology. Based on the research conducted in Freiburg, Haecker published his first book, *Praxis and Theory in the Cell- and Fertilization Studies,* in 1899. Two of his later books, *Bastardization and the Formation of Gamete* (1904) and *About Memory, Heredity and Pluripot*ency (1914), were dedicated to Weismann's 70th and 80th birthdays and resumed his research of the Freiburg period as well (Fig. 1).

In 1900, when Haecker was 36 years old, he became the Chair of Zoology at the Technical University in Stuttgart as a successor of Carl Benjamin Klunzinger (1834–1914). In Stuttgart, he lectured for students of agriculture and veterinary medicine and became interested in Mendelism. During this period, Haecker evaluated the Radiolaria collection of the Valdivia-expedition (1898–1899) with Carl Chun (1852–1914). In 1903 Haecker married Johanna Lucia Anna Kühn. The couple had two children: a daughter, Hertha, and a son, Rudolf. In 1908 Haecker joined the editorial board of the newly founded journal *Zeitschrift für Induktive*

Fig. 1 Valentin Haecker as Rector at Halle University (University Archive Halle, Rep. 40, Nr. I, HI 36)

Abstammungs- und Vererbungslehre (ZIAV). His research interests in Stuttgart focused on axolotls neoteny. In 1909 he moved to Halle as a Full Professor of zoology at the Philosophy faculty. He was elected as a member of the Leopoldina Academy in 1910.

While teaching at the University of Halle, Haecker supervised Bernhard Rensch (1900–1990) and Gerhard Heberer (1901–1973), two PhD students who later became leading figures of the Modern Synthesis in Germany (Reif et al. 2000). In 1922 Rensch published his paper on dwarfism and gigantism in the domestic fowl. Two years later Heberer published his PhD thesis devoted to the study of sperm formation in copepods. Among all Haecker's students, Rensch was especially well trained in the analysis of speciation and phylogeny. Rensch recognized early on the potential and the great theoretical importance of new systematics. His studies significantly contributed to a new field of genetics created by Haecker, the so-called "Phenogenetics" (developmental genetics), devoted to analyze developmental processes resulting in different phenotypic characters. On the other hand, Haecker was convinced that there were strong evidences in favor of nongenetic, Lamarckian mechanisms of modification explaining, for instance, "the geographic color differences of birds and mammals – more brownish in Western and more grayish in Eastern Europe" (Rensch 1998, p. 294). In 1911 the first edition of Haecker's *General Genetics* (*Allgemeine Vererbungslehre*) came out of print, and in 1918 his major work *Developmental Genetics* (*Phänogenetik*) was published. Besides, jointly with the philosopher Theodor Ziehen (1862–1950), Haecker wrote a

book on the inheritance and development of musical talents (Haecker and Ziehen 1923). Finally, Haecker summarized his views on Goethe's morphological works in a book (Haecker 1927a).

During the First World War, Haecker was preoccupied with the harmful effects of the war on the environment. For example, he pointed out that several plant species had shown the ability to regenerate after being effected by battles. He believed in spontaneous regeneration and hold that nature was a regulatory force which could preserve and rejuvenate the German nation. In 1923, Haecker lectured on human racial and family issues. Richard W. Darré (1895–1953), who advocated the restoration of the peasantry (Weindling 1989, p. 330), attended his lectures on agriculture.

In 1926 Haecker became the rector of Halle University. His inaugural lecture was entitled "Environment and Heredity." A year later, on December 12, 1927, he suddenly died from a stroke in Halle (Hoßfeld et al. 2017, 2018).

Work

Barthelmess and Harwood have shown that the interest in Mendelism grew rapidly in Germany around 1900 (Barthelmess 1952; Harwood 1993). The "rediscovery" of Mendel's laws in 1900 is seen worldwide as a turning point in the development of modern genetics. In the first half of the twentieth century, it was generally held that the "rediscovery" took place several times. Four (not three) European biologists – the German plant physiologist Carl Correns (1864–1933), the Dutch botanist Hugo de Vries (1848–1935), the Austrian plant breeder Erich von Tschermak-Seysenegg (1871–1962) along with his brother, the Austrian physiologist Armin von Tschermak-Seysenegg (1870–1952) – independently and simultaneously rediscovered the Mendelian laws (Simunek et al. 2011a; Simunek et al. 2011b).

In the years immediately following the rediscovery of Mendel and until the First World War, the three Mendelian laws were modified and supplemented to explain more complex patterns of inheritance. The quantitative analysis of inheritance was extended to include both plants and animals. The Danish geneticist Wilhelm Johannsen (1857–1927) coined the term "gene" in his book *Elemente der exakten Erblichkeitslehre ("Elements of exact Genetics")* to refer to discrete hereditary units located with the cell, as well as the notions of genotype and phenotype (Johannsen 1909).

In 1901 Carl Correns began to teach the new "science of heredity" (Vererbungswissenschaft), first in Tübingen, and later in Leipzig and Münster. Other German biologists such as Erwin Baur (1875–1933), Richard Goldschmidt (1878–1958), and Valentin Haecker followed him since 1910 (Harwood 1993, p. 35). In parallel, a variety of genetic textbooks came out of print: Johannes Paulus Lotsy (1867–1931) with Vorlesungen über Deszendenztheorie ("Lectures in Evolutionary Theory", 1906, 1908), Erwin Baur with Einführung in die experimentelle Vererbungslehre ("Introduction to Experimental Genetics", 1911), Richard Goldschmidt with Einführung in die Vererbungswissenschaft ("Introduction to General Genetics", 1911), V. Haecker with Allgemeine Vererbungslehre ("General Genetics," 1911), Ludwig Plate (1862–1937) with Vererbungslehre ("Genetics," 1913), Arnold Lang (1855–1914)

with Die experimentelle Vererbungslehre in der Zoologie seit 1900 ("Experimental Genetics in Zoology since 1914"), Heinrich Ernst Ziegler (1858–1925) with Die Vererbungslehre in der Biologie und in der Soziologie ("Genetics in Biology and Sociology", 1918), or Johannes Meisenheimer (1873–1933) with Vererbungslehre ("Genetics", 1923). In addition, several new biological journals were founded such as *Archiv für Rassen- und Gesellschaftsbiologie* (*Archive for Race and Society Biology*), *Archiv für Zellforschung* (*Archive for Cell Research*), *Zeitschrift für Pflanzenzüchtung* (*Journal of Plant Breeding Research*), or *Zeitschrift für induktive Abstammungs- und Vererbungslehre* (*Journal for the Inductive Study of Evolution and Heredity*). As a result of this development, several geneticists with an interest in botany or zoology acquired chairs at different German universities. Haecker moved to Halle (1909), Correns went to Leipzig (1902) and later to Münster (1909), Plate (1909) became Haeckel's follower in Jena, and Baur received a chair in Berlin (1911). Haecker was a key figure in the growth of genetics of that time, but he also had interests in various fields of biology such as ornithology, animal physiology, marine biology, developmental genetics, and philosophy (Haecker 1965; Immelmann 1965a, b; Rensch 1965; Osche 1965; Heberer 1964; Heberer 1965; Kosswig 1965). Along with R. Goldschmidt (see for instance Goldschmidt 1927), he was the second German geneticist interested in the early stages of ontogenesis. Haecker evidently influenced Conrad Hall Waddington (1905–1975), who appealed to Haecker's hypotheses in his article "The Epigenotype" (1942) or, without citing him, in his book Genetics and Development (1962). Waddington noted:

> For the purpose of a study of inheritance, the relation between phenotypes and genotypes can be left comparatively uninvestigated; we need merely to assume that changes in the genotype produce correlated changes in the adult phenotype, but the mechanism of this correlation need not concern us. Many geneticists have recognized this and attempted to discover the processes involved in the mechanism by which the genes of the genotype bring about phenotypic effects. The first step in such an enterprise is [. . .]to describe what can be seen of the developmental processes. For enquiries of this kind, the word 'phenogenetics' was coined by Haecker. The second and more important part of the task is to discover the causal mechanisms at work, and to relate them as far as possible to what experimental embryology has already revealed of the mechanics of development. We might use the name 'epigenetics' for such studies, thus emphasizing their relation to the concepts, so strongly favourable to the classical theory of epigenesis, which have been reached by the experimental embryologists. We certainly need to remember that between genotype and phenotype, and connecting them to each other, there lies a whole complex of developmental processes. It is convenient to have a name for this complex: 'epigenotype' seems suitable. (Waddington 1942, p. 18)

Haecker's Lamarckism

Many German biologists, in particular zoologists and paleontologists, defended Lamarckian evolutionary mechanisms between 1900 and 1940. They believed in the inheritance of acquired characters and a direct effect of the environment on organism's inheritance. For example, the zoologist and geneticist Ludwig Plate (1862–1937) campaigned for a revival of the original Darwinism. His research

program, which he labeled "old-Darwinism," proclaimed the synthesis of selectionism with "moderate Lamarckism" and orthogenesis (Rensch 1983, 1998; Harwood 1993; Levit and Hoßfeld 2006). Plate defined the inheritance of acquired characters as follows: "The inheritance of an acquired character means only that a newly occurred character was in the first generation somatogenic whereas in the subsequent generations it becomes blastogenic" (Plate 1913, p. 439). In modern terms, this means that there is a variety of features, which have been phenotypic in a certain generation and became inheritable in all subsequent generations (Levit and Hoßfeld 2006). Plate attached great importance to the idea that the inheritance of acquired characters should not necessarily be combined with the Lamarckian idea of use or disuse of a certain organ: "It is no matter whether somatic modifications are caused by a use or disuse of an organ or by temperature, nutrition or other factors" (Plate 1913, p. 440).

Haecker, as well as Plate, advocated Lamarckian evolutionary mechanisms, but by contrast to Plate, he concentrated on the description of the phenotype and paid little attention to selectionism; there is not a single reference to natural selection in Haecker's book of 1918. At the cell biology level, he paid much attention to the chromosome theory:

> Haecker acknowledged the importance of the chromosome theory but remained dissatisfied with several of its features throughout his life. Many of his criticisms were also voiced by Bateson: the cytological evidence for crossing-over was inadequate; vastly different kinds of organisms sometimes possessed the same number of chromosomes; sex chromosomes were less likely to be the cause of sex differences than merely indicators of the real cause; and the evidence for purity of the gametes was unconvincing (Harwood 1993, p. 42).

In his *Allgemeine Vererbungslehre* ("General Genetics"), Haecker postulated that speciation was caused by the selection of hereditary varieties in August Weismann's sense. Haecker was convinced that the inheritance of acquired characters should be regarded as improbable, because a chain of causal events which phenotypically altered the soma and the genes could never be identical with a causal chain leading from altered genes over embryonic stages back to the characteristics of the soma (Rensch 1983, p. 32). He argued that "If one assumes that different 'virtual possibilities of individual development' exist, then 'a parallel activation' (*Parallelaktivierung*) of latent general potentialities in genetic and somatic cells by means of the altered chemical processes takes place" (Haecker 1921, p. 154). He also believed that "constitutional concussion" of genetic and somatic cells could cause a parallel reduction of the resistance against illness. Thus, in 1918 he postulated that a "parallel induction" of somatic and germ cells would be possible only "when characteristics acquired by the parents preexisted already in the virtual potential of the plasma" (Haecker 1918, p. 324).

Haecker saw as a candidate for a neo-Lamarckian evolutionary mechanism the so-called "parallel-induction," namely, the simultaneous impact of the environment on soma and germ cells, (1921, 150 ff.) as well as the "ideocynesis," defined as the influence of external stimuli on the ideoplasm (1921: 163 ff. and graft (1921, p. 170).

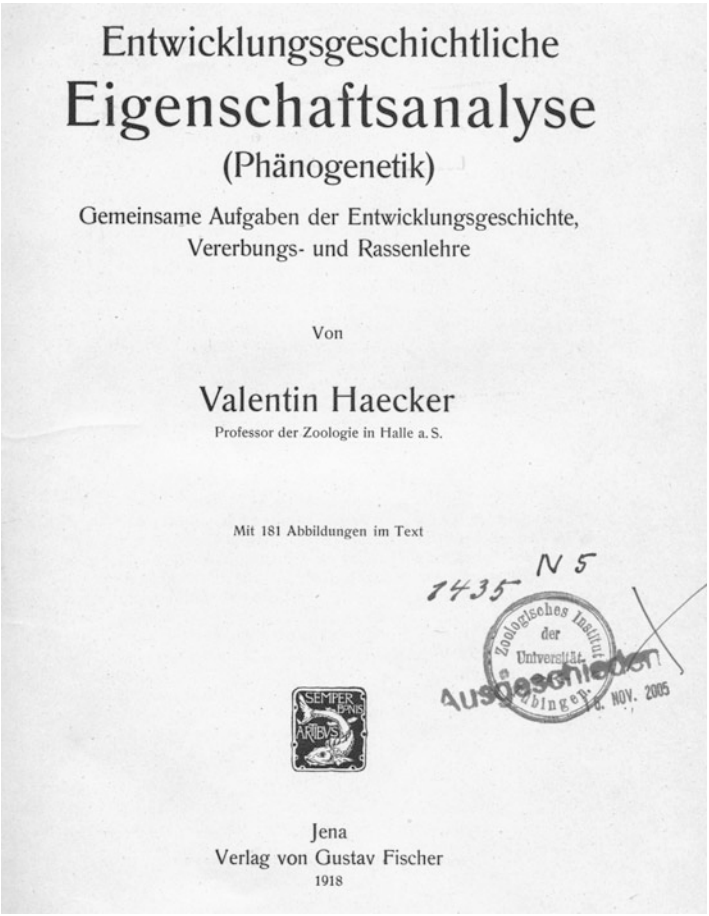

Entwicklungsgeschichtliche
Eigenschaftsanalyse
(Phänogenetik)
Gemeinsame Aufgaben der Entwicklungsgeschichte,
Vererbungs- und Rassenlehre
Von
Valentin Haecker
Professor der Zoologie in Halle a. S.
Mit 181 Abbildungen im Text
1435 N 5
Zoologisches Institut der Universität Tübingen
Ausgeschieden
6. NOV. 2005
SEMPER IDEM ARTIBUS
Jena
Verlag von Gustav Fischer
1918

Fig. 2 Title Page (1918)

The final version of Haecker's Lamarckism can be found in two books of him (1914, p. 8, 1921, p. 148) (Fig. 2).

Haecker's Phenogenetics

One of Haecker's objections to the Mendelian chromosome theory was its failure to bridge the gap between hereditary units and phenotypic traits. His research program, which he called *Phänogenetik* ("phenogenetics"), had precisely the objective to build this bridge between the genotype and the phenotype. He outlined this program in a study of 1918. In the preface, Haecker described a new research field that should explore "in terms of morphogenetics and

developmental physiology" the appearance of the organism's "external characteristics" (Haecker 1918, p. 4; Deichmann 1996, p. 164).

According to Haecker, phenogenetics always begins with the so-called "differential diagnostics," i.e., with histological, morphological, and physiological studies of differences between species or races. All his phenogenetic investigations were descriptive, such as color differences in different races of mammals, axolotls, birds, and plants (Chaps. 7–13) as well as the stripe patterns in various species (Chap. 14), or the growth of the skin (Chaps. 16–18). This "phenoanalysis" is followed by a "phenogenetic descriptive" investigation of variations of a certain trait. The traits in question are traced back to a "phenocritical phase," the point of bifurcation in which the developmental stage manifests an initial divergence of the trait. Phenogenetics in the narrow sense is the study of diverging developmental pathways, and it must penetrate into the deeper ("phenocritical") causes of the observed divergence (Haecker 1918, p. 4 ff.). This process can proceed epigenetically externally or internally, and in that sense phenogenetics is a subdiscipline of developmental mechanics (*Entwicklungsmechanik*) and developmental physiology (*Entwicklungsphysiologie*).

In 1918, Haecker also discussed the problems of asymmetry, such as handedness in humans, which he considered an inherited trait. He also looked at other phenogenetic problems in the anomalies of extremities in animals and humans such as polydactyly, syndactyly, and brachydactyly. Haecker was also particularly interested in the phenogenetics of skull form and face shapes and was most involved in the study of abnormalities of the lower jaw. From a developmental history perspective, he viewed skull and face shape, as well as certain regions of the face, as "compound characters" (*komplex-verursacht*) in William Bateson's sense (Haecker 1918, p. 279), i.e., the development of these regions is seen as a product of the interaction of various developmental processes that occur under the influence of the neighboring organs (Lehmann 1965) (Fig. 3).

The Problem of Pluripotency

The study of pluripotency (*Pluripotenz*) and its evolutionary causes was one of the tasks of phenogenetics. Haecker first used the term *Pluripotenz des Artplasmas* ("Pluripotency of species specific plasm") in his book *Über Gedächtnis, Vererbung und Pluripotency* (1914, p. 40) and explained the hypothesis in detail in his later work on "The manifestations of pluripotency" (*Pluripotenzerscheinungen*) (Haecker 1925). In general, Haecker's theory of pluripotency postulated a relatively plastic concept of hereditary material suitable to account for the inheritance of acquired characters (Harwood 1993, p. 131 – Haecker 1918, Chap. 25; 1911/1921 Chaps. 14–17). In 1925 he published the most complete definition of the term:

> In a narrow (evolutionary) sense, *I understand by pluripotency* an *essential ability* of every organism (not only of species and races, but also of any individual germ and any cell at the embryonic stage of any individual) to develop under certain circumstances in directions *deviating from the basic typ*e. Therefore, pluripotency is a presence of a greater, *although not*

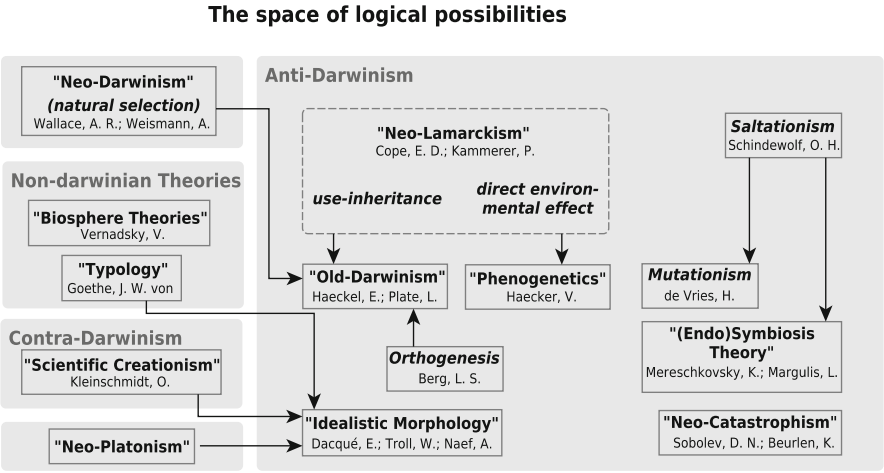


Fig. 3 The whole panoply of alternative evolutionary theories available in the first half of the twentieth century. Some of these theories persisted into the 1950s and 1960s. Neo-Lamarckism, saltationism, typology, and orthogenesis played major roles in the discussion of evolutionary mechanisms and were very influential in paleontology. Names in the boxes are prominent proponents of each theory (Levit and Hoßfeld 2011, 2013)

> *unlimited number*, of potencies or developmental possibilities than determined by a *property grounded in a normal, material, structural constitution of a species specific - but for the most part common to many species - plasm* (1925, pp. 1–2. Haecker's italics. Our translation).

Under the term *Artplasma* ("species specific plasm"), Haecker understands a hereditary material in the very broad sense, whereas the germ plasm was for him identical with nuclear substance of germ cells and germ line cells. Pluripotency enters the stage when the *Artplasma* jumps into another state of equilibrium. If a pluripotency occurs in the germ cells, it is called a "germ plasmatic pluripotency," while if it takes place during the ontogenesis of embryonic organs, it is a "somatic pluripotency." In that sense, Haecker maintained that transitions from heritable variations of germ cells to nonheritable variations of soma were possible (1925, p. 3). In such cases as atavisms and rudiments, pluripotency manifests itself in the ontogenetic development. The Russian botanist Nikolai I. Vavilov (1887–1943) spoke in this respect on *homologous* series and formulated his famous "Law of Parallel Variation," establishing a parallel variability of homologous characters in taxonomically near species (Kupzow 1975), however, without citing Haeckers paper of 1924 (Haecker 1924).

Contributions to General Biology

In addition to his work in genetics and developmental biology, Haecker also conducted research in the field of reproductive biology with a focus on copepods.

He was also interested in ornithology as well as in the study of Radiolari. From his work in reproductive biology, his Titisee study from 1901 is most worth mentioning. Here he made a significant contribution to understanding the complex geographical and ecological distribution as well as the reproductive cycle of zooplankton in mountain lakes (Haecker 1901, Elster 1965, Hoßfeld 1996). The study of ornithology also played an important role in Haecker's life and work. In fact, he produced 22 publications on the subject (Immelmann 1965a, pp. 71–72). His book *Der Gesang der Vögel, seine anatomischen und biologischen Grundlagen* (*The bird song and its anatomical and biological foundations*), published in 1900, was based upon comparative phylogenetic perspectives. Upon looking at the phylogenetic development of song, Haecker was able to determine the phylogenetic basis of these sounds as being dependent upon different factors such as season or changes in the thyroid gland and then illuminated the connection between this and the birds' reproductive life and flight-songs. Haecker also produced a number of works on bird migration and their feathers as well as the correlation between the bird colors and phenogenetics (1921, 1924, 1927b). In 1908, he presented a comprehensive evaluation of a collection of Radiolari materials, edited in volume no. 14, which had been gathered from 1898 to 1899 by a German deep-sea expedition in Valdivia, led by Carl Chun (Haecker 1908).

Legacy

Valentin Haecker numbers among the crucial figures in the history of German genetics of the first half of the twentieth century along with Richard Goldschmidt, Erwin Baur, and Ludwig Plate. His contributions to developmental physiology and experimental biology in general are indisputable as well.

Haecker himself championed the idea that experimental biology was of great importance for applied biomedical research and anthropology. This was due to the fact that experiments are possible in almost all fields of biology, whereas they might be difficult in medicine. Haecker was arguably the first who realized the importance of genetics for medical research.

His main contributions to genetics were the foundation of the theory of phenogenetics and his conceptual work on pluripotency. Although his phenogenetics was a neo-Lamarckian theory, it bounded together the findings of various biological disciplines and answered the questions on the relationships between the phenotype and the genotype. Therefore, Haecker's phenogenetics was indispensable not only for the growth of developmental genetics, but also in the field of evo-devo. Although neo-Lamarckian in its essence, Haecker's phenogenetics reflected on many important aspects of relationships between phenotype and genotype and, in this sense, contributed to the growth of developmental genetics. From today's viewpoint, Haecker's phenogenetics appears as an experimental embryology which, using such methods as alteration or switching off and on of certain genes in order to test the reactions of various organs to different genetic perturbations, appears as a pioneer of contemporary developmental genetics (Freye 1965a, b; Hoßfeld et al. 2017). Haecker recognized

very clearly that genetics should closely cooperate with morphology, physiology, and experimental embryology. Currently, one proceeds from the assumption that the interplay of the genotype and environmental factors determines the phenotype. Haecker as well as current biologists rejected any influence of phenotype on genotype. His neo-Lamarckism was constrained solely to the idea of direct environmental influences on development. The latter makes his approach akin to modern epigenetics. Today it is known that environmental impact can simulate genetics effects (phenocopy) (Hallgrímsson and Hall 2011; Hendrikse et al. 2007; Graw 2010).

Cross-References

- ▶ Conrad Hal Waddington (1905–1975)
- ▶ Developmental Plasticity and Evolution
- ▶ Eco-Evo-Devo
- ▶ Richard Goldschmidt (1878–1958)

Bibliography

Haecker V (1889/90) Über die Farben der Vogelfedern. Dissertation. Arch f mikroskop Anat 35:68–87
Haecker V (1899) Praxis und Theorie der Zellen- und Befruchtungslehre. Jena, Gustav Fischer
Haecker V (1901) Über die Fortpflanzung der limnetischen Copepoden des Titisees. Ber Naturf Ges Freiburg i Br 12:1–33
Haecker V (1904) Bastardierung und Geschlechtszellenbildung. (Ein kritisches Referat). Zool Jb (Suppl 7):161–260
Haecker V (1908) Tiefsee-Radiolarien. Ergebn. d. Dt. Tiefssee-Exp. 14. Jena, Gustav Fischer
Haecker V (1911) Vererbungslehre. Jena, Gustav Fischer, 3rd edn 1921
Haecker V (1914) Über Gedächtnis, Vererbung und Pluripotenz. Jena, Gustav Fischer
Haecker V (1918) Entwicklungsgeschichtliche Eigenschaftsanalyse (Phänogenetik). Gemeinsame Aufgaben der Entwicklungsgeschichte, Vererbungs- und Rassenlehre. Jena, Gustav Fischer
Haecker V (1920) Die Annahme einer erblichen Übertragung körperlicher Kriegsschäden. Archiv für Frauenkunde 4:1–15
Haecker V (1921) Weitere phänogenetische Untersuchungen an Farbenrassen. Z indukt Abstamm u Vererbungslehre 25:177–184
Haecker V (1924) Über die Innervierung der Vogelsyrinx. Phaenogenetische Untersuchungen über Parallelerscheinungen. Z Morph u Anthropol 24:47–58
Haecker V (1925) Pluripotenzerscheinungen. Synthetische Beiträge zur Vererbungs- und Abstammungslehre. Jena, Gustav Fischer
Haecker VU, Erbgut R (gehalten beim Antritt des Rektorats der Vereinigten Friedrichs-Universität Halle-Wittenberg am 12. Juli 1926). Halle (Saale)
Haecker V (1927a) Goethes morphologische Arbeiten und die neuere Forschung. Jena, Gustav Fischer
Haecker V (1927b) Phänanalytische Untersuchungen über Hochgebirgs- und Tieflandvögel mit besonderer Berücksichtigung der Schilddrüse. Z indukt Abstamm u Vererbungslehre 43:121–170
Haecker R (1965) Das Leben von Valentin Haecker. Zool Anz 174:1–22
Haecker V, Ziehen T (1923) Zur Vererbung und Entwicklung der musikalischen Begabung, Leipzig: Verlag von Johann Ambrosius Barth

References

Barthelmess A (1952) Vererbungswissenschaft. Freiburg/München, Karl Alber
Baur E (1911) Einführung in die experimentelle Vererbungslehre. Borntraeger, Berlin
Deichmann U (1996) Biologists under Hitler. Harvard University Press, Harvard
Elster H-J (1965) Valentin Haecker und die Erforschung der Fortpflanzungsbiologie des limnischen Zooplanktons. Zool Anz 174:22–37
Freye H-A (1965a) Valentin Haecker (1864 bis 1927) – Leben und Werk. Hercynia 2:327–337
Freye H-P (1965b) Valentin Haecker (1864-1927) und die Phänogenetik. Zool Anz 174:401–410
Goldschmidt R (1911) Einführung in die Vererbungswissenschaft. W. Engelmann, Leipzig
Goldschmidt R (1927) Physiologische Theorie der Vererbung. Julius Springer, Berlin
Graw J (2010) Genetik, Springer, Heidelberg
Hallgrímsson B, Hall BK (2011) Epigenetics. Linking genotype and phenotype in development and evolution. University of California Press, Berkeley
Harwood J (1993) Styles of scientific thought. The German genetic community 1900–1993. The University of Chicago Press, Chicago and London
Heberer G (1964) Valentin Haecker – Klassiker der Genetik. FAZ vom 15
Heberer G (1965) Valentin Haecker und das Problem der Chromosomen-Reduktion, unter besonderer Berücksichtigung der Copepoden. Zool Anz 174:91–116
Hendrikse JL, Parsons TE, Hallgrímsson B (2007) Evolvability as the proper focus of evolutionary developmental biology. Evol Dev 9:393–401
Hoßfeld U (1996) Ruderfußkrebse (Copepoden) – ein Versuchsobjekt der klassischen Vererbungszytologie zu Beginn des 20. Jahrhunderts. Biologisches Zentralblatt 115:91–103
Hoßfeld U, Watts E, Levit GS (2017) Valentin Haecker (1864–1927) as a pioneer of phenogenetics: building bridge between genotype and phenotype. Epigenetics 10:247–253
Hoßfeld U, Levit GS, Watts E (2018) Genetik des Äußeren. Valentin Haecker und sein Konzept der „Phänogenetik“ (1918). Biologie in unserer Zeit 48(4):239–245
Immelmann K (1965a) Die ornithologischen Arbeiten Valentin Haeckers Teil 1. Zool Anz 174:53–74
Immelmann K (1965b) Die ornithologischen Arbeiten Valentin Haeckers Teil 2. Zool Anz 174:450–467
Johannsen W (1909) Elemente der exakten Erblichkeitslehre. Jena, Gustav Fischer
Kosswig C (1965) Valentin Haeckers Vererbungslehre aus dem Jahre 1911. Zool Anz 174:441–445
Kupzow A (1975) Vavilov's law of homologous series at the fiftieth anniversary of its formulation. Econ Bot 29:372–379
Lang A (1914) Die experimentelle Vererbungslehre in der Zoologie seit 1900. Jena, Gustav Fischer
Lehmann W (1965) Die Bedeutung Valentin Haeckers für die Humangenetik. Zool Anz 174:74–91
Levit G, Hoßfeld U (2006) The forgotten "Old-Darwinian" synthesis: the theoretical system of Ludwig H. Plate (1862–1937). Internationale Zeitschrift für Geschichte und Ethik der Naturwissenschaft, Technik und Medizin (NTM), N.S. 14, pp 9–25
Levit G, Hoßfeld U (2011) Darwin without borders? Looking at "generalised Darwinism" through the prism of the "hourglass model". Theory Biosci 130:299–312
Levit GS, Hoßfeld U (2013) A bridge-builder: wolf-Ernst Reif and the Darwinisation of German palaeontology. Hist Biol Int J Paleobiology 25:297–306
Lotsy JP (1906, 1908) Vorlesungen über Deszendenztheorie. Gustav Fischer, Jena
Meisenheimer J (1923) Vererbungslehre. Gustav Fischer, Jena
Osche G (1965) Über latente Potenzen und ihre Rolle im Evolutionsgeschehen. Zool Anz 174:411–440
Plate L (1913) Vererbungslehre. Gustav Fischer, Jena
Reif W-E, Junker T, Hoßfeld U (2000) The synthetic theory of evolution: general problems and the German contribution to the synthesis. Theory Biosci 119:41–91
Rensch B (1965) Valentin Haeckers tierpsychologische Arbeiten. Zool Anz 74:445–450

Rensch B (1983) The abandonment of Lamarkian explanations: the case of climatic parellism of animal charcteristics. In: Grene M (ed) Dimensions of Darwinism. Cambridge University Press, Cambridge, pp 31–42

Rensch B (1998) Historical development of the present synthetic neo-Darwinism in Germany. In: Mayr E, Provine WB (eds) The evolutionary synthesis, 4th edn. Harvard University Press, Cambridge, MA/London, pp 284–303

Simunek M, Hoßfeld U, Thümmler F, Breidbach O (eds) (2011a) THE MENDELIAN DIOSKURI. Correspondence of Armin with Erich von Tschermak-Seysenegg, 1898–1951. Pavel Mervat, Praha

Simunek M, Hoßfeld U, Wissemann V (2011b) 'Rediscovery' revised – the co-operation of Erich and Armin von Tschermak-Seysenegg in the context of the 'rediscovery' of Mendel's Laws in 1899–1901. Plant Biol 13:835–841

Waddington CH (1942) The Epigenotpye. Endeavour 1:18–20

Waddington CH (1962) New pattern in genetics and development. Columbia University Press, New York/London

Weindling P (1989) Health, race and German politics between National Unification and Nazism 1870–1945. Cambridge University Press, Cambridge

Ziegler HE (1918) Die Vererbungslehre in der Biologie und in der Soziologie. Jena, Gustav Fischer

Richard Goldschmidt (1878–1958)

Yawen Zou

Contents

Abstract

Richard Goldschmidt was known for his work on sex determination, physiological genetics, and macroevolution. Goldschmidt's personal life was plagued by two world wars, and his academic life was full of controversies. He first took on a battle with the Mendelian geneticists over the nature of genes and later with neo-Darwinian scholars over evolutionary issues such as whether evolution was gradual. Goldschmidt's work on homeotic mutants and "hopeful monster" contributed to the understanding of macroevolution. Goldschmidt was considered a heretic by many of his peers, but he is currently revisited by evo-devo biologists because of his insistence on integrating development and physiology into genetics and evolution.

Keywords

Evo-devo · Physiological genetics · Sex determination · Macroevolution · Macromutation

Y. Zou (✉)
Center for Biology and Society, Arizona State University, Tempe, AZ, USA

The Chinese University of Hong Kong, Shenzhen, Shenzhen/Guangdong Province, P. R. China
e-mail: yzou20@asu.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_24

Life

Richard Benedict *Goldschmidt* was born in an upper middle-class Jewish family on April 12, 1878, in Frankfurt-am-Main, Germany. His relatives included bankers, professors, lawyers, and businessmen, and his father was a wealthy merchant, running a successful confectionery shop. Goldschmidt studied Latin, French, and mathematics for 9 years, Greek for 6 years, and natural history for 3 years at the local gymnasium. Goldschmidt's parents wanted him to become a doctor, so he entered the University of Heidelberg in 1896 as a medical student. He took foundation courses in medicine from the anatomist Karl Gegenbaur (1826–1903) and the zoologist Otto Bütschli (1848–1920), whom Goldschmidt admired very much and was the reason he had chosen the University of Heidelberg. Two years later, Goldschmidt passed the premed exam, but he did not want to be a doctor anymore, so he transferred to the University of Munich to study zoology in the lab of the famous zoologist Richard Hertwig (1850–1937) at the Zoological Institute at the University of Munich. A former student of Goldschmidt's at Hertwig's lab, Karl von Frisch (1886–1982), a Nobel Prize winner in physiology, was amazed by the amount of work that Goldschmidt produced and his efficiency (Frisch 1980). In Hertwig's lab, he started to conduct experiments related to the morphology of *Ascaris* and *Amphioxus*. In 1899, he returned to the University of Heidelberg to study in Otto Bütschli's lab, and in 1900, he published his first scientific paper on the development of the tapeworm *Echinococcus* (Goldschmidt 1900). Goldschmidt finished his doctoral dissertation entitled "Fertilization and Early Development of the Trematode Polystomum" in 1902. After graduation, Goldschmidt served his compulsive time in the German army for a year.

Richard Goldschmidt's portrait. (Reprinted by permission from Springer Nature: Nature Reviews Genetics, Richard Goldschmidt: hopeful monsters and other "heresies," Michael R. Dietrich, Copyright 2003)

In 1903, Goldschmidt returned to Hertwig's lab at the University of Munich, working as an assistant to oversee the experimental courses, remaining there until 1914. His students at the time included Hans Nachtsheim (1890–1979) and Jakob

Seiler (1886–1970), who later became prominent zoological geneticists in Germany. A year later, he married Else Kuhnlein with whom he had two children, Ruth and Hans, born in 1907 and 1908, respectively.

In 1909, Goldschmidt became a lecturer (Privatdozent) at the University of Munich. Later, he started his genetic experiments with two kinds of moths, the nun moth and the Gypsy moth, *Lymantria dispar*, which he would study for decades afterwards. Goldschmidt took a Mendelian approach and conducted cross experiments on the Gypsy moth to study a sex determination pattern, which received much attention. In 1911 he published a book entitled *Einführung in die Vererbungswissenschaft* (*Introduction to Genetics*) and became a proponent of the emerging field of genetics in Germany.

In 1914 Goldschmidt was appointed director of the Animal Genetics Department of the newly founded Kaiser Wilhelm Institute for Biology in Berlin-Dahlem. This position did not require him to teach, so he was free to focus on research. While the buildings of Kaiser Wilhelm Institute were under construction, on January 4, 1914, he headed to Japan to collect Asian species of the Gypsy moth through the Albert Kahn Travelling Fellowships, which were awarded to scholars in Germany, France, Britain, Japan, and the USA to promote cultural understanding (Richmond 2015). He used these Asian specimens to cross them with European species to study their sex determination and evolution. Goldschmidt enjoyed traveling around the world for the rest of his life, visiting places such as Korea, China, Russia, and Polynesia because of his interest in Oriental art and collecting art objects.

Because of the outbreak of the First World War, Goldschmidt was not able to return to Germany from Japan because of the embargo on German ships, and in 1914 he had to go to the USA. He first disembarked in California and stayed at the Zoology Department of the University of California, Berkeley. Believing that he might find a way back home more easily on the East Coast, he headed to New York. However, he was unsuccessful and struck in the USA longer than he expected. He was accepted as a visiting professor in Ross Harrison's lab at Yale, and his family later joined him. Because he worked on Lymantria, a pest species restricted by the USDA, he had to move to Harvard's Bussey Institution at Woods Hole to breed his moths. However, when the USA entered the war in May 1918, he was sent to an internment camp because of the anti-German sentiment. Eight months later, he was released and returned to Germany, where he resumed his old post in the Kaiser Wilhelm Institute for Biology in Berlin-Dahlem.

As a physiological geneticist, Goldschmidt investigated the problem of how genetic material controlled development. He published a small book consisting of a few essays on his work on *Lymantria dispar* in 1920 and a summary of other embryologists' research on the link between genetics and development. During the 1920s, Goldschmidt taught at the Imperial Tokyo University for 2 years and developed good relationships with Japanese scientists. Goldschmidt published *Lymantria* in 1933 in which he synthesized his work on sex determination and geographical variation in the Gypsy moth. Both books were well received, both in Germany and abroad, and established his solid status as a prominent geneticist. Due to the purge of Jews in Germany, Goldschmidt was forced to resign, and, once

again, he migrated to the USA in 1935. At that time, he was one of the most prominent geneticists in Germany, but not in the USA. Moving to the USA for refuge was a hard transition for him because he had to adapt to a different language, culture, and academic atmosphere. In 1936, he managed to secure a professorship at the University of California in Berkeley, but this new position required him to take on teaching obligations. For his first year in the USA, he was a lecturer for a large lower-level course in animal biology for 500 students, and only later he started teaching higher-level courses. From 1939 to 1940, he gave the famous Silliman Memorial Lectures on evolution at Yale University. In 1940 he published *The Material Basis of Evolution*, which was not received well by its critics, especially the neo-Darwinian scholars, who were attempting to forge a unified theory of evolution and interpreted Goldschmidt's theory as disrupting their consensus. Goldschmidt was viewed as an iconoclast for his evolutionary theory, although he was able to popularize the distinction between macroevolution and microevolution. Goldschmidt was elected a fellow of the National Academy of Sciences in 1947.

After Goldschmidt retired from UC Berkeley in 1948, he continued to publish a stream of theoretical papers to defend himself against neo-Darwinians. He delivered the Presidential address to the 9th International Congress of Genetics in Bellagio, Italy, in 1953. In contrast with the experimental and statistical characteristics of genetics at the time, Goldschmidt published *Theoretical Genetics* in 1955, in which he summarizes his experience in issues such as the nature of the genetic material and how the genetic material controls development (Goldschmidt 1955). Goldschmidt died on April 24, 1958, after a heart attack, in Berkeley.

Work

Sex Determination of the Gypsy Moth

When Goldschmidt first joined Hertwig's lab in 1898, he was exposed to the problem of sex determination, the main focus of the lab, but more from a morphological perspective. After the rediscovery of Mendel's work, scientists, including Hertwig's lab members, participated in a race on conducting Mendelian cross experiments where if one knew the alleles of the parental generation, one could predict the ratio of the phenotypes of the offspring generation by calculating the combination of alleles. However, when Goldschmidt crossed the Gypsy moth to observe the sex determination pattern, he was not able to use the Mendelian theory to explain his results, and this observation raised his skepticism about the Mendelian genes. He did not accept particulate "genes" as determiners of characters but had a developmental notion of genetics in which factors acted quantitatively (similar to enzymes) in development to determine characters.

When conducting cross experiments on the European species and Japanese species of the Gypsy moth, Goldschmidt observed that not all moths displayed definitive sexuality. Instead, some displayed both female and male traits. He used the terms "intersexes" and "intergrades" to refer to those moths that displayed traits

of the opposite sex in different developmental stages. For example, an intersex moth might first display male traits and, later in development, experience sex reversal by developing female traits. Goldschmidt observed that nongenetic factors could also influence sex determination, and by manipulating the temperature and other environmental factors, he was able to produce intersexes in an orderly way. Goldschmidt concluded that the sex of the moth was not a qualitative trait but rather a quantitative one because, first, a moth could show both female and male characters and, second, the sex of the moth was determined by the quantity of female and male determiners.

To account for the production of intersexes, Goldschmidt proposed what he referred to as "the balance theory of sex determination" (Goldschmidt 1923). He used the word "determiner" instead of "chromosome" or "gene" to name the hereditary material that determined sex. He departed from Mendelian genetics in arguing that sexuality in the Gypsy moth was determined by one female determiner (denoted as F) and one or two male determiners (M or MM). He further argued that the determiner controlling sexuality was quantitative and that some determiners were weaker and some determiners were stronger. Through development, the male determiner(s) and the female determiner had different strengths and competed with each other. For example, if an offspring had a strong F and a weak M (FsMw), it would be a female; however, if it had a weak F and two strong Ms. (FwMsMs), it would be a male. However, if it had a strong F and two weak Ms. (FsMwMw), it could be an intersex female and would experience sex reversal. Using this theory, Goldschmidt was able to produce the intersexes in the rate he predicted.

To link his balanced theory of intersexuality with physiological and developmental processes, Goldschmidt proposed what he called "the time law of intersexuality" (Goldschmidt 1923). Goldschmidt treated the sex determiner as a gene for the simplicity of analysis, but he thought that, in reality, sex determination involved many genes that controlled different developmental processes. He used a graph to explain the time law, in which he plotted the strength of the male and female determiners as two intersecting curves. For intersexes, when the strength of female determiners was higher than that of the male determiners, the intersexes were female and vice versa. The intersecting point of the two curves was the turning point, which explained the sex reversal point of the intersexes. Goldschmidt's critics received his balance theory better than the time law, criticizing the latter for its theoretical nature. Therefore, during the 1930s, the time law became mostly obsolete.

Physiological Genetics

Goldschmidt summarized and generalized almost two decades of his work on the Gypsy moth, as well as other scientists' investigations of how genes controlled development in his 1927 book, *Physiologische Theorie der Verebung* (*Physiological Genetics*), which was translated into English in 1938 (Goldschmidt 1927). Goldschmidt investigated variation, heredity, physiology, development, and evolution all in this one book and argued the importance of bringing development and physiology back to genetics. In fact, according to many nineteenth century

biologists' theories, such as August Weismann (1834–1914) and Hugo de Vries (1848–1935), heredity, development, and evolution were inseparable phenomena (Allen 1974). However, following the Mendelian and Morgan school, heredity could be approached without considering either development or evolution. While this approach had proved to be experimentally successful, Goldschmidt argued that understanding the transmission of genes was only one side of understanding heredity. The other side was "to understand how the gene, whatever it is, acts in controlling typical development to the adult form showing all the hereditary traits" (Goldschmidt 1938, p. 1).

Central to Goldschmidt's theory was the concept of *rate*, as he argued that genes determined traits by changing the rate of development, because the function of mutations was changing the rate of the developmental processes and the time of the onset of genes. He argued that genes acted like an "autocatalyst" to regulate developmental processes, as opposed to the predominant notion of transmission genetics, championed by the Morgan School, which proposed that genes, like beads-on-a-string, were discrete particles. Goldschmidt attacked this corpuscular notion of the gene and claimed that genes were not corpuscles in a linear sequence, but, instead, they had a hierarchical organization (Goldschmidt 1938; Dietrich 2003). He publicly announced that "the theory of the gene is dead" and predicted that in a few decades, scientists would no longer use the word "gene" (Allen 1974).

Goldschmidt's *Physiological Genetics* was not received well by the Morgan School, including Alfred Henry Sturtevant (1891–1970), Calvin Bridges (1889–1938), and Thomas Hunt Morgan (1866–1945), although the book referenced many of its members. Some of the disagreements referred to empirical issues. For example, Morgan did not accept Goldschmidt's notion of genes as enzymes (Morgan 1926). But, as argued by Marsha Richmond, there were "fundamental philosophical differences" between the views of the Morgan School and those of Goldschmidt in that Morgan held a corpuscular notion of genes and Goldschmidt insisted on the quantitative notion of genes (Richmond 2007). Although Goldschmidt did not indicate what exactly the gene was and in some of his writings he described it as similar to an autocatalyst or an enzyme, he clearly pointed out that genes were not linearly arranged particles in chromosomes as treated by the Morgan School. Instead, Goldschmidt believed that "the gene has not necessarily a definite limitation" (Allen 1974, p. 49). It is precisely because he believed that heredity and evolution could only be understood by studying the physiological and developmental processes involved, that he held a quantitative and dynamic rather than a qualitative and static view of the gene. This quantitative and dynamic approach attempted to "understand general phenomena in terms of genic action and developmental systems with all their consequences of interaction, embryonic regulation, and integration" (Goldschmidt 1954, p. 703).

Goldschmidt's work was better received in the UK (Richmond 2007), probably because many British biologists had introduced similar concepts as those of Goldschmidt's. For example, the idea that the speed of developmental processes can influence the phenotype was similar to the notions of "rate genes" and "allometric growth" used by Julian Huxley (1887–1975) and the "heterochrony" concept

used by Gavin de Beer (1899–1972). As embryologists, they all realized the importance of incorporating development in evolution. For example, Huxley, who met Goldschmidt in 1916 at Woods Hole and remained in contact with him until 1955, was receptive of Goldschmidt's approach of combining evolution, development, and genetics, and he viewed Goldschmidt's physiological genetics as almost as important as the transmission genetics advocated by the Morgan School (Huxley 1942; Richmond 2007). Huxley was very familiar with all the writings of Goldschmidt and cited his research on the Gypsy moth extensively in his 1940 book, *Evolution: The Modern Synthesis* (Huxley 1942).

Microevolution and Macroevolution

The most contentious aspect of Goldschmidt's research concerned evolution, especially with regard to speciation. As early as in 1933, in a meeting of the American Association for the Advancement of Science held in Chicago, Goldschmidt presented his insights on evolution (Goldschmidt 1933b). He argued that an often-neglected issue in the theory of evolution was understanding the nature of the developmental system of the organism which underwent evolutionary change (Goldschmidt 1933b). Goldschmidt never questioned evolution itself as a fact. Rather, what he had skepticism about was how evolution changed the genetic material. He believed that simply changing individual genes could not result in species change; rather, macroevolutionary change must be based on other processes.

A hotly debated topic among population geneticists then was whether geographical isolation was the starting point of speciation. Goldschmidt opposed this view and endorsed the concept of "preadaptation" to refer to those mutations that had no adaptive value in the original environment but became adaptive when introduced into a new environment. Goldschmidt argued that geographical isolation was not a starting point but a means that allowed preadaptive traits to become adaptive. Through his study of the Gypsy moth, he concluded that subspecies did not become separate species because of geographic isolation; they just adapted to different areas, and their differences were in degree not in quality. Instead, he maintained that new species formed in the same area along with old species, and profound developmental changes happened not gradually but suddenly (Goldschmidt 1933a). Therefore, he denied the existence of incipient species.

His research on how genetic material evolved culminated in the publication of *The Material Basis of Evolution* (1940). The book had two parts: one on microevolution, the evolution within a species based on accumulative small mutations, and the other one on macroevolution, the evolution at or above the level of species. Neo-Darwinians usually assumed that only small accumulative variations, or microevolution, led to speciation. In contrast, Goldschmidt argued that microevolution only explains evolution after a species is formed, like geographic variations which allows subspecies to adapt to different niches, but that there is a "bridgeless gap" between species, and that big changes must occur rather rapidly to bridge that gap. Goldschmidt used the term macromutation to describe a mutation that has a large

phenotypic effect in contrast with micromutation, which refers to a mutation that involve only one single gene locus. Macroevolutions resulted in profound changes in genetic systems, and thus the development systems, which he also referred to as reaction systems.

To explain how these changes happened, Goldschmidt proposed two types of macromutations, since they are the material basis of evolution. The first was "developmental mutation," which referred to the macromutation that happens in a developmentally important locus or in early embryonic processes. To find empirical evidence for macromutations, Goldschmidt studied the problem of homeosis, or the transformation of one organ into another organ, in *Drosophila* in the 1940s and 1950s (Dietrich 2000). For example, he studied a homeotic mutant podoptera, which can transform wings into leglike structures, and tetraltera, which can transform wings into halteres, a pair of dumbbell-shaped organs for keeping balance. The second type of macromutation was "chromosomal mutation" or "systematic mutation," which referred to large-scale systematic rearrangement of chromosomes as a mechanism of speciation. His proposition of chromosomal mutation is derived from Morgan's study of the linkages of alleles, the doubling of the chromosomes in plants, as well as Hermann Joseph Muller (1890–1967)'s study of the effects of X-ray radiation and Alfred Sturtevant (1891–1970)'s discovery of the position effect. He argued that several successive repatterning of the chromosomes needed to reach a threshold for a systematic mutation to happen (Goldschmidt 1940).

In Goldschmidt's view, the result of macromutations was mostly lethal to the organism, which he called "monsters," but in very rare circumstances, it was able to produce a new species with large changes, which he dubbed "hopeful monsters." Although homeosis served as evidence for "developmental mutations," he lacked both empirical evidence and genetic evidence of "systematic mutations." Thus, his theory was in a disadvantageous position compared with the neo-Darwinian approach to evolution that was being established by several scholars at that time. However, Goldschmidt argued that the lack of direct empirical evidence on macroevolution was not a problem to his theory: "It would be very cheap criticism, indeed, to say that nobody has ever witnessed a process. Neither has anyone witnessed the production of a new specimen of a higher taxonomic category by selection of micromutations" (Goldschmidt 1952, p. 97).

Legacy

For some biologists, Goldschmidt's greatest contributions lie in his work on sex determination in *Lymantria* and his contributions to physiological genetics (Caspari 1980). Although the term "gene" has not disappeared from scientific practice as preconized by Goldschmidt, in philosophy of biology, the nature of the gene has been hotly debated, and Goldschmidt's account has some merit in today's view of the gene. The discovery of introns, exons, and alternative splicing of genes suggests that gene expression is regulated by many factors, indicating that genes are not corpuscular but rather quantitative, as Goldschmidt thought (Griffiths and Karola

2006). Goldschmidt delved into a variety of experiments, but the most notable one was his study on the nervous system of nematodes. This work did not bear much fruit in Goldschmidt's life but influenced Sidney Brenner, who was awarded the Nobel Prize in 2002 for his work on the neural development of nematodes (Ankeny 2001).

According to Stephen Jay Gould (1941–2002), Goldschmidt was not received as a serious figure in evolutionary biology by his contemporaries despite Goldschmidt's prominence as a geneticist. In Gould's words, it almost became fashionable to ridicule Goldschmidt's work (Gould 1982). Some historians suggest that Goldschmidt was not afraid of creating controversy because he wanted to get attention in the USA (Dietrich 2011). When *The Material Basis of Evolution* was published, it met objections from some prominent neo-Darwinians who were forging the new evolutionary synthesis, and Goldschmidt threatened the unification of evolutionary biology. Goldschmidt jokingly said that he "had struck a hornet's nest" (Goldschmidt 1960, p. 324).

Mayr's book, *Systematics and the Origin of Species* (1942), referenced Goldschmidt many times, and the book shows that Mayr was very familiar with Goldschmidt's work on sex determination of the Gypsy moth and opposed him fiercely, although personally the two were friends. The fundamental difference between the two was that Mayr held that geographical isolation was a necessary condition for speciation, while Goldschmidt believed that chromosomal differences could also induce sexual isolation that leads to the formation of a new species, and geographical isolation was not necessary. Mayr criticized Goldschmidt for basing his notion of speciation largely on the data from *Lymantria*. In addition, Mayr criticized Goldschmidt for his lack of population thinking, arguing that the "hopeful monster," even when produced, could not survive in a population (Mayr 1997, p. 32).

Theodosius Dobzhansky (1900–1975) had heard Goldschmidt's Silliman Lectures and referenced Goldschmidt dozens of times in his own book *Genetics and the Origin of Species*. Dobzhansky wrote a review of *The Material Basis of Evolution* in which he argued that Goldschmidt's theory fell into catastrophism and criticized it for it rejecting evolution (Dobzhansky 1940). He thought that Goldschmidt's understanding of genetics was different from that of most other geneticists, and he referred to the "hopeful monster" metaphor as just "a belief in miracles." Goldschmidt defended himself, saying that he was not against evolution as suggested by Dobzhansky but instead embraced the major tenets of Darwinian theory (Goldschmidt 1952).

Similarly, compared with Goldschmidt's physiological genetics, Huxley was less receptive of his evolutionary theory. A firm defender of gradualism, Huxley thought that it was hard to draw a definitive line between species or subspecies, so he did not believe in "bridgeless gaps." George G. Simpson (1902–1984) believed that homeosis, as an example for macromutation, differed from micromutation only in degree, and a few such mutations were not enough to create a new species. It might require the accumulation of many homeotic mutants for speciation. Moreover, Goldschmidt needed to explain how macromutation affected a population instead of an organism (Simpson 1944).

Other evolutionary biologists received Goldschmidt's ideas more favorably. Conrad H. Waddington (1905–1975) remarked that "Goldschmidt's book is one of the most important recent contributions to the theory of evolution" because Waddington himself criticized neo-Darwinians for reducing the evolution of phenotypes to the evolution of discrete units of genes (Waddington 1941). Another example was Sewall Wright (1889–1988), one of the founders of the modern synthesis. In his review of *The Material Basis of Evolution*, Wright agreed with the existence of "developmental mutations" by pointing out that a single mutation could give rise to repetitive homologous structures (Wright 1941). Although a gradualist, who assumes that evolution results from slow and accumulative processes, Wright acknowledged that evolution could take place at different speeds. However, he rejected systematic mutations by arguing that chromosome rearrangement was mostly detrimental, and, if not lethal, it would have less of an effect than a single mutation or no effect at all. He also thought the hypothesis of "bridgeless gaps" between species was a too radical claim.

Although Goldschmidt's contribution was often looked down upon by many of his peers, Goldschmidt himself was optimistic about his work and expected it to be rediscovered by others in the future, just like Mendel's work had been. In his autobiography, published posthumously in 1960, he remarked that "I am confident that in 20 years my book, which is now ignored, will be given an honorable place in the history of evolutionary thought" (Goldschmidt 1960).

In terms of Goldschmidt's contributions to neo-Darwinism perceived after the 1980s, there are differing views. Although Ernst Mayr (1904–2005) claimed that Goldschmidt's ideas actually failed to influence major neo-Darwinians (Mayr 1997, p. 31), the historian Michael Dietrich has argued instead that Goldschmidt's heresy "helped define the neo-Darwinian orthodoxy" (Dietrich 1995). Similarly, Manfred Laubichler has argued that Goldschmidt was an important figure in the modern synthesis because he provided a target for neo-Darwinians and spurred many scientific debates (Laubichler 2009).

As Goldschmidt indeed predicted, his work was revived by Gould in the 1980s (Dietrich 1995). Gould learned about Goldschmidt's theory of macroevolution and speciation while taking Mayr's classes as a graduate student (Dietrich 1995). Gould declared that he had sympathy for Goldschmidt because the latter was ridiculed by people who had not read his work, and thus when *The Material Basis of Evolution* was republished in 1982, Gould wrote the Preface (Gould 1982). According to Dietrich, *The Material Basis of Evolution* has received more citations after the 1980s than at the time when Goldschmidt was still alive (Dietrich 2011).

Gould's theory of punctuated equilibrium assumed that species stayed in a relatively stable status with few genetic changes for a long time, and then significant changes happened rapidly; thus one species could split into two. This theory supports saltation, which assumes that evolution happens through sudden changes, instead of gradualism. Gould also opposed the neo-Darwinian view that the gradual accumulation of small variations could account for all of evolution. He was receptive of Goldschmidt's developmental mutations and yet critical of his systematic mutations; he defended Goldschmidt, maintaining that he did not represent the opposite

of Darwinism as neo-Darwinians tried to argue by pointing out that Goldschmidt acknowledged the importance of small genetic changes as well (Gould 1977).

Dietrich has argued that after Gould revisited Goldschmidt's work on evolution, Goldschmidt was viewed widely as a heretic by many historians and scientists, including himself (Dietrich 2011, 2003). Goldschmidt popularized the distinction between microevolution and macroevolution, and his argument fell into the larger debate between saltationists and gradualists. This debate ended with the dominance of the neo-Darwinian scholars supporting gradualism while Goldschmidt was still alive. However, the debate between saltation and gradualism and between micro-evolution and macroevolution has continued, through scholars like Gould revisiting Goldschmidt. For example, some evolutionary biologists proposed the concept of "neo-Goldschmidtian saltation" and argue for gradualism in the evolution of genetic sequence and punctuated equilibrium in morphology (Bateman and DiMichele 2002).

Goldschmidt's contributions to evolutionary theory have received more attention after the emergence of evo-devo, not just because of Gould but also because of advancements in developmental genetics. Laubichler has argued that, ironically, although development is often overlooked in the modern synthesis, developmental genetics has provided new results regarding the origin of variations (Laubichler 2009). The discovery of *Hox* genes in 1978, which are the genes underlying homeosis, is an example of changes in a few loci that could make big phenotypic alterations (Lewis 1978). To many, evo-devo began with the discovery of *Hox* genes and, therefore, since the 1980s, modern biologists and historians have frequently revisited Goldschmidt's theory about macroevolution and hopeful monsters (Hall 2003).

Some biologists are even more hopeful about the "hopeful monsters." Günter Theißen argues that they can be useful in explaining the origin of innovations that cannot be explained by gradual evolutionary processes. He describes some scientific evidence of quantitative trait loci analyses that show that a few genes can have large effect, among which the most prominent example are the *Hox* genes (Theißen 2006). Olivier Rieppel argues that turtles might be descendants of surviving hopeful monsters because turtles have a very unusual shell structure that does not resemble any of their phylogenetic relatives. The innovation of turtle shell might be due to a macromutation instead of gradual evolution (Rieppel 2001). Eva Jablonka and Marion J. Lamb endorse hopeful monsters from a different perspective, maintaining that environmental changes might produce epigenetic inheritance that affects many genes, giving rise to novelties. They argue that environmental factors can produce epigenetic changes at a population level and dismiss the problem of "lonely" hopeful monsters (Jablonka and Lamb 1999, p. 224).

Cross-References

▶ Conrad Hal Waddington (1905–1975)
▶ Gavin de Beer (1899–1972)

► Heterochrony
► Macroevolution
► Mechanisms of Pattern Formation, Morphogenesis, and Evolution
► Micro-Evo-Devo
► Stephen Jay Gould (1941–2002)

References

Allen GE (1974) Opposition to the Mendelian-chromosome theory: the physiological and developmental genetics of Richard Goldschmidt. J Hist Biol 7:49–92
Ankeny RA (2001) The natural history of Caenorhabditis elegans research. Nat Rev Genet 2(6):474
Bateman RM, DiMichele WA (2002) Generating and filtering major phenotypic novelties: neo-Goldschmidtian saltation revisited. In: Bateman RM (ed) Developmental Genetics and Plant Evolution. Taylor & Francis, New York, pp 109–159
Caspari EW (1980) An evaluation of Goldschmidt's work after twenty years. In: Piternick LK (ed) Richard Goldschmidt: controversial geneticist and creative biologist. University of Washington, Seattle, pp 19–23
Dietrich MR (1995) Richard Goldschmidt's "heresies" and the evolutionary synthesis. J Hist Biol 28(3):431–461
Dietrich MR (2000) From hopeful monsters to homeotic effects: Richard Goldschmidt's integration of development, evolution, and genetics. Am Zool 40(5):738–747
Dietrich MR (2003) Richard Goldschmidt: hopeful monsters and other 'heresies. Nat Rev Genet 4(1):68–74
Dietrich MR (2011) Reinventing Richard Goldschmidt: reputation, memory, and biography. J Hist Biol 44(4):693–712
Dobzhansky T (1940) Catastrophism versus evolutionism. Science 97:356–358
Frisch KV (1980) Introduction. In: Piternick LK (ed) Richard Goldschmidt: controversial geneticist and creative biologist. University of Washington, Seattle, pp 19–23
Goldschmidt RB (1900) Zur Entwicklungsgeschichte der Echinococcuskopfchen [The development of Echinococcus]. Zool Jahrb Anat 8:467–94
Goldschmidt RB (1923) The mechanism and physiology of sex determination. Methuen & Co, London
Goldschmidt RB (1927) Physiologische theorie der vererbung [physiological genetics]. J. Springer, Berlin
Goldschmidt RB (1933a) Lymantria. Nijhoff, Leiden
Goldschmidt RB (1933b) Some aspects of evolution. Science 78(2033):539–547
Goldschmidt RB (1938) Physiological genetics. McGraw Hill Book Company, New York
Goldschmidt RB (1940) The material basis of evolution. Yale University Press, New Haven
Goldschmidt RB (1952) Evolution, as viewed by one geneticist. Am Sci 40:84–135
Goldschmidt RB (1954) Different philosophies of genetics. Science 119(3099):703–710
Goldschmidt RB (1955) Theoretical genetics. University of California Press, Oakland
Goldschmidt RB (1960) In and out of the ivory tower: the autobiography of Richard B. Goldschmidt. University of Washington Press, Seattle
Gould SJ (1977) The return of hopeful monsters. Nat Hist 86(6):22–30
Gould SJ (1982) The uses of heresy: an introduction to Richard Goldschmidt's *The material basis of evolution*. The material basis of evolution. Yale University Press, New Haven, Connecticut
Griffiths PE, Karola S (2006) Genes in the postgenomic era. Theor Med Bioeth 27(6):499–521
Hall BK (2003) Evo-devo: evolutionary developmental mechanisms. Int J Dev Biol 47(7/8):491–496
Huxley J (1942) Evolution: the modern synthesis. Allen & Unwin, London

Jablonka E, Lamb MJ (1999) Epigenetic inheritance and evolution: the Lamarckian dimension. Oxford University Press, London
Laubichler MD (2009) Form and function in evo devo: historical and conceptual reflections. In: Maienschein J, Laublicher M (eds) Form and function in developmental evolution. Cambridge University Press, Cambridge, pp 10–46
Lewis EB (1978) A gene complex controlling segmentation in Drosophila. Nature 276(5688):565–570
Mayr E (1942) Systematics and the origin of species, from the viewpoint of a zoologist. Harvard University Press, Cambridge
Mayr E (1997) Goldschmidt and the evolutionary synthesis: a response. J Hist Biol 30(1):31–33
Morgan TH (1926) Genetics and the physiology of development. Am Nat 60:489–515
Richmond ML (2007) The cell as the basis for heredity, development, and evolution: Richard Goldschmidt's program of physiological genetics. In: Maienschein J, Laublicher M (eds) From embryology to evo-devo: a history of developmental evolution. The MIT Press, Cambridge, pp 169–211
Richmond ML (2015) A scientist during wartime: Richard Goldschmidt's internment in the USA during the first world war. Endeavour 39(1):52–62
Rieppel O (2001) Turtles as hopeful monsters. BioEssays 23:987–991
Simpson GG (1944) Tempo and mode in evolution. Columbia University Press, New York
Theißen G (2006) The proper place of hopeful monsters in evolutionary biology. Theory Biosci 124(3–4):349–369
Waddington CH (1941) Evolution of developmental systems. Nature 147(3717):108–110
Wright S (1941) The material basis of evolution. Sci Mon 53:165–170

Sergey S. Chetverikov (1880–1959)

Giulia Rispoli

Contents

Abstract

Sergey Sergheevich Chetverikov (1880–1959) was one of the most influential scientists of the Russian school of evolutionary genetics. Zoologist by training, Chetverikov contributed to the general development of biological sciences of the twentieth century. He is known for having promoted a synthesis of Mendelian genetics and Darwin's evolutionary theory. In this framework his work is associated, among others, to those of J. B. S. Haldane, Ronald Fisher, and Sewall Wright in Britain and the United States. Throughout his life, Chetverikov mainly focused on the experimental study of the hereditary properties of animal populations, studying especially butterflies and *Lepidoptera*. His major work, titled *On Certain Aspects of the Evolutionary Process from the Viewpoint of Modern Genetics* (1926), paved the way to the establishment of population genetics and the synthetic theory of evolution. Although his contributions remained unappreciated until after his death, as Chetverikov was one of the victims of Trofim Lysenko's crusade against genetics; he is now recognized as a pioneer in population genetics, which, in turn, is likely to play a crucial role in the development of evo-devo.

G. Rispoli (✉)
Max Planck Institute for the History of Science (MPIWG), Berlin, Germany
e-mail: grispoli@mpiwg-berlin.mpg.de; giuliarispoli@gmail.com

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_49

Keywords

Systematics · Entomology · Population Genetics · Evo-Devo

Life

Born on April 24, 1880, in Moscow, Sergey Chetverikov grew up in a well-educated merchant family of manufacturers and received his primary education at home. His father was an entrepreneur and a member of the Progressive Party, while his mother came from a rich family of business owners. Chetverikov had two brothers and one sister. One of his brothers, Nikolay, became a mathematician and helped Sergey enhance his understanding of biometrics and statistics in the later years of Sergey's scientific career. After his elementary education, Chetverikov enrolled in a private technical secondary school, from which he graduated in 1897 at the top of his class. Chetverikov's father wanted him to study engineering and economics, but Sergey soon realized that he had a great passion for the natural sciences and aspired to become a professor of zoology. After spending some time in Kiev, he eventually entered the Department of Physics and Mathematics at Moscow University, against his father's will, in 1900. There, he attended zoology classes led by Professor Nikolay Yu Zograf (1854–1919), studying mainly butterflies and moths (*Lepidoptera*). Two years later, he would publish his first scientific article illustrating butterfly population waves.

While studying in Moscow, Chetverikov became involved with a commission for the study of the fauna in the Moscow Province through the Moscow Society of Naturalists, Anthropologists and Ethnographers. After being arrested in 1901 for participating in student protests, he published in 1902 a report of his researches for the Society's commission, which was headed by the leading zoologist and conservationist Grigory A. Kozhevnikov. That same year, Chetverikov took part in a huge zoological expedition to the West Sayan Mountains, which gave him the opportunity to study and collect many butterflies. In the following year, he published two more articles in which he named new species and subspecies he had discovered during that mission.

In 1905, Chetverikov joined a second expedition to Zaisan Lake and the Tarbagatay Ridge with the biogeographer Petr P. Sushkin (1868–1928) and his wife Anna Ivanovna, who later became Chetverikov's wife. During that expedition, Chetverikov mainly worked on analyzing butterfly material, preparing further articles on *Lepidoptera*. Later that same year, he was arrested again for his involvement in student protests at Moscow State University and spent 2 months in prison. Chetverikov took advantage of this time to focus on university class examinations that he later passed successfully.

When Chetverikov graduated in natural sciences in 1906, he was already a distinguished researcher who had published several papers in the fields of entomology and general biology, and he was also an active member of prestigious scientific

societies. A key work that came out during this period was *Volny Zhizni* (Waves of Life) in which Chetverikov described fluctuations in the size of populations in nature and how these fluctuations play a role in genetic drift.

In 1911, he successfully defended his dissertation in zoology at the Department of Comparative Anatomy at Moscow University with highest honors. His thesis, focusing on the anatomy and the morphology of the freshwater isopod crustacean *Asellus aquaticus* analyzed and described the adaptive functions performed by the external skeleton of this organism. The work, which featured a Darwinian approach, was published in German in 1910 in the *Bulletin of the Moscow Society of Naturalists*. By 1914, Chetverikov had become a member of this Society, as well as the founder of the Moscow Entomological Society.

By the time Chetverikov graduated in zoology, he was already committed to teach entomology at Women's Higher Educational Institution. He held this position until 1917, while in 1919 he was employed at the Moscow University as a docent of experimental zoology dealing with general issues of biology, evolutionary theory, and genetics. At the same time, he kept studying and collecting species of butterflies from different parts of the country. Interestingly, the department where he was appointed was headed by his distant relative Nikolay K. Kol'tsov (1872–1940), the eminent biologist who had founded the Institute of Experimental Biology in Moscow in 1917. Chetverikov had previously worked under Kol'tsov in 1909 joining the Zoology Department of the Beztuzhev Courses (a higher education institution for women) as a laboratory assistant in entomology.

At the Institute of Experimental Biology, which he joined in 1921, Chetverikov ended up becoming the head of the Department of Genetics where many great scientists received their training being influenced by his approach and methods. Graduate and undergraduate students with whom Chetverikov worked at Kol'tsov's Institute included, among others, Nikolai V. Timofeeff-Ressovsky (1900–1981), Dmitry D. Romashov (1899–1963), Boris L. Astaurov (1904–1974), Elizaveta I. Balkashina (1899–1981), Elena A. Fiedler (1898–1973), Sergey R. Tsarapkin (1892–1960), and Nikolay K. Beliaev (1899–1937).

Labeled as a Mendelist-Morganist, in 1929 Chetverikov fell victim of Lysenko's crusade against genetics and lost his academic position. He was arrested and imprisoned in Moscow and a few months later was deported to a remote corner of the Urals. After 3 years of exile in the city of Sverdlovsk, near Yekaterinburg, he became ill and almost blind (Medvedev 1979). Chetverikov never became aware of the exact reasons for his arrest. One of the causes, however, may have been a conspiracy that was planned against him involving the suicide of Paul Kammerer (1880–1926), an Austrian biologist who had set up his laboratory in Moscow in the 1920s to demonstrate the inheritance of acquired characteristics in salamanders. Kammerer had killed himself after his scientific results were invalidated by Hans Przibram in 1926. After Kammerer's death, his obituary in the newspaper *Izvestija* mentioned the existence of a postcard signed by Chetverikov in which he was congratulating the Academy on Kammerer's suicide. Chetverikov declared that he had never written such a message and said that it was probably signed by the same person who informed the authorities of its existence (Adams 1982, 2008).

During his exile between 1929 and 1932, Chetverikov was able to organize several excursions around Sverdlovsk and enrich his collection of butterflies. Even though he had no permission to leave the city, he managed to discover more than 60 new species. In addition, he started working on theoretical aspects of biometrics with the help of his brother Nikolay, who inspired his project of building an objective taxonomy of organisms. After a 3-year period of exile, Chetverikov was forced to leave Sverdlovsk in the summer of 1932, but he was not yet allowed to return to Moscow. His freedom was limited for another 3 years, as he lost his right of residence in Moscow, Leningrad, and in the Urals. He was neither allowed to go to other regions such as Ukraine, Byelorussia, or in several Central Asian republics. Eventually, he got the chance to move to Vladimir, a small town not far from Moscow.

Between 1935 and 1948, Chetverikov became the head of the Department of Genetics and Selection at Gorky University (now Nizhny Novgorod State University), teaching both genetics and entomology. This period is mainly characterized by his research on silkworms, whose cultivation was demanded by the Ministry of Agriculture because of silk's importance in the manufacture of parachutes. During World War II, Chetverikov was appointed dean of the faculty and later on received several military awards. In 1945, he earned a PhD in biological sciences without needing to defend a dissertation, solely on the basis of his reputation as an accomplished scientist. In August 1948, after new reforms involving the teaching of biology were established by Lysenko, Chetverikov was forced to leave the university but kept studying butterflies despite a heart attack and progressive eyesight degeneration (Medvedev 1979). In the late 1950s, with the resurrection of Soviet biology, Chetverikov was elected honorary member of the Entomological Society. He was awarded the Darwin Medal in 1959 by the German Academy of Sciences Leopoldina, an award given to those scientists who have contributed to the development of Darwin's evolutionary theory. Chetverikov died of a hemorrhagic stroke on July 2, 1959, in Gorky.

Chetverikov's Ideas About Genetics and Evolution

Although historiographical accounts of the development of early twentieth-century evolutionary biology have at times been restricted to scientists working in the United States and Western Europe, scientific efforts that led to a synthesis of genetics and evolutionary theory had been enjoying widespread enthusiasm among Russian biologists since the turn of the twentieth century. Chetverikov was one of those who greatly contributed to these efforts. Unfortunately, Lysenko's suppression of genetics in the 1930s made it difficult for most of these works to cross the Iron Curtain and reach the broader international community. The path of the Russian branch of evolutionary biology was in fact very short. It was destroyed during the Stalinist purges between 1936 and 1938 when Lysenko came to power. In 1938 the Soviet agronomist was appointed the President of the All-Union Academy for Agricultural Sciences of the Soviet Union (VASKhNIL), a position previously

held by the most renowned victim of Lysenko's unbridled power: the leading botanist and geneticist Nikolay Vavilov (1887–1943). From this period on, new studies and researches in genetics were considerably undermined and just few scholars – Ivan I. Schmalhausen (1884–1963) among them – raised their voice against Lysenkoism.

Gifted with foresight, Chetverikov began to study the nature of evolutionary processes when experimental and theoretical population genetics were not yet developed. Seeking to connect three main research fields – genetics, evolutionary theory, and entomology – he aimed to fill the gap between Darwin's theory and genetics by laying down the principles of evolutionary genetics. Chetverikov had learned about Darwin's theory from his geography teacher Vladimir P. Zykov (also a professor at Moscow State University) when he was young, before 1895. After that, he became passionate about butterflies and received some training in systematics and entomology. Later, when he entered the Moscow Natural History group under the supervision of Professor Nikolay Yu. Zograf (1852–1920), Chetverikov decided to focus on invertebrates, specifically on *Lepidoptera*. Thanks to his 1902 studies on how to collect butterflies, conducted as part of his research conducted for the Commission for the study of the fauna in Moscow Province, he discovered that naturalists had erroneously classified the same biological species as three different ones. In this way, Chetverikov gained the reputation of being one of the leading experts of butterfly systematics, and his collection was praised as the most comprehensive of the country. However, Chetverikov's interests reached far beyond systematics. Throughout his career, he sought to investigate processes of dispersion, isolation, and spreading of biological populations over time. For instance, the aforementioned essay *Waves of Life* was based on long observations of the irregular flows characterizing *Lepidoptera* population trends. This study was one of the first to bring into focus the implications of fluctuating population size in nature, and was published in 1905, at a time when population genetics was far from being a defined area of scientific inquiry (Adams 2008). In 1915, Chetverikov published a crucial paper on "The Fundamental Factor of Insect Evolution" (translated in English in 1920), which was presented at the meeting of the Moscow Entomological Society the year before. In this paper, he explained that the chitinous external skeleton of insects is the reason why they have evolved toward smaller size compared to vertebrates, which have evolved toward larger forms. Moreover, he believed that the smallness of insects had been the driving force for their evolution. Such a small size allowed these organisms to disseminate and proliferate in a rich variety of forms.

Chetverikov's involvement in genetic research reached its peak between 1919 and 1929 when he joined the Kol'tsov institute and developed an original theory of the evolution of natural populations from the point of view of a synthesis between genetics and evolution. During this decade, he lectured in both general entomology and fundamental theoretical systematics, a new course he designed.

From 1921 on, Chetverikov's work at the Institute of Experimental Biology in Moscow focused on the genetics of wild populations of *Drosophila*. Under Kol'tsov's direction, the geneticist Dmitrij Romashov (1899–1963) had already attempted to produce mutations in *Drosophila* through the use of X-rays, but the experiments failed

and Chetverikov was invited to take over the research. Notwithstanding his lack of knowledge in genetics (as Chetverikov was specifically a zoologist), he was offered to join Kol'tsov's team based on his deep knowledge about insects.

In August 1922, Hermann J. Muller (1890–1967), attracted to Communist Russia, visited the institute and the department of genetics bringing with him cultures of *Drosophila* mutants that he would leave in Kol'tsov's laboratory (Dobzhansky 1982; Gaissinovitch 1980). These fruit flies were very famous in the United States, where Thomas Morgan was establishing a new school focused on the study of causes and conditions of their genetic mutations. At the Kol'tsov's Institute between 1922 and 1924, Chetverikov's group worked following Morgan's genetics, combining practical and experimental research with theoretical and informal reading seminars (sometimes taking place at Romashov's house), during which all the participants discussed Western scientific articles. In that context, the group did not only improve its knowledge in genetics but also produced successful experimental results based on the theoretical knowledge gained. This laboratory was carrying out the major Soviet work on *Drosophila* genetics and was by then headed by Chetverikov (Gaissinovitch 1980).

In 1925, Chetverikov initiated his course of genetics and set up new seminars for geneticists and "drosophilists." In 1926, he published his most famous paper "On Certain Aspects of the Evolutionary Process from the Viewpoint of Modern Genetics." The article appeared in 1927 in the *Journal of Experimental Biology*, edited by Kol'tsov. In this work, considered to be one of the first attempts to sketch out an evolutionary synthesis, Chetverikov sought to highlight the connection between some aspects of the evolutionary process and the new concepts in genetics, therefore dealing with evolution and genetics as two interwoven fields (Chetverikov 1961). Moreover, he focused on genetic data as the basis for variability, as well as the key element to understanding how evolution works. In this respect, he believed that mutations could be regarded as a source for new variability in natural populations. Far from being a special effect of domestication or laboratory experiments, mutations constitute – according to Chetverikov "the very raw material of evolution under natural conditions" (Levit 2015, p. 3). Chetverikov sought to connect Darwin's theory with the genetic laws of heredity by studying how mutations – "genovariations" in his own words – occur in nature (Gaissinovitch 1980). This attempt was particularly innovative at that time, when the relevance of genetics to evolution was not clearly acknowledged. Assuming that genetics and evolution were two distinct fields, several scientists were mainly working on producing mutations in their laboratory under a given set of fixed conditions. Those mutations did not manifest in natural populations, which, on the contrary, appeared rather homogeneous in their phenotypic traits. Chetverikov pointed out that biologists were not able to produce at will persisting, durable, and beneficial variations in artificial environments. He showed that mutations produced in the laboratory – ad hoc – did not survive in the descendants, disappeared very quickly, or became almost harmful. In this context, Chetverikov made also clear that many mutations, which occur randomly in populations, are not selectable, meaning that they do not have any biological or evolutionary role and can be considered almost neutral.

Chetverikov's studies on inheritance and mutations had deep consequences on the Soviet debate on the origin of variations, on which Lamarckists and Morganists had two opposite views. Lamarckists believed that characteristics acquired in one generation by adaptation to the environment could be inherited by offspring. Morganists, on the contrary, held that variations were the result of an internal developmental process determined by genetic mutations that was independent of external circumstances. Chetverikov attempted to reconcile both perspectives within a new scientific framework (evolutionary and population genetics) of which he is now regarded one of the founding fathers (Adams 1968). Results from his research on the role of mutations in evolution were presented in September 1927 during the Fifth International Genetic Congress in Berlin and published a year later.

Within the framework of population genetics, Chetverikov addressed some issues on biometrics in relation to populations of fruit flies. He showed that in populations with no migration and in absence of selection, emerging variations would be maintained in a large population at a constant frequency by the process of free interbreeding (Adams 2008). This issue was connected to the importance of population size, since mutations in large populations would have a better chance to be maintained, spreading throughout the whole population. Contrary to Jenkin Flaming's ideas that variations were swamped by free-crossing, Chetverikov was following the lines put forward by G. H. Hardy's law of equilibrium under free-crossing and Karl Pearson's law of stabilizing crossing formulated in 1908 and 1904 (Adams 2008). Most importantly, he showed that in a large size population there is a better chance that new phenotypic expressions of mutations would appear. He also pointed out that advantageous mutants will eventually be distributed among all the members of the species. In drawing these conclusions, he made use of his knowledge of *Lepidoptera* systematics and took inspiration from the table elaborated by the mathematician Henry Tertius James Norton (1886–1937) and included as an appendix in Reginald Punnett's *Mimicry in Butterflies* (1915). This table helped him depict some relevant deductions on the role of natural selection, which seems to favor recessive mutations instead of dominant ones that disappear quickly. Chetverikov was very interested in understanding the way in which natural selection works, and believed that, far from being a narrow mechanism, it played a creative role in the evolutionary process. He also assumed that most characters do not emerge in the organism for specific adaptive reasons; rather, systematics shows us that most mutations are biologically neutral. This led Chetverikov to assert that the role of natural selection has at times been overrated when it comes to the explanation of evolutionary processes. It seems that isolation, instead of selection, is the cause of speciation (Chetverikov 1961). The fact that mutations tend to spread in large size populations through free-crossing led Chetverikov to conclude that when groups of the same population are isolated from each other for some time, this separation can lead to differentiation (Adams 2008, 1968). Isolation, under the conditions of a process of continuous accumulation of mutations, is indeed a process that might cause a genetic rift within a species. With time, in fact, isolated colonies of one species will manifest differences in individual morphological traits. Chetverikov argued that in most cases we speak about geographical races for this reason.

However, in the absence of isolation, the species will never split into two. In Chetverikov's view, a new speciation could occur only through isolation. As he declared in his famous 1926 paper, isolation, not selection, is the actual source of the origin of species. Nonetheless, according to Chetverikov, natural selection does have an important role in evolution because, by selecting specific traits of a given genotype, it selects also the whole genotypic *milieu* along with its correlated variability.

Chetverikov's Importance in the History of Evolutionary Biology

Chetverikov was one of the first biologists who attempted to conjugate two important biological processes: adaptation and speciation. Moreover, he provided a novel picture of the relationship between genetic differentiation and evolution.

If Fisher, Haldane, and Wright are considered the founders of the Modern Evolutionary Synthesis in the West, Chetverikov complements this development on the side of the Soviet Union. Although their scientific results converged on several points, the two schools followed distinct paths: Chetverikov's studies stemmed from systematics and zoology, while Fisher, Wright, and Haldane began with genetics and animal breeding, supporting their study with very sophisticated mathematics (Adams 2008, 1982). As Dobzhansky pointed out, despite their different origins and educational backgrounds, it seems that "by the 1920s, the mutation-natural selection theory was definitely in the air, and four persons independently seized it" (Dobzhansky 1982, p. 242).

Chetverikov's research had a great influence, especially on the work of Soviet biologists such as Nikolay V. Timofeev-Ressovsky (1900–1982), who was one of his students, Boris L. Astaurov (1904–1974), and Nikolay P. Dubinin (1907–1998). It also inspired the work of the famous Ukrainian geneticist and naturalized American Theodosious G. Dobzhansky (1900–1975), who was among the founders of the school of evolutionary genetics in the United States. Dobzhansky, who produced in 1959 a short translation of a 1926 Chetverikov paper, emphasized the historical importance of Chetverikov's research for the development of modern genetics and evolutionary theory as a field of investigation that was primarily rooted in Darwin's teaching. In his view, Chetverikov's work on *Drosophila melanogaster*, a species of drosophila that was less difficult to cultivate and handle, offered one of the first demonstrations of genetic variation in natural populations. More specifically, Chetverikov initiated the study of a phenomenon that would later be known as "genetic load" (Dobzhansky 1982). Dobzhansky visited Chetverikov's laboratory in 1922 where he first learned about genetic modifications on drosophila, then he moved to New York in 1927 where he started working with Morgan at Columbia University, bringing with him the stock of fruit flies he originally obtained at the Russian laboratory.

Chetverikov's 1926 paper, "On Certain Aspects of the Evolutionary Process from the Viewpoint of Modern Genetics," can be considered in retrospect as one of the cornerstones of the Modern Synthesis. His work proved to be pioneering, as he

elucidated issues concerning the origin of species seen as a problem of population isolation that would be later examined in detail by Ernst Mayr. In this way, he contributed not only to the foundation of population genetics by investigating how variations distribute and how differentiation occurs in biological populations, but also to establishing a confluence between population genetics and evolutionary theory. As Mayr later noted, the naturalists, Chetverikov among them, might claim credit with equal justification for having initiated population genetics (Mayr 1982).

As noted by Adams (2008), Chetverikov's 1926 paper discussing the genetic dimension of interspecific variation, the role of isolation in speciation, and the implications of population size set the agenda for later works in evolutionary genetics, especially those authored by Timofeev-Ressovsky, Dobzhansky, Huxley, and Mayr between 1935 and 1950. Most importantly, the idea that all phenotypic traits must not appear for specific purposes anticipated Stephen Jay Gould and Richard Lewontin's critique of the adaptationist program, epitomized by their 1979 joint article "The Spandrels of San Marco and the Panglossian Paradigm." Chetverikov claimed in fact that there was a wealth of examples of nonadaptive characters in the biological organisms that can be considered neutral (1961). Moreover, his theory of the neutrality of many genes which are not subject to selection foreshadowed the theory of neutral evolution advanced by Motoo Kimura in 1968. Along these lines, Chetverikov dedicated a part of his 1926 article to the analysis of the "genotypic milieu" that he described as a system of interactions of all the genes. This complex system, the genotype of an organism, is responsible for the expression of all phenotypic traits which are indeed the result of the interconnected activity occurring among genes within the genotypic environment. In this way, he insightfully introduced a systemic perspective in genetics by stressing that single genes do not have any role taken in isolation; rather, they depend on each other, forming a whole collective pattern (Chetverikov, 1961). Interestingly, Chetverikov's idea of gene interaction summarized in the theory of "genotypic milieu" anticipated in some aspects the phenomenon that would be later known as *epistasis*. First introduced by William Bateson in 1909, contemporary studies on epistasis refer to the effect of gene interaction on phenotypic expressions and how it can influence the evolvability of phenotypic traits.

It seems that the interaction between development and evolution, which is notably at the core of evo-devo, finds an interesting premise in Chetverikov's attempts to conjugate genetics with evolutionary pathways at the level of the organisms and species. Importantly, Chetverikov's work was also connected to that of scholars who were actively involved in the experimental study of development and growth. For example, in 1922 when Chetverikov was working at the Institute of Experimental Biology, the embryologist Dmitry Petrovich Filatov (1876–1943) joined the Kol'tsov team, setting up a laboratory on developmental mechanisms (Mikhailov 2012). Filatov was interested in the analysis of tissue interrelations, especially focusing on limbs, and later he turned his attention to morphogenetic interactions that have a regulatory influence on the formation of the adult organism's morphology. In this connection, he sought to integrate "the processes of ontogenetic and evolutionary organogenesis" (Mikhailov 2012, p. 16).

At the Institute of Experimental Biology, Chetverikov tried to pursue an integrated program in genetics and evolution, promoting the convergence of previously separate research lines, such as mutation studies in drosophila, population genetics, comparative morphology, and evolutionary biology. His novel approach inspired further researches undertaken by his former students in Russia as well as abroad. Nikolaj V. Timofeev-Ressovskij (1900–1981), for instance, who worked at Kol'tsov Institute under Chetverikov's supervision, moved to Germany around 1930 to conduct fly genetics research on genetic constraints and variation that would play an important role in the history of evo-devo (Olsson et al. 2010).

Chetverikov's contributions surely deserve to be placed at the forefront of genetics and evolutionary biology. By investigating mechanisms of gene interaction as well as how genetic mutations spread in natural populations, he contributed to the establishment of developmental genetics, which in turn would play a crucial role in setting up the current agenda of evo-devo. The dissemination of Chetverikov's work was unfortunately blocked for almost three decades, and this probably explains why it is poorly known in non-Soviet countries. However, rediscovering the wealth of his scientific production can help us illuminate the path that led to his synthesis between genetics and evolution, contributing to our understanding of the twentieth-century history of population genetics and evolutionary biology more generally.

Cross-References

- ▶ Epistasis
- ▶ Evolvability
- ▶ Ivan I. Schmalhausen (1884–1963)
- ▶ Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics

Selected Bibliography

Chetverikov SS (1905) Volny zhizni. Dnevnik zool. Ob-a liubitelei estestvoznaniia, antropologii i etnografii, T. 3, N. 6, pp 106–111

Chetverikov SS (1915) Osnovnoi faktor evoliutsii nasekomykh. In: Izvestiia Moskovskogo entomologicheskogo obshchestva, 1 pp 14–24, published in English as The Fundamental Factor of Insect Evolution. Smithsonian Report for 1918, no 2566 (Washington, 1920), pp 441–449

Chetverikov SS (1961) On Certain Aspects of the Evolutionary Process from the standpoint of modern genetics. Translation by M. Barker and I. M. Lerner Proc. Amer. Phil. Soc. 105:167–195. O nekotorykh momentakh evoliutsionnogo protsessa s tochki zreniia sovremennoi genetiki (1968) Klassikisovetskoigeneliki. Leningrad

References

Adams MB (1968) The founding of population genetics: contributions of the Chetverikov school 1924–1934. J Hist Biol 1(1):23–39. Springer

Adams MB (1982) Serghey Chetverikov, the Koltsov institute, and the evolutionary synthesis. In: Mayr E, Provine B (eds) The evolutionary synthesis: perspectives on the unification of biology. Harvard University Press, Cambridge, MA, pp 242–278

Adams MB (2008) Chetverikov, Sergei Sergeevich, Complete Dictionary of Scientific Biography. http://www.encyclopedia.com/doc/1G2-2830905054.html

Dobzhansky T (1982) The birth of the genetic theory of evolution in the soviet union in the 1920s. In: Mayr E, Provine B (eds) The evolutionary synthesis: perspectives on the unification of biology. Harvard University Press, Cambridge, MA, pp 229–242

Gaissinovitch AE (1980) The origin of soviet genetics and the struggle with Lamarckism 1922–1929. J Hist Biol 13(1):0001–0051

Gould SJ, Lewontin RC (1979) The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptionist programme. Proc R Soc Lond Ser B 25(1161):581–598

Levit GS (2015) Sergei Chetverikov (1880–1959). In: eLS. Wiley, Chichester. https://doi.org/10.1002/9780470015902.a0025066

Mayr E (1982) Prologue: some thoughts on the history of the evolutionary synthesis. In: Mayr E, Provine B (eds) The evolutionary synthesis: perspectives on the unification of biology. Harvard University Press, Cambridge, MA, pp 1–48

Medvedev Z (ed) (1979) Soviet Science. Oxford University Press, Oxford

Mikhailov AT (2012) Russian comparative embryology takes form: a conceptual metamorphosis toward “evo-devo”. Evol Dev 14(1):9–19. https://doi.org/10.1111/j.1525-142X.2011.00518.x

Olsson L, Levit GS, Ossfeld U (2010) Evolutionary developmental biology: its concepts and history with a focus on Russian and German contributions. Naturwissenschaften 97:951–969. https://doi.org/10.1007/s00114-010-0720-9. Springer

Punnett R (1915) Mimicry in butterflies. Cambridge University Press, Cambridge

Ivan I. Schmalhausen (1884–1963)

Giulia Rispoli and Flavio D'Abramo

Contents

Abstract

Ivan I. Schmalhausen (1884–1963) was a Ukrainian geneticist and zoologist nowadays recognized as a central figure in the development of the modern evolutionary synthesis. He studied with Alexey Nikolaevich Severtsov, a leading biologist and the founder of the Russian school of evolutionary morphology, who made relevant contributions in the field of comparative anatomy. Following Severtsov's teaching, Schmalhausen became an expert in evolutionary morphology of animals and directed his attention towards experimental zoology.

Author of over 150 scientific papers on the study of patterns of growth, correlation, and evolutionary theories, his most important book, *Factors of Evolution*, was published in Russia in 1947 and translated into English in 1949 with a foreword by Theodosius Dobzhansky.

At the core of Schmalhausen's scientific production is the theory of the organism as a whole and the notion of stabilizing selection, which places his

G. Rispoli (✉) · F. D'Abramo
Max Planck Institute for the History of Science (MPIWG), Berlin, Germany
e-mail: grispoli@mpiwg-berlin.mpg.de; giuliarispoli@gmail.com; fdabramo@mpiwg-berlin.mpg.de

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_29

work next to that of the British biologist Conrad Waddington. Schmalhausen has been the recipient of several scientific awards and appointed member of both the Ukrainian Academy of Sciences (1922) and the Academy of Sciences of the USSR (1935).

Keywords

Stabilizing selection · Evolutionary morphology · Correlative systems · Cybernetics · Waddington

Life

Ivan Ivanovich Schmalhausen was born on the 23rd April of 1884 into the family of a professor of botany at the University of Kiev, also director of the botanical garden and corresponding member of the Russian Academy of Sciences. Ivan spent his childhood in the garden where his father owned an apartment and learned very young to classify plants and manage the herbarium collection. After his father's death, which happened before he turned 10, Ivan moved to a new house with his brother and sister. In spring 1894, he entered the gymnasium in Kiev and a few years later graduated from the high school with highest results. In 1902, Schmalhausen enrolled in the Natural Sciences Department of the Faculty of Physics and Mathematics of the University of Kiev. However, a strike that occurred in the early autumn led to the closure of the University with the result that Schmalhausen lost the entire academic year. Once readmitted the year after, he started working under the guidance of the assistant professor Michail M. Voskoboynikov (1873–1942). Schmalhausen became actively interested in biological sciences and quickly learned to master the microscopic technique. Following the suggestion of Professor Alexey Nikolaevich Severtsov (1866–1925), he undertook research on fetal lung development in snakes whose results, obtained in only 3 months, led him to take up new research on vertebrate limb development (Fig. 1).

During the years of his undergraduate studies, Schmalhausen did not stay out of political life but actively participated in the revolutionary student movement, which caused him the loss of another university year. He completed his studies in the spring of 1907, and by this time, he had already published an article on the development of lungs in snakes (1905) as well as a brief report about the development of limbs in amphibian *Anura*. At the same time, he began his teaching career first as an assistant to professor Voskoboynikov at the University for Women (1905–1906) and the following year by assisting professor Severtsov. The latter moved to Moscow a few years later in 1911, while Schmalhausen remained in Kiev, where he continued teaching at the University and the Kiev Polytechnic Institute until he received Severtsov's invitation to work as a senior researcher at the Institute of Comparative Anatomy of Moscow University. There, Schmalhausen became teaching assistant and was instructed to conduct a large workshop on the comparative anatomy of vertebrates.

Fig. 1 I. I. Schmalhausen. (Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Herald of the Russian Academy of Sciences, A consistent evolutionist, E. I. Vorob'eva, N. Yu. Feoktistova, 2009)

In 1914, Schmalhausen defended his Master's thesis: *Neparnye plavniki pyb i ich filogeneticheskoe razvitie* ("Unpaired fins in fishes and their phylogenetic development"). This work was fleshed out with an extensive experimental study on the function of fish fins carried out at the Naples Zoological Station in the summer of 1914.

In 1916, he defended his doctoral dissertation: *Razvitie konechnostej amfibij i ich znachenie v voprose o proischozhdenii konechnostej nazemnych pozvonochnych* ("The development of the amphibians' limbs and their importance to the question of the origin of the limbs in terrestrial vertebrates"). The first work contains a wealth of materials about the development of the skeleton and muscles of amphibians and provides new insights into the evolution of fins, especially the caudal fins.

In 1917, Schmalhausen was appointed professor at Tartu University in Estonia. However, due to the German occupation of the Baltic states, he remained in Moscow until after the evacuation of Tartu University, which was transferred to Voronezh (central Russia) 1 year later. At the new University, Schmalhausen headed the Department of Zoology and Comparative Anatomy of Vertebrates and also gave lectures to students from the Embryology department. In 1921, he came back to the University of Kiev to teach Embryology, General Biology and Evolutionary Theory. During the last year of his stay in Kiev, Schmalhausen taught also Genetics. Additionally, he became a senior researcher at the Faculty of Physics and Mathematics of the University of Kiev, and in 1922 was elected a full member of the Ukrainian Academy of Sciences, where he established the Department of Experimental Zoology. Later on, Schmalhausen was appointed the head of the Department of Zoology, where his work mainly focused on the origin of terrestrial vertebrates. He then turned his attention to the study of fetal growth patterns, being able to prove

the existence of periodic changes of growth and established a bond between these changes and periods of intense differentiation.

In Kiev, Schmalhausen was chairman of the Society of Naturalists and the Scientific Council of the Karadag Biological Station. In 1930, he organized the Fourth All-Union Congress of Zoologists, Anatomists, and Histologists. In 1935, Schmalhausen was elected member of the Academy of Sciences of the USSR, and after Severtsov's death in 1936, he ended up heading the Institute of Evolutionary Morphology, moving definitely to Moscow in the spring of 1937. There, Schmalhausen continued his theoretical study of individual factors of development and their implications for evolution. A couple of years later, he was appointed professor and head of the Department of Darwinism that he had established himself at the Moscow State University. He held this position until 1948. As a result of the extensive work he conducted with his colleagues and students, the volume *Problemy Darwinizma* ("Problems of Darwinism") was released in 1946.

While working on the evolution of animals, Schmalhausen paid particular attention to genetics. He notably was one of the Soviet biologists railing against Trofim Lysenko at the 1948 August session of the Lenin All-Union Academy of Agricultural Sciences (VASKhNIL) in Moscow. On that occasion, Lysenko opened his speech targeting the reactionary genetics and the defense of the Malthusian theory invoked by Western scientists. He then criticized Schmalhausen's ideas for having nothing in common with dialectical materialism (Birstein 2004). Lysenko castigated Mendelian genetics as well as Weismann's germinal theory as opposed to the theory of the inheritance of acquired characters. As a result of Schamalhausen's criticisms and strident opposition to Lysenko, he was labelled a supporter of Mendelism and Morganism, and a few years later expelled from his job.

In 1948, the academician Evgeny N. Pavlovsky (1884–1965) invited Schmalhausen to join the Embryology Laboratory of the Zoological Institute of the USSR Academy of Sciences where he returned to comparative anatomy and his research on the origin of terrestrial vertebrates. He studied the evolutionary transformations extensively in fishes and terrestrial vertebrates especially in the lower skull, the axial skeleton, and the respiratory and circulatory systems.

In 1955, Schmalhausen signed collectively with three hundred scientists a Letter denouncing Lysenkoist dictatorship in biology. In 1958, he was appointed honorary member of the German Academy of Natural Scientists "Leopoldina," the German Academy of Sciences and the Academy of Zoology in Agra (India). In 1961, he became seriously ill and finished the manuscript of the book *Regulyatsiya formoobrazovaniya v individual'nom razvitii* ("The regulation of morphogenesis in individual development") in the hospital. After leaving the clinic in 1962, he continued working hard in spite of poor health, and between 1962 and 1963, he managed to accomplish another volume titled *Proischozdenie nazemnych pozvonochnych* ("The origin of terrestrial vertebrates"), initiated before his illness. This work provides a clear explanation of the transition of vertebrates from aquatic to terrestrial life.

Based on the relevance of the 28 works he published between 1950 and 1960, the Presidium of the Academy of Sciences of the USSR awarded Schmalhausen the prestigious Ilya Metchnikov Prize in 1963. During the same year, he began writing a

new book on the use of cybernetics in biology which promised to be very innovative in the framework of his research in evolutionary morphology and biology. He did not manage to complete the book, which was posthumously published in 1968 with the title *Kiberneticheskie voprosy biologii* ("Cybernetic problems of biology").

In the autumn of 1963, his disease worsened, and on October 7, he died. After his death, Schmalhausen's works got repeatedly published both in Russia and abroad. In 1995, the Presidium of the Russian Academy of Sciences established a prize in memory of the life and career of this eminent zoologist.

Work

Schmalhausen published his most renowned book, *Faktory Evolyutsii*, in 1946. An English translation (*Factors of Evolution: The Theory of Stabilizing Selection*) edited by Theodosius Dobzhansky (1900–1975) appeared in the West in 1949 and a reprint in 1986 with a new foreword by David B. Wake (Dobzhansky 1949). This work contains an analysis of evolutionary processes through the lenses of comparative embryology, comparative anatomy, and mechanisms of development. The Russian biologist, who mastered genetics, aimed to integrate embryology in the Modern Synthesis. He tried to make a synthesis of evolution, genetics, and embryology, an attempt that was hindered by the Mendelian-chromosomal genetic theory through which embryological concerns were put aside (Amundson 2000, p. 336). Schmalhausen sought to strengthen the Modern Synthesis because he lamented the exclusion from it of functional morphology and embryology (Gilbert 1994). Severtsov, the founder of the Russian school of evolutionary morphology and Schmalhausen's mentor during his education, thought that population genetics was a pivotal discipline within the Modern Synthesis, but useless in explaining macro-evolution, and Schmalhausen aimed to remedy this deficiency (Gilbert 1994).

In particular, with Thomas H. Morgan, genetics was meant to consider biological inheritance solely as *transmission* and not as *development* of inherited traits. In effect, "Heredity came to refer to the genetic material of the parent (structurally arranged on chromosomes), the transmission of that material to the offspring, and the eventual appearance in the offspring of the inherited trait" (Amundson 2000, p. 339). Neither the historical origins of parental genetic material nor the ontogeny of the phenotypic trait in the offspring were of relevance. Transmission genetics was embedded in the Modern Synthesis, whereas both embryology and developmental genetics were ignored. In contrast, Schmalhausen highlighted the necessity to consider the developmental processes which create new phenotypes such as new species. In other words, Schmalhausen advanced the argument according to which evolutionary phenotypic changes can only occur within changes in the developmental process:

> [. . .] resistance to poison and parasitic diseases, immunity, resistance of plants to frost and drought [. . .] [which are] adaptations to definite conditions of existence [. . .] are often adaptive and are of considerable importance in the formation or races and species (Schmalhausen 1949, p. 162).

Stabilizing Selection as a Factor of Evolution

Schmalhausen thought of natural selection not only as a directional factor (i.e., able to produce new adaptations to new environmental circumstances) but also as a stabilizing factor: if a phenotypic trait of a species causes it to be well adapted, then such a morphological and developmental trait is stabilized by natural selection so as to be not influenced by internal or external disturbances that would reduce the fitness of the organism:

> The *stabilizing form of selection* is based upon the selective advantage under definite and, especially, fluctuating conditions possessed by the normal organization over variations from the norm. It is associated with the elimination of most variations and the establishment of more stable mechanisms of normal morphogenesis. (Schmalhausen 1949, p. 73)

Schmalhausen sorted out the environmental changes, which most of the organisms' experience, into local, seasonal, and chance variations. For instance, for what regards the seasonal forms of plants:

> the development of shade and light forms of leaves is sometimes induced primarily by external factors [. . .] [i.e.] illumination received by the axillary buds during the past vegetation season [. . .], and then, is fully regulated by internal developmental mechanism. This behavior shows the manner in which the adaptive morphogenesis of the tree is protected against erroneous responses to the changing conditions of spring illumination. (Schmalhausen 1949, p. 81)

Selection, necessary to elicit stabilized developmental trajectories, is produced amidst a gamut of natural conditions which might connote the evolution of a species. When extreme environmental conditions are present, then the organism produces a high genetic variance which might breach the stabilized function of selection. For Schmalhausen, the heightened genetic variation is the effect of the environment which reveals *latent* genetic differences that can eventually be selected. One of the instances of stabilizing selection in a varying external environment proposed by Schmalhausen is represented by a flowering plant called "shepherd's purse" (*Capsella bursa-pastoris*) which was introduced by man in the mountains of Erdshias-dagh (Asia Minor). Because of this relocation, the plant developed alpine characteristics such as deep roots, low stems, and the development of tiny hairs which, by breaking the wind and by reducing the air flow, decrease the rate of evaporation – all characteristics which facilitate the survival in an environment with little liquid water and frost. When the shepherd's purse is relocated again in the lowland, even after four generations, it shows the low stem. Therefore, argues Schmalhausen, the processes of low stem formation have been stabilized, that is they have become autonomous. In this and other specific cases analyzed by Schmalhausen, some of the morphological changes developed by organisms in response to changes in the environmental conditions are transmitted across generations, that is to say, they are inherited:

> This *partial* hereditary fixation of variations which previously were dependent, i.e., adaptive, modifications is very common. It includes many ecologic and geographic forms of plants and animals, which revert incompletely to the former phenotype when they are transferred to other conditions that are similar to the original ones. The ability to revert is evidence of the importance of modifications in the appearance of certain forms. That this reversion is only partial, or in specific traits, indicates that the given phenotype was exposed to natural selection – a stabilizing selection since the selection occurred within the limits of the already established modifications of the norm. [. . .] If the alpine habit develops in mountains among many plants, then most typically alpine plants retain their alpine traits in part even after they have been transferred to the lowlands. Apparently, their morphogenesis has become more stable as a result of frosts and drought severely eliminating all individuals, which developed the erroneous lowland modifications of long stems and short roots during an accidentally mild and humid spring. (Schmalhausen 1949, pp. 83–84)

Schmalhausen's notion of "hereditary fixation" to refer to characteristics organisms acquire in their interaction with environmental changes is also present in the work of Conrad H. Waddington (see chapter ▶ "Conrad Hal Waddington (1905–1975)"). Differently from Schmalhausen, who drew on experimental, geographical, or ecological knowledge produced by other scientists, Waddington *empirically* developed this idea through the notion of "genetic assimilation." In an article published in 1953, Waddington showed results of the experiment done with Drosophila exposed to heat shock which eventually elicited a phenotype where a small vein normally present on the wing is not formed. Once the flies showing the phenotypic mutation were selected to mate, after the 16th generation they showed the crossveinless character even in the absence of the heat shock: that phenotypic character elicited by an environmental change was *genetically assimilated* (Waddington 1953).

In Schmalhausen's account, the organism's sensitivity to react to "normal" environmental changes (i.e., those changes that do not affect the developmental trajectory of the viable organism) by means of physiological and developmental adjustments is sifted by natural selection. The notion of genetic assimilation implies an inherited (genetically controlled) reduction in the developmental flexibility of the organism which makes it more resistant to environmental and genetic perturbations. The reduction of the organisms' plasticity implied by the process of genetic assimilation was named by Waddington as *canalization*, and it also refers to the increasing determination of the cells during development, from the totipotent cells to specialized cells (Waddington 1942; see chapter ▶ "Canalization: A Central but Controversial Concept in Evo-Devo").

The Whole Organism as a Cybernetic System

A pivotal role in Schmalhausen's education was no doubt played by Alexey N. Severtsov, whose work in comparative morphology initiated Schmalhausen into the study of the organism's integrity. It is important to note that Severtsov saw the development of the organism through the theoretical lens of dialectical materialism, a theory which strongly influenced Schmalhausen in the study of evolutionary biology (Wake 1986). This philosophical perspective, based on the assumption

that each layer of matter – physical, chemical, or biological – is irreducible to the level immediately below in its overall development, provided, in his view, a theoretical benchmark for understanding the evolutionary processes as well as the interdependence of the organism's integrity with its ontogenetic and phylogenetic development (Vorob'eva and Medvedeva 1982). According to Severtsov, dialectical materialism was also meant to provide a philosophical explanation for the connection between ontogenesis, morphogenesis, and environmental factors.

Drawing on Severtzov's teachings, Schmalhausen's book *Organizm kak tseloe v individual'nom i istoricheskom razvitii* ("The organism as a whole in its individual historical development") published in 1938, summarized researches he had conducted in Kiev, especially on the relations of morphogenetic processes and their role in the development of the organism's whole organization. The book withstood two editions but due to a lack of circulation did not receive much attention by Western researchers.

In this work, Schmalhausen mapped out the biological development of the organism through processes of differentiation and integration. Moreover, he emphasized that the processes taking place during development are interconnected and have to be interpreted in the light of the organism's historical development. Questioning the idea that the structure and function of the organism can be analyzed as two separate dimensions, Schmalhausen stressed that the formation of the organism depends on the correlations of its parts and functions. He introduced the idea of the organism as a correlative system which develops under the influence of external environmental factors. In this connection, he claimed that the inputs coming from the outer environment generate morphogenetic reactions in the correlative system.

In the final stage of his career and while studying the correlative basis and the dynamic coordination of development, Schmalhausen found himself attracted by cybernetics. He believed that evolutionary processes could be explained by cybernetics because every biological organism is a self-regulating system to be studied as a whole evolving unity. Schmalhausen' ideas explaining evolution through cybernetics appeared in various articles and were summarized in the posthumously published book *Biologicheskie voprosy kibernetiki* 1968) ("Cybernetic Questions of Biology") and also used in another volume, also published posthumously: *Regulyatsiya formoobrazovaniya v individual'nom razvitii* ("Regulation of Formation in Individual Development," 1964). The application of cybernetic notions, communication, and information theories – research fields developing extensively during the 1950s – helped him look for a more solid explanation of the dialectic between evolutionary patterns and individual development. Cybernetics allowed Schmalhausen to explain the mechanisms underlying biological processes at all levels (subcellular, cellular, tissues, organs, whole organism, and population) and even at a supra-organismal level, namely, at the level of "biocenosis," which explains the correlative and coevolutionary relationship between ecosystems and biological communities as a cohesive organizational complex.

The perspective that applies cybernetic and system principles to the study of biocenosis became known in the West as systems ecology and has in George Evelyn Hutchinson and Eugene Odum its pioneers in the United States. Schmalhausen went

even further in his cybernetic reformulation of evolutionary theories. He employed, for instance, the notion of "biogeocenosis" put forward by Vladimir N. Sukachev (1880–1967), a prominent geo-botanist and geographer who is widely acknowledged in Russia for his comprehensive studies of phytocenology and biogeography (Sukachev 1944). In particular, the study of biological communities in relation to the entire biosphere and the geological components of the earth provided Schmalhausen with the possibility to further scale up his cybernetic analysis, cutting across the biological level to the planetary one. Sukachev was not the only one to suggest Schmalhausen a useful theoretical framework to be applied in his investigations. Another central source he relied upon was the mineralogist Vladimir I. Vernadsky (1863–1945), the founding father of biogeochemistry and the modern notion of the biosphere as a global system of circulation of matter and energy (Vernadsky 1926). Vernadsky's work is on backstage of many further developments, and especially the notion of the earth-system, which would be prominent in the rise of global ecology, earth system science and biosphere studies in the 1970s.

Sukachev's biogeocenosis and Vernadsky's biosphere offered Schmalhausen a profitable mean to extend the applicability of his theory of stabilizing selection to incorporate regulatory processes that occur at the level of the population and the environment (Schmalhausen 1968).

Cybernetics inspired Schmalhausen to describe evolution as a process of "automatization," a regulative phenomenon in which the organism or the population is seen as a "primary evolving entity." The biogeocenosis operates indeed as a regulating mechanism in this process (Schmalhausen 1949; Levit et al. 2006; Levit 2007, p. 142; Vorob'eva 2006).

Innovatively, Schmalhausen's take on evolution represents a complex body of self-regulating systems such as the organism as a whole, the population, and the biogeocenosis. Along these lines, he tried to integrate different structural levels of organization in his analysis of ontogeny and evolution which can be seen as united by mean of a large homeostatic process (Vorob'eva 2010).

Legacy

As Vorob'eva has pointed out, Schmalhausen's strategy has been that of merging evolutionary theory and genetics with embryology and morphology for the first time during the spreading of the Modern Synthesis. He conceived of the problem of the organism's integrity as connected with both phylogeny and ontogeny, a particularly important insight when it comes to evo-devo (Vorob'eva 2006, 2010; Wake 1996). Through the connectedness of all the organism's parts, every genetic change could not only give rise to a plural phenotypic expression (pleiotropy) but it also could manifest itself in the embryo as a multistage change of the whole chain of morphogenetic correlations.

According to Richard Lewontin and Richard Levins, *Schmalhausen's Law* indicates that organisms living within a normal range of environmental conditions

show genetic perturbations which have little or no effect on their physiology and development. On the contrary, when organisms experience severe or unusual general stress conditions, then even small genetic or environmental differences might elicit major effects (Lewontin and Levins 2007). Lewontin and Levins have shown the relevance of Schmalhausen's Law in pointing the "danger of predicting the outcome of perturbations using the results of experiments on *single factors* under *controlled conditions*" (2007, p. 77). A good instance might be the danger of considering climate changes, such as a one-degree change in temperature and its effect on malaria distribution, without considering the complex web of the vector mosquito, its natural enemies and competitors. All the species within this complex web might indeed be at the border of their distribution so as to reveal their sensitiveness to small environmental changes, that in turn might lead some species to become locally extinct and others to a great expansion. Under conditions of any kind of stress, small differences have big effects, so that a normal model in which are assumed "same" conditions for all the population might be just misleading. Therefore, in Lewontin and Levin's account, the "Schmalhausen Law" points to the importance of the "historical relation of a population with its environment, the responsiveness of the physiology to familiar and to new stressors, and the inherent variability of both organisms and environments" (Lewontin and Levins 2007, p. 80). According to Mary Jane West-Eberhard, Schmalhausen assigned to plasticity an important role in evolution (see chapter ▶ "Developmental Plasticity and Evolution"). However, she has criticized both Schmalhausen and Waddington to have conceived evolution, by means of *stabilising selection* and *genetic assimilation,* as a process "liberating the organism from the determining influence of the environment" (West-Eberhard 2003 p. 499). West-Eberhard conceives instead the regulatory systems of organisms as a complex of devices which "link stimuli from the external environment to genomic function" and where gene expression is "mediated within cells by hormones and hormonelike substances" (2003, p. 499). In West-Eberhard's account, the developmental processes are more important and prevail upon evolutionary adaptive dynamics to substantially determine evolutionary processes themselves.

Since his first studies and the publication of *The organism as a whole* (1938), Schmalhausen always tried to connect the manifold developmental aspects of the organism with the evolutionary processes. When he was a student doing experiments on lung development, he drew particular attention to the relations of phylogenetic changes and development. With time, he tried to integrate these two levels within a more comprehensive cybernetic and organizational framework, in accordance with the most recent scientific developments of his time. Schmalhausen is nowadays recognized for having played a major role not only in the establishment of the Russian school of evolutionary biology but also for his contribution to the worldwide development of biological sciences, genetics, and embryology. His systemic and cybernetic understanding of the interplay between development and evolution reveals a sophisticated attempt to interlace evolutionary morphology, population genetics, embryology, and ecology (Gilbert 2002). This attempt makes evident the importance of Schmalhausen's legacy

not only in the light of evo-devo but also in prefiguring some of the issues that will develop later, with the discipline nowadays known as ecological evolutionary developmental biology, or eco-evo-devo (see chapter ▶ "Eco-Evo-Devo"). Schmalhausen's explanation of evolutionary patterns as systems of correlative and self-regulating dynamics that are biological, embryological, ecological, and even biospherical according to different levels of organization, echoes Gilbert's description of Eco-Evo-Devo as the study of the organism's evolution embedded in a developmental environment, making evident the anticipatory potential of Schmalhausen's theorizations. In emphasizing the importance of ecology, Schmalhausen found largely support in Sukachev's notion of the biogeocenosis and Vernadsky's biogeochemistry but his merit has been that of having applied them to problems of development and evolution. He employed notions, ideas, and approaches that biologists of the Modern Synthesis were utterly disregarded (Vorob'eva 2006). Moreover, he had an exceptional foresight in trying to legitimize his integrative analysis of ontogeny, phylogeny, and ecology through a cybernetic framework. Schmalhausen's key notion of stabilizing selection has also contributed to the contemporary debate on *robustness*, namely the persistence of an organismal trait under perturbations that ensures the stability of the phenotypic traits exposed to perturbing factors

wSchmalhausen's contribution to the development of biological sciences has been manifold and unique. He bridged disciplines and perspectives that before his attempts were isolated and treated apart from each other. He produced an understanding of developmental biology as intertwined to evolutionary biology. He reflected on the different levels of biological organization, looking at the "whole organism" as embedded in a larger environmental setting of which it is nothing but a part contributing to its overall stability. The systemic and overarching investigation that Schmalhausen provides in his works – many of them unfortunately still unknown to Western historians and biologists – makes his contributions undoubtedly exceptional to the history of evo-devo. Schmalhausen has been one of those scientists who thought outside the box. His interest in organizational theory and cybernetics as well geography and biosphere studies, as additional perspectives that fuelled his work in morphology and zoology towards an interdisciplinary pathway, gives us the chance to place him in a broader framework than evo-devo and its legacy. For these reasons, Schamlhausen deserves his preeminent place in the history and philosophy of science.

Cross-References

▶ Canalization: A Central but Controversial Concept in Evo-Devo
▶ Conrad Hal Waddington (1905–1975)
▶ Developmental Plasticity and Evolution
▶ Eco-Evo-Devo

Bibliography

Schmalhausen II (1923) Osnovy sravnitel'noj anatomii pozvonochnykh. GIZ, Moscow/Petrograd
Schmalhausen II (1926) Problema smerti i bessmertiya. Seria Proroda i Kul'tura, Moscow
Schmalhausen II (1938) Organizm kak tseloje v individualnom i istoricheskom razvitii. AN SSSR, Leningrad
Schmalhausen II (1939) Puti i zakonomernosti evoliutzionnogo protsessa. AN SSSR, Moscow/Leningrad
Schmalhausen II (1946a) Problemy Darwinizma. Sovetskaja Nauka, Moscow, (2nd edition: 1969)
Schmalhausen II (1946b) Faktory evolyutsii: teoriya stabiliziruyuschevo otbora (1946) AN SSSR. Leningrad, Moscow, (2nd, 1968)
Schmalhausen II (1949) Factors of evolution: the theory of stabilizing selection. Blakiston Co., Philadelphia, (reprinted in 1986 by Chicago University Press)
Schmalhausen II (1964a) Proischozhdenie nayemnykh pozvonochnykh. Nauka, Moscow
Schmalhausen II (1964b) Regulyatsiya formoobrazovaniya v individual'nom razvitii. Nauka, Moscow
Schmalhausen II (1968) Kiberneticheskije voprosy biologii (cybernetic questions in biology). Nauka, Novosibirsk
Schmalhausen II (1983) Izbrannyje trudy. Puti i zakonomernosti evoliutzionnogo prozessa. Nauka, Moscow

References

Amundson R (2000) Embryology and evolution 1920–1960: worlds apart? Hist Philos Life Sci 22(3):335–352
Birstein VJ (2004) The perversion of knowledge, the true story of soviet science. Westview Press, London
Dobzhansky T (1949) Foreword. In: Schmalhausen II (ed) Factors of evolution: the theory of stabilizing selection. The Blakiston Company, Philadelphia
Gilbert S (1994) Dobzhansky, Waddington, and Schmalhausen: embryology and the modern synthesis. In: Adams MB (ed) Evolution of Theodosius Dobzhansky: essays on his life and thought in Russia and America. Princeton University Press, Princeton, pp 143–154
Gilbert SF (2002) The genome in its ecological context. Philosophical perspectives on interspecies epigenetics. Ann N Y Acad Sci 981:202–218
Levit GS (2007) The roots of Evo-Devo in Russia: is there a characteristic "Russian tradition"? Theory Biosci 126(4):131–148. https://doi.org/10.1007/s12064-007-0013-9
Levit GS, Hossfeld U, Olsson L (2006) From the "Modern synthesis" to cybernetics: Ivan Ivanovich Schmalhausen (1884–1963) and his research program for a synthesis of evolutionary and developmental biology. J Exp Zool B Mol Dev Evol 306(2):89–106. https://doi.org/10.1002/jez.b.21087
Lewontin R, Levins R (2007) Biology under the influence: dialectical essays on the coevolution of nature and society. NYU Press, New York
Sukachev VN (1944) O prinzipakh geneticheskoj klassifikatzii v biogeotsenologii. Zh Obtschei Biol 5:213–227
Vernadsky VI (1926) Biosfera. NHTI, Leningrad
Vorob'eva EI (2006) Problems of organism integrity and its perspectives. Biol Bull 33(5):427–436. https://doi.org/10.1134/S1062359006050025
Vorob'eva EI (2010) Evo devo and the I.I. Schmalhausen concept of the evolution of ontogeny. Biol Bull 37(2):106–113
Vorob'eva EI, Medvedeva IM (1982) Akademik Ivan Ivanovich Schmalhausen i Problema Zelostnosti v biologii. In: Schmalhausen II (ed) Izbrannye trudy. Nauka, Moscow

Waddington CH (1942) The canalization of development and the inheritance of acquired characters. Nature 150:563–565. https://doi.org/10.1038/150563a0

Waddington CH (1953) Genetic assimilation of an acquired character. Evolution 7(2):118–126. https://doi.org/10.2307/2405747

Wake DB (1986) Foreword. In: Schmalhausen II (ed) Factors of evolution. The University of Chicago Press, Chicago/London, pp v–xii

Wake DB (1996) Schmalhausen's evolutionary morphology and its value in formulating research strategies, systematic biology as an historical science. Memor della Soc Ital di Sci Natur e del Museo Civico di Storia Naturale di Milano 27:129–132

West-Eberhard MJ (2003) Developmental plasticity and evolution. Oxford University Press, New York

Gavin de Beer (1899–1972)

Yawen Zou

Contents

Abstract

Gavin de Beer (1899–1972) was an evolutionary embryologist considered by many as a forerunner of modern evolutionary developmental biology (evo-devo). This entry discusses de Beer's works with a special focus on his contributions to evo-devo. De Beer was trained in zoology and later became interested in comparative and evolutionary embryology. De Beer joined the attack on Ernst Haeckel's biogenetic law and argued that ontogeny did not recapitulate phylogeny, but ontogeny caused phylogeny instead. Influenced by the advancements of the Modern Synthesis and the rise of genetics, de Beer advocated for the integration of embryology, heredity, and evolution and emphasized on the importance of embryology in evolutionary theory in many of his writings. Although de Beer failed to make a significant impact on the Modern Synthesis, his work on heterochrony influenced modern evo-devo biologists such as Stephen Jay Gould, and his views on homology are still revisited today.

Y. Zou (✉)
Center for Biology and Society, Arizona State University, Tempe, AZ, USA

The Chinese University of Hong Kong, Shenzhen, Shenzhen/Guangdong Province, P. R. China
e-mail: yzou20@asu.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_20

Keywords

Gavin de Beer · Evo-devo · Biogenetic law · Heterochrony · Homology

Life

Gavin Rylands de Beer was born on November 1, 1899, in Malden, England, but he spent his childhood in France until he was 13 years old because his father worked as a correspondent for a telegraph company based in France. Partly due to his bilingual upbringing, later in his life, he managed to speak and write in French, Italian, and Swiss-German. After returning to England in 1912, de Beer went to Harrow School and became interested in biology. In 1917 he entered Magdalen College, Oxford to study zoology, which at the time was dominated by embryology. At Oxford, de Beer had three famous teachers (the members of the so-called Oxford School) with whom he kept a lifelong relationship: Edwin Goodrich (1868–1946), Julian Huxley (1899–1980), and John Burdon Sanderson Haldane (1892–1964).

After graduating in 1921 with first-class honors, de Beer was elected a Christopher Welch Scholar and Edward Chapman Prizeman and started to teach zoology at the Merton College, Oxford. He visited the Stazione Zoologica in Naples and studied Hans Spemann's experimental embryological techniques in Freiburg University. De Beer's early research concerned the comparative anatomy of somites, gill slits, cranial nerves, and the pituitary body. In 1926 he published two books: *An Introduction to Experimental Embryology* and *The Comparative Anatomy, Histology and Development of the Pituitary Body* (De Beer 1926). Both works proved to be very successful textbooks, which established de Beer's status as an experimental and comparative embryologist (Brigandt 2006). De Beer was an industrious writer, not just in his scientific research but also in documenting his European travels in the Alps (De Beer 1930b, 1932).

De Beer moved to the University College London in 1938 where he became a professor of embryology. In 1940 he was elected Fellow of the Royal Society. After the Second World War broke out, he served in the British Army, initially as an intelligence officer. After that, he worked on psychological warfare and participated in the Normandy landing. When the war was over, he returned to the University College London and resumed his career as a professor of embryology. From 1947 to 1949, de Beer was president of the Linnean Society. In 1950 he was elected director of the Natural History Museum in London, where he worked until retiring in 1960. During that period, he oversaw many exhibitions educating the public about evolutionary theory. He was knighted in 1954. His contribution to evolutionary theory was recognized with the awarding of the Darwin Medal in 1957.

After his retirement, de Beer continued working on popularizing evolutionary theory in works such as *Charles Darwin*: *Evolution by Natural Selection* in 1963 and *Atlas of Evolution* in 1964 (De Beer 1963, 1964). In 1965, he settled in Switzerland, where he spent time pursuing humanistic studies. In particular, he read the works of Voltaire, Gibbon, and Rousseau, of whom he published a biography in 1972

(De Beer 1972). De Beer returned to England in 1971 and died of a heart attack at Alfriston, Sussex, on June 21, 1972.

Work

Heterochrony

Karl Ernst von Baer (1792–1876) had argued that in embryonic development, general characters develop before special characters, and, as an animal develops, it diverges from other animals. In addition, the embryo stage of a higher-level animal only resembles the embryo stage, not the adult stage, of lower level animals. With the advent of evolutionary theory, von Baer's laws were overshadowed by Haeckel's biogenetic law or the recapitulation theory, which states that the development of an embryo is determined by its phylogenetic history. Ernst Haeckel (1834–1919) argued that evolution occurred only in adult forms, through terminal addition of traits at the end of development (Haeckel 1866). Haeckel also recognized two kinds of exceptions to the biogenetic law, when ontogeny does not recapitulate phylogeny: "heterochrony," or variation in timing that causes acceleration or delay of traits, and "heterotopy," defined as the displacement of organs caused by the variation in location (Haeckel 1905, p. 10). The biogenetic law was influential from its formulation in 1866 until the end of the nineteenth century. In the 1920s and 1930s, recapitulation was dismissed by many, including de Beer's teacher Goodrich, but was still supported by some such as Ernst MacBride (1866–1940). De Beer's ambition was to use his own theory "to replace on the rails laid by von Baer the train of biological thought which was shunted off them by Haeckel" (Barrington 1973, p. 70).

In 1930, de Beer published a short book, *Embryology and Evolution*, where he argued that ontogeny did not recapitulate phylogeny. Instead, de Beer claimed that modifications of ontogeny resulted in phylogenetic transformations. The book was a success and was expanded in its third edition to become *Embryos and Ancestors*, first published in 1940 and later republished several times. As recognized by Stephen Jay Gould (1941–2002), *Embryos and Ancestors* "dominated English thought on the relationship between ontogeny and phylogeny for more than 40 years" (Gould 1977, p. 222). In this book, de Beer argued that internal factors (such as hormonal influences, as opposed to external factors such as gravity) modify the ontogeny in ancestors, and then ancestors pass down these factors to descendants. In this view, phylogeny plays no role in determining ontogeny; instead it is determined by ontogeny. De Beer argued that the theory of recapitulation thwarted the search for the causal study of ontogeny, and he counted on genetics to explain development.

De Beer proposed that ontogeny was modified through *heterochrony*, a mechanism by which the timing of development determined the size and shape of body parts. Key to his theory of heterochrony were the notions of *paedomorphosis*, the retention of juvenile traits in adult organisms, and *gerontomorphosis*, the

specialization of adult traits of ancestors that decreases the ability of descendant organisms to further specializing:

> Characters present in the early stages of ontogeny have (provided that they are not too specialized) played an important part in evolution by paedomorphosis, resulting in large structural changes without loss of plasticity. Characters present in the late stages of ontogeny have played an important part in evolution by gerontomorphosis, resulting in relatively small structural change with loss of plasticity (de Beer 1940, p. 96).

Both the idea and the term "paedomorphosis" actually came from Walter Garstang (1868–1949), who had previously shown that adaptation also exists in the larval, and not just in the adult, stages (Hall 2000). Garstang and de Beer shared many similarities: they both studied embryology in Oxford, and they both claimed that the modification of ontogeny was the cause of phylogeny.

De Beer further classified heterochrony into eight types: cenogenesis, deviation, neoteny (and paedogenesis), reduction, adult variation, retardation, hypermorphosis, and acceleration. Some authors have pointed out that de Beer's taxonomy is too complicated and difficult to understand. According to McNamara and McKinney (1991), only four of them (viz., neoteny, pedogenesis, hypermorphosis, and acceleration) can be properly called heterochrony in the modern sense (see Brigandt 2006, for a detailed explanation of these concepts). Neoteny and paedogenesis are the phenomena in which characters in the young stage of ancestors appear in the adult stage of descendants. Acceleration is the phenomenon in which adult characters of ancestors appear in the young stage of descendants. If the development of some traits in descendants is delayed, the descendants will add characters on to those traits, resulting in hypermorphosis. In a more extreme critique, Gould rejected the eight types of heterochrony that de Beer proposed altogether and argued that all these types "can be reduced to the two aspects of a single process—acceleration and retardation" (Gould 1977, p. 222).

Embryology, Genetics, and Evolutionary Biology

Although in the 1930s biologists still had not determined whether protein or DNA was the physical carrier of inheritance, genetics had already received considerable attention. De Beer was deeply influenced by the work of Thomas Hunt Morgan (1866–1945), particularly by his book *Embryology and Genetics* (1934), which he often referenced in his own writings. De Beer was also influenced by Richard Goldschmidt's (1878–1958) work on the factors that can determine the sex and color of caterpillars in the gypsy moth (Hall 2003). The results of Goldschmidt's experiments on the gypsy moth were translated and published into English in 1923 and were discussed by Huxley and de Beer (Richmond 2007). Goldschmidt formulated the balance theory of sex determination to explain that the sex of the moth was determined by the speed of the developmental processes of two or three sets of what he called female and male determiners (Goldschmidt 1923). De Beer was amazed that Goldschmidt was able to determine the sex of the gypsy moth by experimentally

manipulating the developmental processes involved in sex determination. This further encouraged him to claim that ontogeny should be the cause of phylogeny. De Beer was among the first generation of embryologists who realized the power of genetics. In this context, he published a paper entitled "Genetics, The Center of Science" (de Beer 1966) and, in the preface of *Embryos and Ancestors*, proposed that embryology, evolution, and genetics should come together.

De Beer's cooperation with Julian Huxley did not stop after he left Oxford. In 1934 they published a book together titled *The Elements of Experimental Embryology* (Huxley and de Beer 1934). Huxley focused on experimental embryology at the time but later became a major contributor to the Modern Synthesis. The 1930s and 1940s witnessed the huge success of the Modern Synthesis, but it is often said that embryology was not properly integrated as a discipline in the emerging synthetic theory of evolution. In this sense, historians have different views about the role played by embryologists (de Beer and Huxley) in the constitution of the Modern Synthesis. Frederick Churchill has argued that Huxley was quickly convinced by the progress of population genetics and thought it could offer a better causal explanation of development and evolution, while de Beer had become sensitized to avoid connecting phylogeny and ontogeny causally because of the failure of Haeckel's biogenetic law (Churchill 1980). In contrast, Tim J. Horder (2006) has argued that embryology, and particularly de Beer's theory of embryology, did have a crucial role in the Modern Synthesis. Horder pointed out that Huxley's book "mainly concerns microevolution, but in the last two chapters, embryology (according to the Huxley/de Beer model) is a central strand" (Horder 2006, p. 895). Notably, although not referencing to heterochrony directly, Huxley referenced de Beer about allometric growth, a concept similar to heterochrony, several times in his famous book *Evolution*: *the Modern Synthesis* (Huxley 1942). There is probably some truth in both views. Both de Beer and Huxley attempted to incorporate embryology into the Modern Synthesis, but, at that time, only population genetics was a discipline ready to offer explanations at the quantitative and experimental level, which made them easily accepted by the experimenters at the time (see the section on "Legacy").

Homology

Alan C. Love and Rudolf A. Raff have argued that comparative embryology was a key source in current evo-devo (Love and Raff 2003). In comparative embryology, *homology* is an essential concept, and de Beer pursued this topic for more than 30 years, bringing development back into homology (Laubichler 2000). De Beer's earliest writing on homology can be found in a 1938 book titled *Evolution*: *Essays on Aspects of Evolutionary Biology*. The book, edited by de Beer, was a collection of articles written by separate authors, including Julian Huxley, Haldane, Garstang, and de Beer himself. In his article "Embryology and Evolution," de Beer claimed that "homologous characters need not be controlled by identical genes" and that, as a consequence, "homology in phenotypes does not imply homology in genotypes," especially when comparing traits of distantly related species (de Beer 1938, p. 66).

De Beer discussed homology extensively in the article "Embryology and Evolution," probably because homology was a long quest for Goodrich (de Beer 1947), who he considered as "the leading comparative anatomist in the world of his day" (de Beer 1946, p. 112). Goodrich had studied coelomoducts (an excretory and genital duct that is typical of some invertebrates such as annelid worms) and nephridia (the unit of excretory system in many primitive invertebrates such as arthropods and mollusks) for more than 50 years. Goodrich dispelled the common belief that they were homologous by eventually proving that coelomoducts were of mesodermal origin, whereas nephridia were of ectodermal or ectomesodermal origin (Goodrich 1945).

De Beer's last comprehensive writing on homology was a small "pamphlet" of only 16 pages entitled *Homology, an Unsolved Problem* (1971). De Beer first reviewed the history of the concept of homology and then discussed homology in animals and plants, using examples such as ear ossicles and leaves and flowers. He distinguished between two kinds of homology: serial homology and latent homology. Serial homology refers to the similarity of repeated organs, such as the body segments of certain annelid worms. Latent homology refers to the shared structure of two related species, which is not exhibited in their common ancestors. De Beer continued to discuss the relationship of homology to embryology and genetics, including recent findings in genetics. At the end of the booklet, he concludes, as he did in 1938, that "homologous structures need not be controlled by identical genes, and homology of phenotypes does not imply similarity of genotypes" (De Beer 1971, p. 15). After more than 30 years' research on the subject, de Beer concluded that the problem of homology was still unresolved. Even today, homology continues to be a central issue of modern evo-devo (Laubichler 2000; see chapter ▶ "Developmental Homology").

Legacy

In the late 1970s, evolutionary biology started to incorporate embryology to become what is now known as evo-devo. In 1977 Gould published *Ontogeny and Phylogeny*, which played a major role in reviving the synthesis of evolution and development (Gerson 2007). Gould's book was heavily influenced by de Beer's *Embryos and Ancestors.* Horder even claimed that "Gould's *Ontogeny and Phylogeny* is, at heart, a re-statement of de Beer" (Horder 2006). Gould actually spent 11 pages of his *magnum opus* reviewing de Beer's work. Although Gould was the one who popularized the concept of heterochrony, the credit for introducing heterochrony into scientific discussion belongs to de Beer (Wourms 2007).

De Beer's contributions to evo-devo go beyond influencing Gould's concept of heterochrony. For example, de Beer joined Garstang in arguing that paedomorphosis is a main mechanism for macroevolution. In this sense, Rudolph Raff has argued that de Beer's account of heterochrony had a great influence on the discussion of macroevolution as well (Raff 1996). McNamara and McKinney have pointed out that paedomorphosis became a main competing theory to the recapitulation theory (McNamara and McKinney 1991).

The understanding of the genetic basis of developmental processes has confirmed some of de Beer's theoretical claims regarding paedomorphosis, homology, and the role of development in evolution. For example, de Beer claimed that characters present in the early stages of ontogeny played an important role in evolution, resulting in large structural changes without loss of phenotypic plasticity. The major structural changes that result from the modification of *Hox* genes have been interpreted as a confirmation of de Beer's hypothesis (Baguñà and Garcia-Fernandez 2003). De Beer's general claim about homology has also been confirmed: different developmental processes, germ layers, or genetic materials can still produce homologous structures and vice versa (Hall 2000).

One of the factors that might explain the neglect of de Beer's work in the Modern Synthesis concerns the theoretical nature of his evolutionary theory, which was in contrast with the experimental results of neo-Darwinians. In the 1930s and 1940s, de Beer advocated for the integration of embryology, evolution, and genetics, but the causal links between development and evolution were not addressed adequately by de Beer at his time. As a result, although de Beer wanted to integrate development into the Modern Synthesis, his endeavor was overlooked by neo-Darwinians.

Ingo Brigandt argues that when evaluating a past biologist's contribution to a biological discipline, it is irrelevant to determine whether that biologist's work under a current view is accurate. Instead, what is more important is deciding whether that biologist has contributed to the problems that a discipline addresses (Brigandt 2006). In this regard, de Beer contributed to the discussion of homology and heterochrony, which are two of the most central topics of evo-devo. Although de Beer's claim that ontogeny determined phylogeny is no longer influential, just as Haeckel's recapitulation theory, because the relationship of ontogeny and phylogeny is more complicated than one determining the other, de Beer's views on evolution, according to Gould, "would form the basis of modern ideas on embryology and evolution" because it spurred many biologists to question Haeckel and investigate the relationship between phylogeny and ontogeny (Gould 1992, p. 164).

Although embryology was largely excluded from the Modern Synthesis, de Beer's legacy lived on through subsequent evolutionary biologists such as Gould and their understanding of de Beer's account of homology and heterochrony. A review of de Beer's work shows that the integration of development into evolutionary theory did not suddenly happen in the 1970s. Rather, the essential concepts and epistemological questions of evo-devo were pursued long before that. Once the limitations were overcome by the advancement of genetics, molecular biology, and genomics, the synthesis of development and evolution was able to bear the fruits that we see today.

Cross-References

- ▶ Developmental Homology
- ▶ Developmental Plasticity and Evolution
- ▶ Explanation in Evo-Devo
- ▶ Form and Function in Evo-Devo

► Heterochrony
► Macroevolution
► Mechanisms in Evo-Devo
► Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics
► Richard Goldschmidt (1878–1958)
► Stephen Jay Gould (1941–2002)

Acknowledgments I am grateful to Manfred Laubichler and Federica Colonna for their encouragement and comments on early drafts of this essay. Comments by the editors Laura Nuño de la Rosa and Daniel J. Nicholson helped to improve this article significantly.

Bibliography

De Beer GR (1926) The comparative anatomy, histology, and development of the pituitary body. Oliver & Boyd, Edinburgh
De Beer GR (1930a) Embryology and evolution. Clarendon Press, Oxford
De Beer GR (1930b) Early travellers in the Alps. Sidgwick & Jackson, London
De Beer GR (1932) Alps and men. Edward Arnold, London
De Beer GR (1938) Embryology and evolution. In: de Beer GR (ed) Evolution: essays on aspects of evolutionary biology. Clarendon Press, Oxford
De Beer GR (1940) Embryos and ancestors, 1st edn. Clarendon Press, Oxford
De Beer GR (1946) Edwin Stephen Goodrich. J Anat 80(2):112–113
De Beer GR (1947) Edwin Stephen Goodrich. 1868–1946. Obituary Notices Fellows Royal Soc 5:477–490
De Beer GR (1963) Charles Darwin: evolution by natural selection. T. Nelson, London/New York
De Beer GR (1964) Atlas of evolution. Thomas Nelson and Sons, Camden
De Beer GR (1966) Genetics: the centre of science. Proc R Soc Lond B Biol Sci 995:154–166
De Beer GR (1971) Homology, an unsolved problem. Oxford University Press, Oxford
De Beer GR (1972) Jean-Jacques Rousseau and his world. Thames and Hudson, London

References

Baguñà J, Fernandez JG (2003) Evo-devo: the long and winding road. Int J Dev Biol 47(7/8):705–716
Barrington EJW (1973) Gavin Rylands de Beer.1899-1972. Biogr Mem Fellows R Soc 19:65–93
Brigandt I (2006) Homology and heterochrony: the evolutionary embryologist Gavin Rylands de Beer (1899–1972). J Exp Zool B Mol Dev Evol 306(4):317–328
Churchill FB (1980) The modern evolutionary synthesis and the biogenetic law. In: Mayr E, Provine WB (eds) The evolutionary synthesis: perspectives on the unification of biology, 1st edn. Harvard University Press, Cambridge, pp 112–122
Gerson EM (2007) The juncture of evolutionary and developmental biology. In: Maienschein J, Laublicher M (eds) From embryology to evo-devo. The MIT Press, Cambridge, pp 435–463
Goldschmidt RB (1923) Mechanism and physiology of sex determination (trans: Dakin W). Methuen & Co, London
Goodrich ES (1945) Memoir: the study of Nephridia and genital ducts since 1895. Q J Microsc Sci 2(343):303–392
Gould SJ (1977) Ontogeny and phylogeny, 1st edn. Harvard University Press, Cambridge

Gould SJ (1992) Heterochrony. In: Keller EF, Lloyd EA (eds) Keywords in evolutionary biology. Harvard University Press, Cambridge, pp 158–165
Haeckel E (1866) Generelle morphologie der organismen, 1st edn. Druck Und Verlag Von Georg Remer, Berlin
Haeckel E (1905) The evolution of man, 5th edn. Watts, London
Hall BK (2000) Balfour, Garstang and de Beer: the first century of evolutionary embryology. Am Zool 40(4):718–728
Hall BK (2003) Evo-devo: evolutionary developmental mechanisms. Int J Dev Biol 47(7/8):491–496
Horder TJ (2006) Gavin Rylands de Beer: how embryology foreshadowed the dilemmas of the genome. Nat Rev Genet 7(11):892–898
Huxley JS (1942) Evolution, the modern synthesis. George Allen & Unwin, London
Huxley JS, de Beer GR (1934) The elements of experimental embryology. Cambridge University Press, Cambridge
Laubichler MD (2000) Homology in development and the development of the homology concept. Am Zool 40(5):777–788
Love AC, Raff RA (2003) Knowing your ancestors: themes in the history of evo-devo. Evol Dev 5(4):327–330
McKinney ML, McNamara KJ (1991) Heterochrony. Springer US, New York
Morgan TH (1934) Embryology and genetics. Columbia University Press, New York
Raff RA (1996) The shape of life: genes, development, and the evolution of animal form. University of Chicago Press, Chicago
Richmond ML (2007) The cell as the basis for heredity, development, and evolution: Richard Goldschmidt's program of physiological genetics. In: Maienschein J, Laublicher M (eds) From embryology to evo-devo: a history of developmental evolution. The MIT Press, Cambridge, pp 169–211
Wourms JP (2007) The relations between comparative embryology, morphology, and systematics: an American perspective. In: Maienschein J, Laublicher M (eds) From embryology to evo-devo: a history of developmental evolution. The MIT Press, Cambridge, pp 215–266

Conrad Hal Waddington (1905–1975)

Flavia Fabris

Contents

Abstract

Conrad Hal Waddington was a paleontologist, embryologist, geneticist, and philosopher. After more than a century, Waddington's studies into the nature of developmental and evolutionary processes are increasingly recognized as a theoretical reference for contemporary evo-devo. Nonetheless, the complexity and the multifaceted impact of his theoretical approach make it difficult to form a clear and unitary picture of his life, works, and heritage. The chapter examines his early studies in experimental embryology and his pioneering work on epigenetics, which offered new insights into the internal connections between embryology, genetics, and evolution. The chapter concludes by examining the contemporary directions of research mostly influenced by Waddington, with particular attention to contemporary epigenetics and the study of the interplay between metaphysics and science.

F. Fabris (✉)
Konrad Lorenz Institute for Evolution and Cognition Research, Klosterneuburg, Austria

Egenis – The Centre for the Study of Life Science, University of Exeter, Exeter, UK
e-mail: fabris.fl@gmail.com; flavia.fabris@kli.ac.at

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_30

Keywords

Embryonic induction · Epigenetics · Epigenetic landscape · Genetic assimilation · Canalization · Theoretical biology

Life

Conrad Hal Waddington was born the 8th of November 1905 in Evesham, England, to Hal Waddington and Mary Ellen Warner. He soon afterwards moved to South India, where his father was employed as a tea plantation manager, a job that kept the parents far from their home country, to which they returned only in 1928. Waddington himself moved back to England only 4 years after the family relocation and, at the age of 5, started living under the custody of his aunt and uncle. He left their house only in 1914 to attend the Aymestrey House Preparatory School in Malvern Link, where he lived with his beloved grandmother (Robertson 1977).

Waddington's interest in the natural world was not long in coming. He immersed himself in the study of geology and the classification and collection of fossils, and, during preparatory school, was initiated in the study of chemistry by his very first

Waddington's 50th birthday photo album – Copyright The University of Edinburgh. (Accessed from https://images.is.ed.ac.uk/luna/servlet/s/dykh3r)

mentor, Eric John Holmyard (1891–1959). Geology and paleontology turned out to be more than a childhood infatuation. Waddington was awarded a scholarship to attend Clifton College, first, and then Sidney Sussex College, Cambridge University, from where he graduated in 1926 with a first-class degree in Geology and Natural Science. Waddington's first introduction to philosophy also came from Holmyard. Under his supervision, Waddington studied Gnostic and Hellenistic philosophy, which eventually paved the way for his future studies of Whitehead's metaphysics of process as well as cybernetics (Waddington 1969: 73–75, 1970: 113–114).

Although his interest in biology grew during his undergraduate studies, mostly thanks to the influence of his friend Gregory Bateson (1904–1980), it is only during his postgraduate studies that it matured into a more robust research project. Waddington started his early career research as a PhD student in paleontology, with a funded project on ammonites. At the same time, he managed to secure additional funding, which he used to deepen his studies in the metaphysics of science. The interdisciplinary approach that characterized his early academic studies soon became the hallmark of his revolutionary contributions to theoretical biology and experimental epigenetics, and granted him a place among the most influential and innovative thinkers of the past century.

By the time Waddington defended his PhD dissertation in 1938, he had already been married twice, first to the artist Elizabeth Lascelles – with whom he had a child – and then, after their divorce in 1936, to Margaret Justin Blanco White – with whom he had two daughters. Following his urge to engage with experimental work, he tried to establish himself as a geneticist. For a while, he enthusiastically and proficiently collaborated with John Burdon Sanderson Haldane (1892–1964) on a study about linkage conditions (Haldane and Waddington 1931). Unfortunately, according to Waddington (1938), he failed in this attempt to pursue a career in genetics due to a basic lack of funding and positions in the UK. Nonetheless, he applied for, and was successfully awarded, a research fellowship to conduct new studies, this time in experimental embryology, at the Strangeways Laboratory of Cambridge University, where he trained in grafting embryonic tissue (Robertson 1977). The results of his studies can be considered the first formative milestone in Waddington's academic career. In fact, his research on experimental embryology led him to collaborate first with Hans Spemann (1869–1941) on what would later be known as the Mangold-Spemann organizer, a cluster of cells that was shown to induce the development of the central nervous system in amphibians. He then delved into the chemical nature of inductive organization by collaborating for 6 years with Dorothy Needham (1896–1987), Joseph Needham (1900–1995), and Jean Brachet (1909–1988), and the result of this preliminary international investigation laid the foundation for his study of tissue differentiation and genetic control in developmental systems. In fact, despite the failure in securing a job as a geneticist, Waddington never left genetics aside. Quite the opposite: he kept cultivating his interest, building mainly on Morgan's theoretical work (Morgan 1934), and managing to offer a novel synthesis of the principles of genetics and the principles of embryology. At the end of the 1930s, he published two important books, *An Introduction to Modern Genetics* (1939) and *Organisers and Genes* (1940), and he managed to secure

funding for a collaboration with Thomas Hunt Morgan (1866–1945), Theodosius Dobzhansky (1900–1975), and Alfred Sturtevant (1891–1970), working in their prominent *Drosophila melanogaster* Lab at Caltech, then Columbia, and finally Cold Spring Harbor (Gilbert 1994). His international work eventually granted him the prestigious Albert Brachet Prize in 1936 for his innovative research on the nature of the organizer.

The decade between 1930 and 1940 saw the second, and perhaps decisive, milestone of Waddington's career. During these years Waddington, together with Joseph Henry Woodger (1894–1981), John Desmond Bernal (190–1971), Dorothy Maud Wrinch (1894–1976), and the Needhams participated in the Theoretical Biology Club, or "Biotheoretical Gathering," a meeting-group active in Cambridge and London from 1932 to 1938 (Peterson 2016). The history of the group was recently reconstructed from notes, correspondences, and oral history, and nowadays it is very well known for having set the stage for "a common theoretical discourse on the epistemological parity and complementarity of the biological and the physical sciences" (Abir-Am 1987: 1). Inspired by the writings of D'Arcy Thompson (1860–1948) and Alfred North Whitehead (1861–1947), the members of the club articulated an interdisciplinary research program by integrating problems and experimental resources from physics, mathematics, and philosophy, thereby encouraging a new rationale for scientific unity and a new synthesis across the institutionalized boundaries of the above disciplines (Abir-Am 1987; Peterson 2016). Albeit the club and its members are primarily known for their scientific contributions, Waddington and his peers were ahead of their time also in exploring the connection between science and its impact on society, politics, and ethics. In *The Scientific Attitude* (1941), Waddington discussed at length the philosophy of dialectic materialism, its Marxist roots, and the interplay between the scientific enterprise and the political ideologies of his time.

It is in the course of these 10 years that Waddington outlined his integrated view of genetics – which he dubbed "Diachronic Biology" – and presented the very first theoretical sketch of what would later be known as epigenetics. By then a prominent and highly recognized academic, Waddington joined the army during the Second World War, only to be appointed, a few years later in 1945, Deputy Director of the National Animal Breeding and Genetic Research Organization under the Agricultural Research Council, then Professor of Animal Genetics at the University of Edinburgh, and finally fellow of the Royal Society in 1947.

The period between 1950 and 1970 can be considered the pinnacle of Waddington's academic production. In 1954, he published *Principles of Embryology*, which contains the first formulation of the epigenetic landscape and of developmental potentials, the discussion of which would later be enriched in *The Strategy of Genes* (1957), where Waddington first describes the phenotype and its plasticity in terms of regulated causal interactions between genes and the chemical tendencies that they produce. In 1962, Waddington completed his project of founding the new field of Diachronic Biology by publishing *New Patterns in Genetics and Development*, in which he presented a novel account of genetic variability in terms of the canalization of development.

Waddington's thriving publishing decades were accompanied by a similarly productive dissemination of his research agenda. He was a well-known scholar in the USA, and in 1959, he became a foreign member of the American Academy of Arts and Sciences and continued to traverse the Atlantic for several years as a visiting professor, mainly staying at the Institute for Advanced Studies at the Wesleyan University, Connecticut, from 1961 to 1967. With the support of the Wellcome Foundation, he then successfully established the Epigenetic Research Group, of which he became Honorary Director in 1965. From 1961 to 1967, he held the position of President of the International Union of Biological Sciences.

It is only after 1967, however, that the foundations of his legacy were built. Between 1966 and 1970, he organized a series of three symposia held at Villa Serbelloni, in Bellagio, Italy. Waddington managed to gather together scholars in physics, embryology, genetics, chemistry, philosophy, and engineering for a period of 1 week to 10 days, to engage in intense interdisciplinary debates on the fundamental dynamic nature of development, and its principles of organization. Waddington aimed to design the core elements of Theoretical Biology, a new field of inquiry with its "own skeleton of concepts and methods" (1968: i). Among the participants, there were distinguished scholars such as the theoretical biologist Brian Goodwin (1931–2009), the neuroscientists Jack D. Cowan and Richard Gregory (1923–2010), the geneticist John Maynard Smith (1920–2004), the physicists David Bohm (1917–1992), Edward William Bastin (1926–2011), Edward H. Kerner (1924–2002), and Howard H. Pattee (1926), the theoretical chemist Christopher Longuet-Higgins (1923–2004), the philosopher Marjorie Grene (1910–2009), the topologist René Thom (1923–2002), the biologist Lewis Wolpert (1929), and the automata theorist Michael A. Arbib (1940). As a result of these intense meetings, four volumes united under the general title *Towards a Theoretical Biology* were published: *Prolegomena* (1968), *Sketches* (1969), *Drafts* (1970), and *Essays* (1972). Today, these volumes represent the core of Waddington's interdisciplinary efforts, and they attest to his theoretical legacy.

In 1970, Waddington left his position at the University of Edinburgh to hold the Chair in Science at the State University of New York. However, the American stay was brief, and Waddington decided to go back to Edinburgh just 2 years later, where he passed away on the 28th of September 1975 at the age of 70.

Work

Early Studies

As mentioned above, Waddington did not have the chance to pursue a career in genetics and instead turned to embryology. Nonetheless, Waddington did not approach embryology merely for reasons of employability. His dissatisfaction with the standard view that posed "preexisting forms of organization" (Waddington 1959a: 2) as explanatory of the regularity of developmental patterns, along with his previous experience with J.B.S Haldane, brought him more closely to the study

of the chemical conditions behind the processes of differentiation and organization that characterize the development of an organism (see Speybroeck 2002). Given that developmental processes apparently "[emerge] from an initially homogeneous material in which no pattern is present to begin with" (1959a: 2) – and that Waddington had discarded as questionable the hypothesis of an order "imposed from the outside" (1959a: 16) – he moved to examine the hypothesis that organisms do, in fact, possess the resources to direct the differentiation and organization of their own development. It is this hypothesis that set the guidelines for his first decade of research in experimental embryology, and that led to his well-known collaborations with Spemann, and subsequently with the Needhams and Brachet.

By the time Waddington approached Spemann, in 1931 (Waddington 1961: 58), the foundations for the mechanics of development had already been sketched by the German embryologists during the previous decades. Spemann was convinced that the emergence of developmental patterns could be traced back to particular physical regions of the embryo which he labelled "organization centers." According to Spemann, these regions acted as chemical inducers, causing a change in other physical regions in their proximity and thus influencing the development of the undifferentiated egg. As Waddington later reported, "the organiser was named as such because it appeared the mesoderm imposed on the reacting ectoderm a certain pattern; the mesoderm appears to determine that the forebrain appears here, the hindbrain there and the neural tube in a certain position. You get a complete orderliness in the body, which appears to be imposed on the ectoderm by the mesoderm" (1959a: 18). In the years that followed, Waddington's experimental work at Spemann Lab did, in fact, confirm Spemann's hypothesis, generalizing his findings beyond amphibians, to chick and rabbit embryos. These experiments led Waddington to extend the mechanics previously sketched by Spemann toward a "less superficial examination" (1959a: 18) and eventually to provide a novel and richer interpretation of the interplay between the inducing role of the organizer and the resultant effect in the cytoplasmic environment.

As mentioned before, Waddington received primary education in philosophy at an early age, focusing in particular on the works of Whitehead. He did not fall prey to the obscurities of the metaphysics in *Process and Reality* (1929). He instead came to the realization that scientific work could not be taken apart and interpreted in isolation from a scientist's metaphysical beliefs; these were not "mere epiphenomena but (things that) have a definite and ascertainable influence on the work he produces" (Waddington 1969: 72). As he himself later reported, "I am quite sure that many of the two hundred or so experimental papers I produced have been definitely affected by consciously held metaphysical beliefs, both in the types of problems I set myself and the manner in which I tried to solve them" (1969: 72). In fact, anticipating trends in the metaphysics of science that only very recently emerged in the philosophical literature, Waddington adopted a toolbox approach to metaphysics (French 2018), shaping Whiteheadian insights to fit the relevant embryology.

Waddington's experimental results obtained at the Spemann Lab, and through his collaboration with Brachet, Joseph and Dorothy Needham (1932–38), suggested that differentiation could not be a matter of chemical induction only, because the

relevant processes bringing the embryo from an undifferentiated homogeneous lump of matter to a differentiated whole often involve the causal role of the organizer combining with the products of its inducing action. While Spemann focused on the biological property of organization as a capacity to induce differentiated stages, Waddington focused on organization as the complex result of the inner capacity of organic matter in the cell to both act and react to specific (inducing) signals. The organizer was not, according to the young embryologist, the only causal condition for the emergence of developmental patterns. Instead, ready-to-be-induced biological regions possessed specific competences, or potentials, to react to inducing signals – dubbed evocation (Waddington 1961: 58–59) – by dynamically exhibiting phases in which a region "switches into one or other of alternative pathways of further change" (1961: 62–63). Nevertheless, at the time, there were no explored theoretical alternatives in biology to the causal reductionism adopted wholeheartedly by Spemann, among others. Waddington remedied this by performing one of the most exciting syntheses between science and metaphysics in the history of science – a synthesis that rightfully places him among the forefathers of contemporary works in metaphysics and biology (Nicholson and Dupré 2018). He put forward an explanation of development in interactionist terms, where causation was understood in terms of mutual, simultaneous interaction – rather than an asymmetrical, linear relation – and where its products were to be interpreted as the result of co-constructive processes between networks of genes and their cytoplasmic environment (see Fabris 2018).

The insertion of Whiteheadian metaphysics was revealing of his experimental results and eventually led to a renovated formulation of the classical embryological notions of competence, induction, individuation, and determination, in terms of a broader theoretical framework connecting genetics and embryology (Gilbert 2000). This framework contained the germs of early cybernetic theory, and anticipated, by almost a century, novel insights in the metaphysics of causation (see, e.g., Mumford and Anjum 2018) and niche construction theory (see chapter ▶ "Evo-Devo and Niche Construction"). This revolutionary investigation kept Waddington busy for many years beyond the decade mentioned above, during which he deepened and articulated his new Diachronic Biology, in which embryology, genetics, and evolution "formed a group whose interconnections are obvious and unavoidable" (in Wilson 1925: 1056) and that culminated with the publication of *Organisers and Genes* (1940). From the early 1940s onwards, Waddington relentlessly pushed biology to the verge of an intellectual revolution so far-reaching that it ended up shaping the future of the discipline. It was the birth of epigenetics.

The Rise of Epigenetics

It is tempting, from a contemporary perspective at least, to read into Waddington the intention to provide a fully formed new theory of biology right from the start. This would be misguided. The intellectual route that led to the formation of

epigenetics was, like Waddington's career, varied and eclectic, and yet not random. It was paved by his work on the notions of canalization of development and genetic assimilation which, in turn, built upon the interactionist framework cultivated during his doctoral studies. Waddingtonian epigenetics can be understood on the basis of these two main theoretical components.

The canalization of development is first and foremost an explanatory account of developmental change. According to the orthodox view of the time, mainly advanced by the architects of the Modern Synthesis, developmental changes were to be explained in preformationist terms: the organism was thought to change through time by unfolding and manifesting features already codified in the genome (Oyama 2000; Lewontin 2000). The biological whole that was the organism was thus reduced to a mere epiphenomenon of its genes (Gilbert and Sarkar 2000). On the contrary, Waddington's early interactionism centered on the reactive role of the genetic products, in relation to both the external and the internal environment (see also Peterson 2016).

Building on his renovated embryological notion of competence, Waddington suggested that at each stage of development, the organism's competences progressively undergo modification, restricting the possible differentiation outcomes, that is, the possible phenotypes. In *Organisers and Genes* (1940), Waddington referred to this phenomenon of the constraint of competences as the canalization of development. We now know that the term is much older than Waddington, as it is rooted in the metaphysics of Bergson (1911) and Whitehead (1929: 129; see Peterson 2016). Waddington's usage of the term, however, is an exquisite example of the toolkit approach to metaphysics recently discussed by French (2018).

Also in *Organisers and Genes* (1940), he put the notion of canalization to work in the explanation of the stability of developmental patterns in the face of atypical perturbative conditions. Although Waddington's experimental results suggested evidence of the reactive role of genetic products, these reactions were clearly not running amok; they instead seemed to be characterized by constancy of some kind. To explain how an organism in the course of its development produces stabilized, repeated phenotypic end-states across a population, Waddington offered a novel model for developmental compensation. This model firstly took the form of a pictorial representation – with the help of his friend and artist John Piper – and secondly – with the help of his friend and topologist René Thom – in mathematical terms, as a multidimensional vector field with time-extended attractors (Waddington 1968: 166–169). The model in question is the famous epigenetic landscape. The landscape is depicted as a system of valleys and pits where the formed pathways represent the connections between a cell in its undifferentiated cytoplasmic state, and its final, fully differentiated state (Waddington 1961: 64). Each route, dubbed a chreod (Waddington 1957, 1977: 105), represents one possible course for the manifestation of a developmental potentiality, achieved by the restriction of competences, the full range of which dubbed the chreodic profile (Waddington 1957, 1961).

By the time Waddington coined the term "canalization" in 1942, the idea of mutual causation in biology was already getting a foothold in the field, mainly

thanks to a new theoretical movement now well-known as organicism, whose members, like Waddington, sought to promote a nonreductive, post-Darwinian understanding of development (Nicholson and Gawne 2015). Unsurprisingly, Waddington was active in the movement and contributed to its expansion, strengthening the conviction that organismic persistence was inherently and irreducibly adaptive. The epigenetic landscape model served its purpose in sparking debate, becoming a popular topic of discussion both within and to some extent outside the movement. However, what was meant to be, at least initially, a heuristic for understanding the complex interplay between networks of genes and their products, developed into a more complete, and contentious, theory as it started to spread outside the den of neo-Darwinian thinking.

It was only by bringing evolution into the picture that Waddington's theory developed into a more mature and unified form, and yet it was met with some resistance. The idea of development exerting a directing control over evolution and inheritance was a natural extension of Waddington's previous work, yet the general principles of neo-Darwinism famously opposed any active role of the organism in creating novel mutations. Mutations were understood in terms of random variation, the appearance of which was best explained by the causal action of natural selection affecting the frequencies of genes in a population with different types of mating systems. This view – also called the Mendelist-Morganist view of heredity (Jablonka and Lamb 2005: 28) – took genes as the units of selection, and interpreted evolution as a change in the genetic composition of populations (Dobzhansky 1951: 11).

Waddington expressed his dissatisfaction with the Modern Synthesis in his *Paradigm for an Evolutionary Process* (1969), among other works. To see how development exerted a directive influence on both inheritance and evolution, one had to overcome the "simplistic" synthetic interpretation of the genome as the only instructor of development (1969: 107). The manifestation of new traits was instead rooted in the whole, time-extended, developmental system that, as a unit of development, actively reads and interprets the genome (Waddington 1968, 1969; see also Wilkins 2008). Waddington extended his notion of canalization to indicate the general capacity of all developmental systems to produce normal phenotypes despite genetic and environmental perturbations (Waddington 1940, 1975). Phenotypes, according to Waddington, are not the mere display of genetic information but rather the result of a compensatory epigenetic process, dubbed homeorhesis (1977), that guarantees "continual change along certain pathways" (1977: 195). While canalization – now understood as a developmental property of the organism as a whole – "acts to ensure that the system goes on altering in the same sort of way that it has been altering in the past, [homeorhesis] ensures the persistence of a given type of modification" (Waddington 1977: 105). It is this balance between the robustness and the plasticity of the system that instructs development and explains the process of the assimilation of acquired characters in a post-Darwinian way. The process by which "phenotypes' responses to environmental stimuli can be incorporated into the genotype through a process of selection" (1975: 59), Waddington labeled genetic assimilation (see chapter ► "Canalization: A Central but Controversial Concept in

Evo-Devo"). Critical for the confirmation of his novel view were Waddington's experiments on *Drosophila melanogaster*, whose results are discussed at length in "Genetic Assimilation of an Acquired Character" (1953), "Genetic Assimilation of the Bythorax Phenotype" (1956), and "Canalization of Development and Genetic Assimilation of an Acquired Character" (1959b). By inducing stress on *Drosophilae*, first in the form of heat shock on pupae (1953), then in the form of ether on larvae (1956), and finally using salt (1959b), he was able to observe phenotypic variation in heterogeneous lines. He then demonstrated that these phenotypic traits, if selected for a certain number of generations in the presence of the same stress, could be inherited through the germline (Piacentini et al. 2014), and therefore could appear again even in the absence of the original environmental inducer (1975: 59). Waddington interpreted acquired characters – in line with the Modern Synthesis – as concealed or hidden behind the robustness of canalized paths (1977). However, in contrast with the Synthesis, he interpreted the acquired characters as adaptive resources that the developmental system uses to control phenotypic changes. The organism is then active in changing the epigenetic landscape toward the formation of new chreods, thereby fostering new developmental possibilities (1975: 73). According to Waddington the developmental system selects genes from a highly variegated gene pool: it develops under their influence, expressed together with a heterogeneous environment. At any given stage of its life history, the ways its genes and its previous environment have acted up to that point have considerable effect on which inducing signals can further affect the system (1961).

The Modern Synthesis views on the extreme reduction of phenotype to genotype, and the complete randomness of evolutionary novelties, were, for Waddington, at the root of its theoretical limitations. And indeed, the Waddingtonian theory of the complex genetic regulation of developmental potentiality was at odds with the idea, promulgated by the architects of the Synthesis, of context-independent randomly generated mutations. In particular, it offended against the strict Weismannian separation between the soma and the germ-line (Jablonka and Lamb 2005). However, the critique that Waddington presented against the dominant scholarship of his time should not be interpreted as a radical fracture with it, but more mildly, as an extension of it.

First, Waddington was not aiming to replace the orthodoxy but rather to extend and enrich its explanatory power. As he nicely puts it, "supposing that at any time we become possessed of a complete scientific explanation of the process of evolution, as that is brought about by natural selection acting on gene mutations, for instance: we shall not then be justified in taking this as a complete general explanation of evolution" (Waddington 1929: 66). Second, Waddington's view on inheritance was in fact significantly different from other scholars, such as Lamarck and Baldwin. Lamarck, more than a century before Waddington, proposed an explanation of evolutionary novelties in terms of acquired characters. However, Lamarck insisted that the environment exerts a direct causal action on the phenotype. By contrast, Waddington maintained that selective pressures only contribute to, instead of exclusively determine, heritable modifications (1959a, 1975). Phenotypes are not in fact reshaped continuously by environmental stimuli, as the Lamarckian approach would

entail, but exhibit an invariance whose explanation could not be satisfactorily provided by modelling inheritance as a solely gene-environment affair (1961). On the other hand, neither was Waddington in agreement with Baldwin. According to Baldwin's theory of Organic Selection, the capacity of the organism to respond to environmental stimuli was not under genetic control (Baldwin 1896; see also Loison 2018). However, Waddington was not looking to reject the role of the genome in inheritance. Instead, according to his theory, the network in which genes are organized plays a prominent role in the regulation of the effects of the local inducing signals on the developmental system (1957).

It is also important to stress that Waddington's aforementioned synthesis between embryology, genetics, and evolution was also simultaneously and independently developed by Schmalhausen (see chapter ▶ "Ivan I. Schmalhausen (1884–1963)"). In his *Factors of Evolution* (1949), Shmalhausen attempted to reincorporate embryology within the Modern Synthesis and to stress the relevance of embryology and the role of the interactions between heredity and environment in producing the phenotype. Like Waddington, the Russian zoologist and evolutionary biologist proposed a similar concept of canalization that he called "autoregulation" (1949) and hypothesized a mechanism that explained the process of genetic stabilization, that is, how physiological induction could be transferred to embryological induction. However, despite the superficial similarities, in Waddington's own admission, Schmalhausen "did not formulate in any precise form the process which has been referred to genetic assimilation" (Waddington 1975: 75). According to Waddington, the type of explanation of the inheritance of acquired characters provided by Schmalhausen was in no way different from the Baldwin Effect and rather far-off his novel approach. The fundamental difference among the two syntheses was recognized by Dobzhansky, who favored Shmalhausen's work over Waddington's one because he considered the former "more suitable and better able to be integrated into the Modern Synthesis" (see Gilbert 1994: 153).

Waddington cannot be legitimately grouped together with Lamarck, Baldwin, or Shmalhausen; nor with Dobzhansky and Morgan. His unique understanding of the organism brought to attention the importance of the phenotype as a core explanatory element of the evolution of developmental systems. It laid the foundation for a renovated version of his earlier Diachronic Biology, a cutting-edge new field of research "that studies the causal interactions between genes and their products which bring the phenotype into being" (1968: 10). It was during the experiments that led to this novel view that Waddington coined the term epigenetics, "the causal study of embryological development" (1957:13). Under this theory, the canalization of development provided the ground for the contemporary understanding of robustness as the organismic ability to actively restore stabilized developmental pathways in the face of perturbations. Waddington's epigenetics provided a pioneering understanding of organisms as active agents capable of modifying their adaptive responses by adjusting their reactivity to external and internal inducing signals, again anticipating by almost a century later trends in evolvability studies (see chapter ▶ "Variational Approaches to Evolvability: Short- and Long-Term Perspectives").

Legacy

Tracing the legacy of Waddington is not an easy task. Given the multifaceted character of his research, his work had an impact in many directions. There are, however, a few lines of research that were, and still are, influenced by his intellectual heritage. Waddington's theoretical and experimental synthesis between genetics, embryology, and evolution profoundly entrenched genetics, ecology, molecular biology, and paleontology into what is nowadays known as evolutionary developmental biology, or evo-devo (Laubichler and Hall 2008). Waddington's contributions lie at the heart of what Hall (2003) individuated as the five tenants of evo-devo research agenda: "(1) the origin and evolution of embryonic development; (2) how modification of development and developmental processes lead to the production of novel features; (3) adaptive plasticity of development; (4) how ecology impacts on development to modulate evolutionary change" (2003: 492).

As a matter of fact, the novel causal analysis of the epigenotype established by Waddington, and his overarching attention to the whole dynamic developmental unit that comprises the genotype and the phenotype, offered the tools for the search of "emergent units and processes between genotype and phenotype and the basis of the evolutionary developmental mechanisms operating within each" (Hall 2003: 494). Within this research program, the epigenetic landscape, which had been marginalized for many decades, had slowly gained renewed attention from dynamic systems theorists who adopt an interactionist framework and seek to advance mathematical models for the study of embryogenesis (Huang 2012; Jaeger and Monk 2014; inter alia).

As mentioned in the former section, Waddington never embraced the Modern Synthesis, and therefore he never became one of its main contributors, nor he was counted among its official ranks (Slack 2002). Along with Goldshmidt (see chapter ▶ "Richard Goldschmidt (1878–1958)"), he is indeed well-known for his critique of neo-Darwinism. Yet, Waddington's critique was never as extreme as Goldschmidt's. By contrast to the latter, Waddington never doubted the existence of individual genes and their role in evolution; his objection was rather directed only against some of the fundamental assumptions of neo-Darwinism. As discussed in the previous section, Waddington's work on canalization and genetic assimilation was in direct contrast with the neo-Darwinian attention to random variation, or as Waddington called it, random search (Waddington 1968; see also Wilkins 2008). The spirit of the Waddingtonian revolution, and his criticism of random variation survives in the emerging framework of the Extended Evolutionary Synthesis, whose proponents, similarly to Waddington, emphasize the organisms' role in evolution and reject the primacy of natural selection (see chapter ▶ "Evo-Devo's Contributions to the Extended Evolutionary Synthesis"). Within this theoretical context, key Waddingtonian concepts of constructive development and reciprocal causation are now resurrected to explain the evolvability in phenomena like genetic assimilation (Pigliucci and Müller 2010), and in reciprocal niche construction and developmental scaffolding.

Likewise, Waddington's experimental line of research is far from being abandoned. According to Waddington, there was a general difference between the experiments on *Drosophila* of 1953 and 1956, and those of 1959. The former suggested an interpretation of genetic assimilation as a threshold phenomenon in which the manifestation of *crossveinless* and *bithorax* phenocopies were regulated by epigenetic mechanisms buffering environmental responses. The latter involved no threshold response; instead, it involved the action of epigenetic processes responsible for the modulation of organisms' adaptability in the abnormal environment with increasing levels of salt content. Contemporary experiments on canalization have benefitted from the discovery of the heat shock protein Hsp90 in *Drosophila*, a chaperone protein which responds naturally to environmental change. And yet scholars have not yet had the final word on which of the two interpretations is to be preferred (Fabris 2018). While one prominent model interprets the data alongside Waddington's experiments of 1953–1956 (Rutherford and Lindquist 1998), another, no less prominent, explains acquired characters as the result of de novo, rather than preexisting, mutations (Specchia et al. 2010). The discussion of whether we should adopt the first mechanistic approach or the second processual one is still open.

Finally, and building on this, the central role of the notion of process in Waddington's studies of development have recently sparked a debate in the metaphysics of science. In fact, his notion of process is not metaphysically innocent. He explicitly stressed that it was not intended in a purely epistemic sense; instead, it provided the correct ontology of living systems. During an era characterized by the expulsion of metaphysical commitments from science, Waddington boldly claims that organisms are "four-dimensional events, rather than aggregates of things" and that "the organisms undergoing the process of evolution are themselves processes" (1969: 72–73). Today, many philosophers of biology take seriously the role of metaphysical commitments behind scientific theories, as well as the interplay between these commitments and experimental methods (see also Mumford and Anjum 2018). Within this debate, two camps can be distinguished. According to the first camp, now usually said to promote a substance view, organisms are to be understood mechanistically, in terms of organized interactive components, and their development in terms of a succession of discrete ordered stages. According to the second camp, which promotes a processualist view, we ought instead to have a dynamic understanding of living entities (Nuño de la Rosa 2010; Nicholson and Dupré 2018). In contrast to the former, which conceives organisms as existing independently, and prior to any form of change or activity, processualists take change to be fundamental and regard static entities as transient stabilities that exist only at the limit of abstraction from continuous processes.

Cross-References

- ▶ Canalization: A Central but Controversial Concept in Evo-Devo
- ▶ Developmental Plasticity and Evolution
- ▶ Developmental Innovation and Phenotypic Novelty

▶ Dispositional Properties in Evo-Devo
▶ Eco-Evo-Devo
▶ Evo-Devo and Niche Construction
▶ Ivan I. Schmalhausen (1884–1963)
▶ Richard Goldschmidt (1878–1958)
▶ Variational Approaches to Evolvability: Short- and Long-Term Perspectives

Acknowledgments I would like to thank Laura Nuño de la Rosa and Gerd B. Müller for the opportunity to participate in this project. I am grateful for comments received on earlier versions of this chapter by Laura Nuño de la Rosa, Daniel J. Nicholson, Yawen Zou, Laurent Loison, and Andrea Raimondi. I would also like to take the opportunity to express my gratitude to Barbara Continenza, Scott Gilbert, Eva Jablonka, Marion Lamb, Paul Griffiths, Jan Baedke, Alan Love, and Laura Fanti for many discussions on Waddington's work.

References

Abir-am PG (1987) The biotheoretical gathering, trans-disciplinary authority and the incipient legitimation of molecular biology in the 1930s: new perspective on the historical sociology of science. Hist Sci 25(1):1–70

Bergson H (1911) Creative Evolution. Henry Holt and Company, New York

Baldwin JM (1896) A new factor in evolution. Am Nat 30(354):441–451

Dobzhansky T (1951) Genetics and the origin of species. Columbia University Press, New York

Fabris F (2018) Waddington's processual epigenetics and the debate over cryptic variability. In: Nicholson DJ, Dupré J (eds) Everything flows, toward a processual philosophy of biology. Oxford University Press, Oxford. 246–263

French S (2018) Toying with the toolbox: how metaphysics can still make a contribution. J Gen Philos Sci/Zeitschrift für Allgemeine Wissenschaftstheorie 49(2):211–230

Gilbert SF (1994) Dobzhansky, Waddington, and Shmalhausen: embryology and the modern synthesis. In: Adams MB (ed) The evolution of Theodosius Dobzhansky. Princeton University Press, Princeton

Gilbert SF (2000) Diachronic biology meets evo-devo: C. H. Waddington's approach to evolutionary developmental biology. Am Zool 40:729–737

Gilbert SF, Sarkar S (2000) Embracing complexity: organicism for the 21st century. Dev Dyn 219:1–9

Haldane JBS, Waddington CH (1931) Inbreeding and linkage. Genetics 16(4):357–374

Hall BK (2003) Evo-Devo: evolutionary developmental mechanisms. Int J Dev Biol 47(7–8):491–495

Huang S (2012) The molecular and mathematical basis of Waddington's epigenetic landscape: a framework for post-Darwinian biology? BioEssays 34(2):149–157

Jablonka E, Lamb MJ (2005) Evolution in four dimensions: genetic, epigenetic, behavioral, and symbolic variation in the history of life. MIT Press, Cambridge, MA

Jaeger J, Monk N (2014) Bioattractors: dynamical systems theory and the evolution of regulatory processes. J Physiol 592:2267–2281

Laubichler MD, Hall BK (2008) Guest editorial Conrad Hal Waddington: forefather of theoretical EvoDevo. Biol Theory 3(3):185–187

Lewontin RC (2000) The triple helix: organism, environment, and evolution. Harvard University Press, Cambridge, MA

Loison L (2018) Canalization and genetic assimilation: reassessing the radicality of the Waddingtonian concept of inheritance of acquired characters. Semin Cell Dev Biol:1–10. https://doi.org/10.1016/j.semcdb.2018.05.009

Morgan T H (1934) Embryology and Genetics. Columbia University Press, New York

Mumford S, Anjum R (2018) Causation in science and the methods of scientific discovery. Oxford University Press, Oxford
Nicholson DJ, Dupré J (2018) Everything flows, toward a processual philosophy of biology. Oxford University Press, Oxford
Nicholson DJ, Gawne R (2015) Neither logical empiricism nor vitalism, but organicism: what the philosophy of biology was. Hist Philos Life Sci 37(4):345–381
Nuño de la Rosa L (2010) Becoming organisms: the organisation of development and the development of organisation. Hist Philos Life Sci 1:289–315
Oyama S (2000) The ontogeny of information. Developmental systems and evolution. Duke University Press, Durham
Peterson EL (2016) The life organic. The theoretical biology club and the roots of epigenetics. University Press, Pittsburgh
Piacentini L, Fanti L, Specchia V, Bozzetti MP, Berloco M, Palumbo G, Pimpinelli S (2014) Transposons, environmental changes, and heritable induced phenotypic variability. Chromosoma 123:345–354
Pigliucci M, Müller GB (2010) Evolution: the extended synthesis. MIT Press, Cambridge, MA
Robertson A (1977) Conrad Hal Waddington. 8 November 1905 – 26 September 1975. Biogr Mem Fellows R Soc 314(1167):575–622
Rutherford SL, Lindquist S (1998) Hsp90 as a capacitor for morphological evolution. Nature 396:336–342
Slack JMW (2002) Conrad Hal Waddington: the last renaissance biologist? Nat Rev Genet 3:889–895
Specchia V, Piacentini L, Tritto P, Fanti L, D'Alessandro R, Palumbo G, Pimpinelli S, Bozzetti MP (2010) Hsp90 prevents phenotypic variation by suppressing the mutagenic activity of transposons. Nature 463:662–665
Van Speybroeck L (2002) From Epigenesis to Epigenetics The Case of C. H. Waddington. Ann. N. Y. Acad. Sci. 981:61–81
Waddington CH (1929) [unpublished] "Philosophy and Biology" (Essay Submitted for Arnold Gerstenberg Prize), Cambridge University, UK. (Waddington papers −University of Edinburgh Library [CHW-UEL], MS 3024.2)
Waddington CH (1940) Organisers and genes. Cambridge University Press, London
Waddington CH (1953) Genetic assimilation of an acquired character. Evolution 7:118–126
Waddington CH (1956) Genetic assimilation of the bithorax phenotype. Evol Int J Org Evol 10:1–13
Waddington CH (1957) The strategy of the genes: a discussion of some aspects of theoretical biology. George Allen & Unwin, London
Waddington CH (1959a) Biological organization: cellular and sub-cellular. Pergamon Press, London
Waddington CH (1959b) Canalization of development and genetic assimilation of acquired characters. Nature 183(4653):55–56
Waddington CH (1961) The nature of life. The Scientific Book Club, London
Waddington CH (1968) Towards a theoretical biology, vol. 1: prolegomena. Edinburgh University Press, Edinburgh
Waddington CH (1969) Towards a theoretical biology, vol. 2: sketches. International Union of Biological Sciences & Edinburgh University Press, Edinburgh
Waddington CH (1975) The evolution of an evolutionist. Cornell University Press, Ithaca
Waddington CH (1977) Tools for thought. Paladin, St Albans
Whitehead AN (1929) Process and reality. The Free Press, New York
Wilkins AS (2008) Waddington's unfinished critique of Neo-Darwinian genetics: then and now. Biol Theory 3(3):224–232
Wilson EB (1925) The cell in development and heredity. Macmillan, New York

John Tyler Bonner (1920–2019)

Stuart A. Newman

Contents

Abstract

John Tyler Bonner made major conceptual and experimental contributions to developmental and evolutionary biology over a career that spanned three-quarters of a century. Most of his work was conducted at Princeton University, where his laboratory and those of several generations of scientific descendants transformed the study of cellular slime molds, in particular *Dictyostelium discoideum*, into a major subfield of developmental biology. Bonner and his associates identified the cell aggregation attractant of *D. discoideum* as cyclic AMP, and the regulator of aggregate spacing as ammonia. His work appears to be the first to demonstrate the existence of a reaction-diffusion biological patterning mechanism with identified molecular components. In his conceptual work on morphogenesis and the evolution of form, expounded in more than a dozen books, he characterized many of the multiscale dynamical mechanisms now understood to function in animal and plant development, as well common principles that unify the understanding of morphological evolution in genetically disparate multicellular organisms. Bonner was an advocate of unifying the study of development and evolution, and his organization of the 1981 Dahlem Workshop on Evolution and Development was a founding event of the field of evolutionary developmental biology.

S. A. Newman (✉)
New York Medical College, Valhalla, NY, USA
e-mail: newman@nymc.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_32

Keywords

Morphogenesis · Cellular slime molds · Cell polarity · Plasticity · Phenotype-first

Life

John Tyler Bonner was born on May 12, 1920 in New York City, but spent most of his childhood in Locust Valley, Long Island, near Paris, at boarding school in Switzerland, and then in London, where his parents, Lilly Marguerite Stehli and Paul Hyde Bonner, a novelist, were part of the literary and social circle that included the novelist Rebecca West. His family returned to the U.S. in 1934 so that he and his brothers could attend Phillips Exeter Academy, in New Hampshire, following which he went on to Harvard, where he received his B.S. degree in 1941. He remained at Harvard for graduate work where he was soon appointed a Junior Fellow of the Society of Fellows. But then World War II intervened and he enlisted in the Air Force, serving as chief of the Biological Specialties Unit of the Aero Medical Laboratory at Wright Field, and conducting research on high altitude physiology and flotation factors in the design of life vests. He worked for a few months in the rainforest of the Barro Colorado Research Station in the Panama Canal Zone during this period, which, as he later wrote, gave him a deep appreciation of the complexities of ecological systems (Bonner 2002; Sunderland 2008).

These early experiences, privileged but cosmopolitan, coupled (from the evidence of his own memoirs and the testimony of colleagues (Brigandt et al. 2019)) with a quizzical and self-reflective temperament, provided Bonner with the confidence required to successfully grapple with some of the major, perennial questions of developmental and evolutionary biology. Though equipped with no paleontology and little genetics, he made his mark in the broader fields by channeling the *umwelt* of an inconspicuous soil microbe, declaring in a scientific memoir, "I have devoted my life to slime molds" (Bonner 1993).

The phylogenetically peripheral nature of his chosen organism (the social amoeba *Dictyostelium discoideum*) relative to the animals and plants that were the mainstream subjects of these fields then, as now, took Bonner down a different path from other founding figures of evo-devo. Unlike Stephen Jay Gould (1941–2002) and his predecessor Gavin de Beer (1899–1972), for example, who were concerned with how alterations in development could lead to evolutionary change, Bonner wondered how evolution gave rise to developmental systems, an arguably more challenging problem. A series of three lectures delivered at University College, London in 1955 was, in fact, titled "The Evolution of Development" (Bonner 1958).

Bonner first encountered *D. discoideum* as an undergraduate in the laboratory of the botanist and mycologist William H. Weston, Jr. (1890–1978) when he read the 1936 thesis of Kenneth B. Raper (1908–1987) a Ph.D. student of Weston's (Bonner 2002). Although Raper described the life cycle of the organism in great detail (Raper 1940) and continued to make important contributions to the biology of the dictyostelids, among other microorganisms, "cellular slime molds" (CSMs) would

almost certainly have remained on the margins of biological science for the next decades had they not been taken up by Bonner, for whom learning of them was an epiphany that spurred an explosive effort to understand the full scope of morphogenesis.

After the interruption by his military service, Bonner returned to Harvard, where he completed his own Ph.D., also with Weston, and also on *D. discoideum*. Receiving his degree in 1947, he spent a summer at the Marine Biological Laboratory (MBL) in Woods Hole, Massachusetts, where he was invited to share an office with an elder of the field he aspired to join, Edwin Grant Conklin (1863–1952). Ross Granville Harrison (1870–1959), another scientist he revered, was also in attendance (Bonner 2002). Neither of the embryologists would have recognized Bonner's research program as akin to theirs. Not receiving a hoped-for offer from Harvard, he took up an assistant professorship at Princeton (Fig. 1), where he remained throughout his career, delivering his last lecture in 2009 (Valenti 2019).

Bonner had an early flush of success based on his lectures on the other-worldly life cycle of *D. discoideum* featuring time-lapse movies he made as a student. One of these eventually became iconic in the field, with more than 300,000 views on YouTube (https://youtu.be/bkVhLJLG7ug) as of mid-2020. This led to coverage in the popular press and a request for a viewing of the movie by Albert Einstein himself. The pinnacle of this early acclaim, however, solidifying his intuition that he had struck gold scientifically, came when Harrison, then in his 80's, told the younger man during a speaking visit at Yale that "if he were starting all over again, he would work with slime molds" (Bonner 2002).

Bonner received several awards and honorary degrees, including two Guggenheim Fellowships (1958; 1971–1972) to work at the University of Edinburgh, election to the American Philosophical Society (1972), the US National Academy of Sciences (1973), and as an Honorary Fellow of the Indian Academy of Sciences (1992). An interest in India was initially stimulated by a spirited correspondence with the theoretical biologist J.B.S. Haldane (1892–1964) who

Fig. 1 John Tyler Bonner in his Princeton Laboratory (ca. 1990). (Courtesy of the Indian Academy of Sciences)

had relocated to Calcutta a few years after the two had met at Bonner's University College lectures (Bonner 2002). This was reinforced by a friendship, beginning in the 1970s, with a Dictyostelium-focused evo-devo scientist of the generation that followed his, Vidyanand Nanjundiah of the Indian Institute of Science. Bonner spent several sabbaticals and extended visits in Bangalore (1990–1991; 1993; 1998) at the invitation of his younger colleague (Brigandt et al. 2019), a further example of his habit of stepping into an unfamiliar world and making himself at home.

Work

In the early days of Bonner's career little could be rigorously established about how distant on the tree of life the social amoebae (or cellular slime molds) were from the embryophytes (land plants) and metazoans (animals). What was clear, however, was that unlike animals they had no soma-germline separation, and unlike plants they generated their forms using individual cellular and globally coordinated multicellular motility. Notably, they developed by aggregation rather than clonally, in contrast to both types of embryo-based systems. Given Bonner's scientific ambitions, the lack of demonstrable phylogenetic affinities between *D. discoideum* and any other complex organisms was an obstacle that he set out to surmount. He had no alternative but to reconceive the evolution of morphogenetically capable multicellular systems.

It is therefore misleading to think of Bonner's scientific contributions, as most appreciations before and after his death have done, as consisting mainly of putting a new "model system" on the map. Although this is certainly part of what he accomplished, his integrative work, largely in the form of conceptually oriented books and monographs that appeared regularly alongside, and somewhat independently of, his experimental research papers, as well as his own statements (cited in Nanjundiah (2019b)), make it clear that Bonner did not conceive of *D. discoideum*, as a model for anything else. As a multicellular life form, though one as exotic as they come, it was worthy of studying on its own terms. But in his unprovincial way, Bonner took this as a major challenge since the principles of its development and origination were so far outside the expectations of the developmental and evolutionary models prevalent in the mid-twentieth century. Because Bonner's experimental work, which established a previously nonexistent branch of developmental biology, and his integrative work on the evolution of morphogenesis, were produced on parallel tracks, they will be summarized separately here.

Biology of the Dictyostelid Life Cycle

The problem agenda of Bonner's empirical research program is embodied in the Dictyostelium life cycle, first described by Raper (1940), but recounted here in his own words in his 1993 memoir *Life Cycles*, refined and embellished by what he had learned himself in the interim:

> The molds begin as encapsulated spores which split open, and out of each spore emerges a single amoeba. This amoeba immediately begins to feed on the bacteria that are supplied as food, and after about 3 hours of eating they divide in two. At this rate it does not take long for them to eat all the bacteria on the agar surface—usually about 2 days. Next comes the magic. After a few hours of starvation, these totally independent cells stream into aggregation centers to form sausage shaped masses of cells each of which now acts as an organized multicellular organism. It can crawl towards light, orient in heat gradients, and show an organized unity in various other ways. It looks like a small, translucent slug about a millimeter long (indeed, this migrating mass of amoebae is now commonly called a 'slug'). It has clear front and hind ends, and its body is sheathed in a very delicate coating of slime which it leaves behind as it moves, looking like a microscopic, collapsed sausage casing.. . . After a period of migration whose length depends very much on the conditions of the slug's immediate environment, the slug stops, points up into the air, and slowly transforms itself into a fruiting body consisting of a delicately tapered stalk one or more millimeters high, with a terminal globe of spores at its tip. (Bonner 1993, pp. 3–4)

There are excellent accounts of Bonner's contributions to understanding the described transitions of the CSM life cycle, including his own monographs on the subject (Bonner 1967, 2009) and an appreciation written shortly after his death by Nanjundiah (Nanjundiah 2019a). In line with the objectives of this Reference Guide therefore, a few high points with important implications for the broader field of evolutionary developmental biology will be described here. The remainder of this section draws heavily on Nanjundiah's review, which can be consulted for references to cited findings.

The first major Dictyostelium-related question Bonner addressed was the means by which the organism's cells communicated with one another in the course of aggregation. Although he was just a graduate student when he came up with the answer, it essentially turned CSM research from a fascinating but obscure pursuit into a new field. A popular idea in the 1940s was one advanced by the Austrian biologist Paul Weiss (1898–1989), in which cells navigated by "contact guidance" whereby they detected features of the solid substratum to which they adhered. Bonner thought another mechanism, chemotaxis, attraction to a diffusible signal, might be at work. Bonner had met Weiss, who tried to persuade him to discard his notion, but he persisted. As he recounted:

> I tried hard to keep an open mind and investigated the possibility that the amoebae were oriented by some sort of electrical force or by some interfacial phenomenon happening on the surface between the moving amoebae and the substratum in an attempt to demonstrate Weiss's contact guidance. . .None of these things worked; all my experiments seemed to rule them out. At the same time I could not prove chemical attraction either. During the course of this work I had developed a way to have aggregation occur on the bottom of a glass dish under a layer of water. One day, to see if a current affected the orientation of the amoebae, I decided to swirl the water very slowly in a circular dish with a bent stirring rod over some aggregates. I left the motor running and after some time glanced through the dissecting scope to see what happened. I was really not expecting much, and what I saw nearly blew me through the roof. The current had produced an asymmetrical aggregation pattern: there were no oriented amoebae upstream of the center; they seemed to be wandering about aimlessly, while the amoebae downstream were perfectly oriented toward the center and moving against the current. In a flash I realized the attraction had to be by diffusion, and the diffusing

> agent had been moved downstream by the current, like wind moving the smoke from a pile of leaves in the fall, with no smoke upwind, and the smoke trailing long distances downwind. (Bonner 2002, pp. 76–77)

Analyzing the later-famous movie that Bonner took of the organism's life cycle, his mathematician colleague L. J. Savage found that the amoebae could sense directionality if the concentration of attractant differed by as little 2% across the cell's length. The cells responded by elongating and moving up the gradient, and then producing substances that caused them to stick together (Bonner 1944, 1947). Since the attractant's identity was unknown, Bonner delved into English literature for a name: "[I]n Edmund Spenser's *Faerie Queene* there is a witch named Acrasia who attracted men and transformed them into beasts. This seemed perfect for me because the chemical attracted the amoebae and they were transformed into stalk cells and spores. So I named the attractant 'acrasin'" (Bonner 2002, p. 78).

Bonner's film showed the cells undergoing periodic bursts of movement as they underwent aggregation, and this was later related to the oscillatory production and release of acrasin, and its relaying from cell to cell and its degradation. When Bonner and his colleagues eventually identified the diffusible signal in *D. discoideum* acrasin as cyclic AMP (cAMP) CSM studies leapt into the emerging field of molecular developmental biology. This was entirely unexpected, however, since in the animal systems that were the paradigms of development cAMP was an intracellular effector – a "second messenger" – of endocrine hormones. The discovery that it was used extracellularly in *D. discoideum* and (as it later became evident) was so distinct from the protein growth factors that serve as the morphogens (diffusible developmental signals) in animal embryos, reinforced the outlier status of the CSMs. However, it had the effect of making their life cycles a theoretical problem in which conditionality and dynamics was more a focus than the machine-like programs thought at the time to underlie animal embryogenesis, which supposedly varied from one species to another, and from their common ancestors, by accumulation of small genetic changes due to adaptive selection.

This alternative, evo-devo-anticipating perspective was reinforced when it was later recognized that CSM species other than *D. discoideum* used different chemoattractants for aggregation. A modified dipeptide is the acrasin in *Polysphondylium violaceum* (Shimomura et al. 1982), for instance, and a derivative of folic acid serves this role in *Dictyostelium minutum* (de Wit and Konijn 1983). This conservation of the formal aspects of key developmental processes despite substitution of molecular identities anticipated "developmental system drift" (True and Haag 2001) and the broader phenomenon of autonomy of the morphological phenotype (Müller and Newman 1999; Newman 2019; see chapter ▶ "Inherency").

Bonner treated the question of the size of the aggregation territory and resulting spacing of fruiting bodies as a problem in biological pattern formation of a kind that later came to be studied in animal and plant systems. (These relationships were not lost on Bonner, as discussed below.) A striking property of the pattern is that it barely changes over a large range of cell densities. A possible explanation for this phenomenon was that a gaseous factor released by each center prevented other centers

from forming close to it. Bonner and colleagues explored this possibility in the dictyostelid *D. mucoroides* and found that ammonia in fact served as such an inhibitor, working in an opposite fashion to cAMP, which functions as an aggregation center activator (Thadani et al. 1977). In the brief paper describing this, the investigators cite Gierer and Meinhardt (1972), a theoretical analysis of reaction-diffusion mechanisms as a possible basis for spacing patterns in developing systems, using hydra as the reference organism. The 1977 paper from the Bonner group appears to be the first example in the literature of this mechanism (which is closely related to Alan Turing's proposed "chemical basis for morphogenesis"; Turing 1952) with chemically characterized morphogens. Turing-type mechanisms have come to be enormously popular models of developmental pattern formation and related evo-devo scenarios in recent years (Kondo and Miura 2010; Newman et al. 2018).

Some early findings by Raper had set the stage for these investigations. In his description of several CSM species Raper had noted that the sizes of the stalk and spore mass (sorus) of very different sized fruiting bodies exhibited a constant proportionality). A hint to how this was regulated was found in experiments that pointed to plasticity of cell fate, i.e., presumptive stalk versus presumptive spore. Raper cut slugs transversely and found that the resulting fragments reorganized into miniature slugs and, with little or no mixing between different regions, could give rise to normal fruiting bodies (Raper 1940). Thus, the fate of a cell at the slug stage of development seemed labile and capable of switching between the two types.

By the early 1950s Bonner had determined that aggregating cells differed in their speed of movement, and that within the migrating slug the relatively faster cells accumulated in the anterior, prospective stalk region (Bonner 1952). The cells in the anterior region were also generally larger (Bonner et al. 1955; Bonner and Frascella 1953). But only when he and his colleagues tracked the presence of cell type-specific markers (histochemically determined, since gene expression methods were decades in the future) could they confirm Raper's observation that the spatial pattern could regulate. Specifically, whatever phenotypic differences were present in the pre-aggregation population, and distributed nonuniformly in the slug, could be overridden.

In a remarkable 1957 review, Bonner integrated these observations on cell heterogeneity and regulation into a theoretical model (Bonner 1957) that resembles nothing so much as present-day multiscale analyses of proportion regulation in animal embryogenesis. Nanjundiah (2019a) characterizes the model as follows: (i) to begin with, the cells that join an aggregate are heterogeneous; (ii) in part, their speeds of movement differ, because of which (relatively) fast cells – presumptive stalk cells – end up in the front of the slug and relatively slow cells in the back; (iii) a factor is supplied by slow cells to fast cells at a rate proportional to the number of slow cells, (iii) the factor is utilized by fast cells at a rate proportional to their own number, (iv) the factor is used by presumptive stalk cells to differentiate terminally into stalk cells and (v) cells that do not become stalk, become spores.

The result is that cell type proportions equal the ratios of the rates at which the relevant processes occur. By combining initial heterogeneity (which leads to non-uniformity in the slug) with intercellular communication (which mediates

regulation), Bonner accounted for both the spatial pattern of cell types in the slug and structural proportions of the fruiting body.

Throughout Bonner's paper he compares observations and experimental results on the CSMs *D. discoideum*, *D. mucoroides*, and *Polysphondylium*, which all differ in the cell type distributions of their slugs and the morphologies of their fruiting bodies. In a manner that either would not have occurred to animal developmental biologists of the time, or considered infeasible to address mechanistically by them, when comparing, say, development of bivalves and snails, or fish and birds, Bonner concludes the article with a section on "Evolution of Cellular Slime Molds." In this section he states first, that assuming an adaptive value of the morphogenetically complex life cycle of CSMs over free-living amoebae is "gratuitous," and that "there is little or no evidence to show that this is the case." He offers a few adaptationist hypotheses about the origin of the fruiting body, but then coming to *Polysphondylium*, which has a fruiting body with many side branches, states that "here the adaptive advantage seems especially hard to grasp." In fact, his model accommodates both classical selectionist scenarios (a perspective that came to be upheld by others in later years; Strassmann and Queller (2011)), but also the possibility of novelties arising incidentally from the dynamics of the developmental system ("the continuous variation turns into a discontinuous one") (see chapter ▶ "Developmental Innovation and Phenotypic Novelty"). And the generation of those novel forms (what he called "neutral morphologies"; Bonner (2013)) need not be gradual, which put him at odds with the prevailing Modern Synthesis, but on the path to evo-devo.

Evolution of Development

Fifty years after the fact, Bonner wrote that the motivation behind his first book, *Morphogenesis* was "to show that the methods and the ideas of the old embryology could be extended to all organisms, and that bacteria, algae, fungi, amoebae and slime molds, and protozoa did as much developing as animal embryos used in conventional embryology" (Bonner 2002, p. 98). This was not well received by some senior figures in the relevant fields: he recounted the dismissive reactions he received to this proposal in conversations with both the French embryologist Boris Ephrussi (1901–1979) and the German-American biophysicist Max Delbrück (1906–1981), both proponents of the emerging gene-centric developmental biology (Bonner 2002).

Bonner was hardly resistant to a role for genetics in the theoretical framework he was attempting to fashion. The Watson-Crick model of the gene did not yet exist, but the legend of Thomas Hunt Morgan's (1866–1945) leaving developmental biology to establish the field of developmental genetics because the problem of heredity was "something easier" than regeneration was the first thing mentioned in the Introduction to *Morphogenesis* (Bonner 1952). His lesson from this was that a "micro-theory" would be necessary to explain development. He firmly asserts his confidence that biological phenomena are fundamentally based in physics and chemistry, but that those fields might have to be "enlarged" to encompass biological phenomena.

Genetics, as it matured, would certainly contribute importantly to the understanding of development in Bonner's view, but he expresses this conviction in a way that is prescient relative to current understanding: "[I]t may be that any micro-theory or micro-theories of development are part of the gene theory of heredity" (Bonner 1952, p. 10). Putting it this way preemptively (though unfortunately, ineffectively) undermined the "genetic program" notion that took hold once the molecular structure of the gene was elucidated. Rather than development being part of heredity (as is increasingly recognized) the standard idea for the following half-century was that heredity was genes and gene–controlled development.

Given the phylogenetic distance of his chosen experimental organism from the mainstream ones of animal and plant developmental biology, Bonner was compelled to take an evolutionary perspective if he was to draw any broadly applicable theoretical lessons from his experimental work. And since it ultimately turned out that the developmentally relevant genes in these highly divergent organisms were largely different, the lack of genetics when he first started out was not a liability. Instead, his conceptual framework was based on tangible physical processes: growth, morphogenetic movements, polarity, and differentiation. All of these pertain to all developing forms, notwithstanding having different molecular underpinnings in different lineages. In the first of three lectures in his 1955 University College series on the evolution of development he notes that "development has not originated once in a common ancestor of all larger organisms, but a number of times" (Bonner 1958), and his framing in terms of the fundamental processes described in his 1952 book and in these talks shows that the components must be generic features of life that are separate from any specific genetic toolkit. This comports with present-day evo-devo recognition of a frequent disconnect between conservation of morphological traits and the genetic processes underlying them (see chapter ► "Developmental Homology").

In the 1952 book, in a trope he revisited throughout his theoretical writings, he presents the concept of what has come to be termed developmental bias. In a characteristically charming and homespun fashion, he writes:

> Development is first divided into two broad categories, which we will call the "constructive" processes and the "limiting" processes. The former are all those that tend to build up, and the latter those which check, guide, and channel the constructive processes. This introduces the idea that development is a result of the interaction of two somewhat opposed processes, one building up, the other checking. It might be compared to the old Scottish game of curling, where the stone is first propelled across the glare ice and its progression is guided and cajoled by the player who frantically roughs the ice in front of the stone with his broom so as to give it just the right speed and direction. (Bonner 1952, p. 7)

But it was in his consideration of the constructive processes of development that he may ultimately have turned out to be most radical. As described in the previous section, in his 1957 dynamical model of CSM development he showed how different rates and ratios of the fundamental processes could lead to abrupt (we would now say nonlinear) changes in morphology, with divergent evolutionary consequences.

In a later book on randomness in evolution, with a nod to the theoretical biologist D'Arcy Wentworth Thompson (1860–1948), Bonner introduced the idea of "neutral phenotypes" (Bonner 2013). By this term he referred to morphological motifs arising from causes (such as inherent organizational processes) which can take hold in an ecological setting independently of a prior history of natural selection. His concept of developmental processes as potentially generating forms without any honed adaptive advantage led Bonner to a heterodox view of the evolutionary process. Regarding D'Arcy Thompson, whose eclipsed work he pointedly kept alive in an increasingly gene-centric environment by editing an abridged edition of *On Growth and Form*, he noted that the author's ideas "were heretic in 1917 and it must be admitted that, for partly different reasons, they remain so today" (Thompson 1961).

One problem with D'Arcy Thompson, the one that led to his dismissal by the neo-Darwinian consensus, was his obliviousness to the role of the gene in the hereditary propagation of phenotypes. How were the forms produced by surface tension, viscous flow, and other mechanical effects incorporated into a species' developmental repertoire when the external conditions of their realization changed? Here Bonner invoked genetic assimilation, citing the work of the mid-twentieth century biologists Ivan I. Schmalhausen (1884–1963) and Conrad Hal Waddington (1905–1975) who suggested, independently, that selection against variability in outcome of developmental pathways which originally depended on particular environments could convert the conditional to the genetically enforced (Bonner 1988). Such "phenotype-first" scenarios for the evolution of complexity, with the implication of nonprogrammed determination of phenotype, have become mainstays of modern evo-devo.

Lastly (although the richness of Bonner's ideas went well beyond the limited treatment possible here) was his conviction that not all kinds of organisms were equally subject to natural selection. This nonuniformitarianism, the idea that while every organism is the product of evolution, the causal basis of the evolutionary process itself has changed, would have made him unpopular with the evolutionists of the 1950s and 1960s, had they been paying attention. But his views, unlike those of most animal and plant biologists of the time, were formed by his engagement with life cycles in which all stages were subject to different ecological settings (as in the CSMs) rather than, primarily, mature phenotypes. They are no longer controversial.

As he writes in his 2002 memoir:

> [U]nder many ecological circumstances a larger organism will have an advantage in the competition for resources over a smaller one. So natural selection is simultaneously favoring a very small stage for managing heredity or dispersal and a very large stage for effectively competing for energy in the form of food. This led me to an important conclusion about the life that development was the inevitable result of sex and size. The life cycle was framed by these twin pressures of natural selection. Obviously these ideas were a direct outcome of my fixation with life cycles. Slime molds, ciliates, algae, insects, human beings—we are all life cycles. It is not just the adult that evolves through natural selection but the entire cycle. (Bonner 2002, p. 115–116)

Throughout Bonner's career he continually revisited the role of increased size (usually a surrogate for multicellularity) in accelerating evolution, arguing that it enabled greater morphological and cell-type disparity, hence more competition and selection. Once nervous systems arose, they caused another shifting of modes by enabling nongenetic transmission of experiences to offspring – in its most advanced form, cultural evolution. Because behavioral inheritance does not typically involve changes to the morphological or physiological phenotype, Bonner considered cultural evolution as relatively autonomous from the individual-based modes. He restated and elaborated on these themes in his last paper, written when he was 98 years old (Bonner 2019). It was published posthumously in a memorial issue in his honor in *Journal of Experimental Zoology* Part B, where it was accompanied by engaged responses from biologists and philosophers from the evo-devo community (Brigandt 2019).

Legacy

John Bonner has a unique legacy in evo-devo. He began with certain handicaps that would have derailed the careers of less adept figures. He styled himself from the start both a developmental and evolutionary biologist, working on an organism that almost no one had heard of, which was unrelated (by far) from any currently under investigation by the powerful senior figures in experimental embryology and their academic progeny. He immediately began to question tenets of the Darwinian orthodoxy during the Cold War 1950s, when anything that hinted at plasticity or saltationism in evolutionary theorizing was treated in the US academy as Communist-tainted heresy (Robison 2018). He embarked on his research program at the threshold of the genetic-program paradigm with no genetics training or orientation, and a disinclination to embrace it any more than other relevant modes of explanation when genes became part of CSM biology. He adopted systems biology ways of thinking before there was such a thing as systems biology.

Yet through scientific brilliance, along with a modest and witty demeanor, he became a popular figure in science, garnering funding, awards, and other accolades. It did not hurt that his chosen organism was (initially) so obscure that he was not perceived as a threat to any established work or ideas. If *D. discoideum* "developed" or "evolved" differently from animals or plants, no problem. So the subfield he founded exploded into a growth industry, producing nearly 10,000 papers from groups throughout the world over approximately 65 years, with CSMs taking their place among the prominent "model organisms," although this was a concept, as we have seen, that Bonner rejected.

It also helped that he was a graceful and prolific writer, spinning out books full of insights, anecdotes and illuminating analogies at the rate of one every few years. These books represented a parallel career of experimentally informed theorizing. Though he did not work on canonical organisms in his laboratory, he wrote about them extensively in his monographs and essay collections, not shying away from

drawing lines and connections, not via genes, but through dynamical processes, between CSMs and other aggregative and other multicellular forms to the animal and plant systems from which they diverged early in the history of life.

He was a conscious propagandist (a strong term for someone who performed the role so mildly, but accurate nonetheless) for a new synthesis of development and evolution, both in the titles of his monographs (*Morphogenesis*, *The Evolution of Complexity*, *Cells and Societies*) and in his edition of D'Arcy Thompson's *On Growth and Form*. The latter treatise, the work of a waning and at the time intellectually marginal figure, coming as it did in 1961, would likely have been perceived as a poke in the eye to the establishment if released by any other 40-year-old scientist perceived as aspiring to centrality in either developmental or evolutionary biology. But Bonner brought it off. As it happened, the book became an inspiration to a new generation of molecular biology-informed theoreticians who were poised to bring physical and mathematical concepts to the study of development in a broad range of organismal systems.

Probably Bonner's most important intervention along these lines was organizing the Dahlem Workshop on Evolution and Development in May of 1981 and editing the resulting report (Bonner 1983). Here he brought together most of the major figures of the time in development and evolution, including Eric H. Davidson (1937–2015), Stephen J. Gould, Antonio Garcia-Bellido (1936–), Pere Alberch (1954–1998), and Rudolf Raff (1942–2019). Many of these scientists may have met for the first time in Berlin, and this meeting probably marked evo-devo's origin as a field.

Based on these accomplishments and activities John Bonner could reasonably be considered the most seminal figure in the creation of evo-devo. Certainly, contemporary developmental biological research, based as it is on cell-cell interactions and signaling, utilizing physical concepts of oscillatory and self-organizational behaviors, resembles the work on CSMs he began in the 1950s much more than the experimental embryology of that era. Moreover, the evolutionary theory of our time has departed from gradualist adaptationism in ways that Bonner anticipated very early on. In this respect, though, however much the obscurity of his chosen experimental system allowed him to work unhindered by ideological fashions, it also kept him out of the limelight and prevented his broader recognition as a scientific genius truly ahead of his time.

Cross-References

- ▶ Conrad Hal Waddington (1905–1975)
- ▶ Developmental Homology
- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Developmental System Drift
- ▶ Evo-Devo's Contributions to the Extended Evolutionary Synthesis
- ▶ Evolution of Complexity
- ▶ Gavin de Beer (1899–1972)
- ▶ Inherency

▶ Ivan I. Schmalhausen (1884–1963)
▶ Stephen Jay Gould (1941–2002)

References

Bonner JT (1944) A descriptive study on the development of the slime mold *Dictyostelium discoideum*. Am J Bot 31:175–182
Bonner JT (1947) Evidence for the formation of cell aggregates by chemotaxis in the development of the slime mold *Dictyostelium discoideum*. J Exp Zool 106:1–26
Bonner JT (1952) Morphogenesis; an essay on development. Princeton University Press, Princeton
Bonner JT (1957) A theory of the control of differentiation in the cellular slime molds. Q Rev Biol 32:232–246
Bonner JT (1958) The evolution of development; three special lectures given at University College, London. University Press, Cambridge, UK
Bonner JT (1967) The cellular slime molds. 2d rev. and augm. edn. Princeton University Press, Princeton
Bonner JT (ed) (1983) Evolution and development: report of the Dahlem workshop on evolution and development, Berlin 1981, May 10–15. Life sciences research report 22. Springer, Berlin/New York
Bonner JT (1988) The evolution of complexity by means of natural selection. Princeton University Press, Princeton
Bonner JT (1993) Life cycles. Princeton University Press, Princeton
Bonner JT (2002) Lives of a biologist: adventures in a century of extraordinary science. Harvard University Press, Cambridge, MA
Bonner JT (2009) The social amoebae: the biology of cellular slime molds. Princeton University Press, Princeton
Bonner JT (2013) Randomness in evolution. Princeton University Press, Princeton
Bonner JT (2019) The evolution of evolution. J Exp Zool B Mol Dev Evol 332:301–306. https://doi.org/10.1002/jez.b.22859
Bonner JT, Frascella EB (1953) Variations in cell size during the development of the slime mold Dictyostelium discoideum. Biol Bull 104:297–300
Bonner JT, Chiquoine AD, Kolderie MQ (1955) A histochemical study of differentiation in the cellular slime molds. Exp Zool 130:133–158
Brigandt I (ed) (2019) Special issue: The Evolution of Evolution – a Tribute to Professor John Tyler Bonner. J Exp Zool Part B 332:295–370
Brigandt I et al (2019) John Tyler Bonner: remembering a scientific pioneer. J Exp Zool B Mol Dev Evol 332:365–370. https://doi.org/10.1002/jez.b.22920
de Wit RJ, Konijn TM (1983) Identification of the acrasin of Dictyostelium minutum as a derivative of folic acid. Cell Differ 12:205–210. https://doi.org/10.1016/0045-6039(83)90029-5
Gierer A, Meinhardt H (1972) A theory of biological pattern formation. Kybernetika 12:30–39
Kondo S, Miura T (2010) Reaction-diffusion model as a framework for understanding biological pattern formation. Science 329:1616–1620. https://doi.org/10.1126/science.1179047
Müller GB, Newman SA (1999) Generation, integration, autonomy: three steps in the evolution of homology. In: Bock GK, Cardew G (eds) Homology. Novartis Foundation symposium 222, vol 222. Wiley, Chichester, pp 65–73
Nanjundiah V (2019a) Individual and collective behaviour in cellular slime mould development: contributions of John Bonner (1920–2019). Int J Dev Biol 63:333–342. https://doi.org/10.1387/ijdb.190272vn
Nanjundiah V (2019b) John Tyler Bonner (1920–2019). Curr Sci 116:1258–1261
Newman SA (2019) Inherency and homomorphy in the evolution of development. Curr Opin Genet Dev 57:1–8. https://doi.org/10.1016/j.gde.2019.05.006

Newman SA, Glimm T, Bhat R (2018) The vertebrate limb: an evolving complex of self-organizing systems. Prog Biophys Mol Biol 137:12–24. https://doi.org/10.1016/j.pbiomolbio.2018.01.002

Raper K (1940) Pseudoplasmodium formation and organization in *Dictyostelium discoideum*. J Elisha Mitchell Sci Soc 56:241–282

Robison SK (2018) Epigenetics and public policy: the tangled web of science and politics. Praeger, an imprint of ABC-CLIO, LLC, Santa Barbara

Shimomura O, Suthers HL, Bonner JT (1982) Chemical identity of the acrasin of the cellular slime mold *Polysphondylium violaceum*. Proc Natl Acad Sci U S A 79:7376–7379. https://doi.org/10.1073/pnas.79.23.7376

Strassmann JE, Queller DC (2011) Evolution of cooperation and control of cheating in a social microbe. Proc Natl Acad Sci U S A 108(Suppl 2):10855–10862. https://doi.org/10.1073/pnas.1102451108

Sunderland ME (2008) John Tyler Bonner (1920–) embryo project encyclopedia. http://embryo.asu.edu/handle/10776/1778

Thadani V, Pan P, Bonner JT (1977) Complementary effects of ammonia and cAMP on aggregation territory size in the cellular slime mold *Dictyostelium mucoroides*. Exp Cell Res 108:75–78

Thompson DW (1961) On growth and form. (Edited and abridged by JT Bonner), 2nd edn. Cambridge University Press, Cambridge

True JR, Haag ES (2001) Developmental system drift and flexibility in evolutionary trajectories. Evol Dev 3:109–119

Turing AM (1952) The chemical basis of morphogenesis. Phil Trans Roy Soc Lond B 237:37–72

Valenti D (2019) Microbiologist John Bonner, leading expert on cellular slime molds, dies at 98. Princeton University Office of Communcations, Princeton

Stephen Jay Gould (1941–2002)

Federica Turriziani Colonna

Contents

Abstract

Stephen Jay Gould contributed to paleontology, evolutionary biology, developmental biology, and the history of science during the second half of the twentieth century. He was also one of the most influential writers of popular science of his generation. Gould's scientific work impacted the evolutionary theory from a philosophical perspective and historically contributed to the establishment of evo-devo.

This chapter discusses Stephen J. Gould's legacy as it relates to evo-devo. In particular, the chapter analyzes the concepts of punctuated equilibria, heterochrony, and neoteny as discussed in Gould's 1977 *Ontogeny and Phylogeny*, as well as the concepts of developmental constraints and exaptation. Finally, this chapter describes Gould's contribution to macroevolution and his proposal of the concept of levels of selection.

F. Turriziani Colonna (✉)
Center for Biology and Society, Arizona State University, Tempe, AZ, USA
e-mail: federica.turrizianicolonna@asu.edu; fturrizi@asu.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_36

Keywords

Neoteny · Developmental constraints · Exaptation · Macroevolution

Life

Stephen Jay Gould was born on 10 September 1941 in New York City. His mother, Eleanor Rosenberg, was an artist, and his father, Leonard Gould, was a court stenographer. Gould recalls visiting the American Museum of Natural History in New York with his father at the age of five and says that after seeing the skeleton of *Tyrannosaurus rex* he decided that he wanted to become a paleontologist. At the age of seventeen in 1958, Gould graduated from Jamaica High School in New York and enrolled at Antioch College in Yellowsprings, Ohio, from which he graduated in 1963 at the age of 22 with a Bachelor's degree in geology and philosophy. When he was at Antioch, Gould took the opportunity of studying abroad at the University of Leeds, England. Also at Antioch, Gould met the artist Deborah Lee, whom he married in 1963. The couple later had two children, Jesse and Ethan.

After graduating from Antioch, Gould enrolled at Columbia University in New York and obtained his doctoral degree in paleontology at the age of 26 in 1967. Upon completion of his graduate degree, Gould became assistant professor of geology at Harvard University in Cambridge, Massachusetts. A few years later in 1971, he received tenure at Harvard University and was then promoted to professor and curator of invertebrate paleontology at the Museum of Comparative Zoology in 1973. About a decade later in 1982, Gould became the Alexander Agassiz Professor of Zoology.

During the 1970s, Gould, along with his colleague Niles Eldredge, developed the theory of *punctuated equilibria*, which states that new species are generated relatively quickly and not through a continuous and cumulative process.

In 1977, Gould published his first monograph, titled *Ontogeny and Phylogeny.* In this book, Gould discusses the history of the interaction between developmental and evolutionary biology and demonstrates that development and evolution are closely related. In addition, Gould analyzes the concept of recapitulation, which holds that developmental or embryonic stages of a particular organism repeat the evolutionary stages of its ancestors.

In 1979, Gould challenged again some aspects of the evolutionary theory that were commonly accepted within the scientific community, with a paper that he co-authored with Richard Lewontin titled "The Spandrels of San Marco and the Panglossian Paradigm: A Critique of the Adaptationist Programme." In this paper, Gould and Lewontin developed an argument against the adaptationist program, which sought to explain all traits and behaviors of organisms as *adaptations* to particular environments.

In the 1970s, Gould argued against sociobiology. In 1975 Gould's Harvard colleague Edward O. Wilson had published a book titled *Sociobiology: The New Synthesis*, in which he claimed that altruism and aggression were to be explained in

the light of evolution (Wilson 1975). Wilson's book provoked a collective reaction (Allen et al. 1975) and Gould warned that such ideas promoted biological determinism, in which one's choices have no role in his or her character. In addition – Gould continued – sociobiology carried racism and sexism as possible consequences.

Throughout all his life, Gould published popular science articles on evolutionary theory, which he later collected in various books, such as *The Panda's Thumb*, *Ever Since Darwin*, *The Mismeasure of Man*, *Wonderful Life: The Burgess Shale and the Nature of History*, *Bully Brontosaurus*, *Eight Little Piggies*, and *Leonardo's Mountain of Clams and the Diet of Worms*. Gould regularly contributed to newspapers and popular science magazines such as *Scientific American* and *The New York Times*. In 1981 *Discover Magazine* named Gould "Scientist of the Year." Gould was also interviewed many times and his interviews appeared in mainstream magazines, including *People* and *Time*.

Within the debate between creationists and evolutionists, in 1982 Gould testified in an Arkansas trial against the incorporation of biblical teachings in the science curriculum. Gould further stressed the importance for science to be independent from religion in a 1997 article titled "Non overlapping magisteria" as well as in his 1999 book *Rocks of Ages: Science and Religion in the Fullness of Life*. In the same year, Gould was diagnosed with mesothelioma, a cancer of the lining of internal organs that is likely caused by asbestos. However, Gould kept pursuing his work. During the following years, Gould was acknowledged with multiple awards including the Medal of Excellence from Columbia University in 1983, the silver medal from the London Zoological Society in 1984, and in 1992 the Linnean Society of London awarded him with the Gold Medal for Service to Zoology. Throughout his life, Gould received over 40 honorary degrees from different institutions. In 1995, Gould divorced his first wife Deborah Lee and married Rhonda Shearer. Starting in 1999, Gould served as president of the American Association of the Advancement of Science. On 20 May 2002, Gould died of a cancer metastasis in the lung. In the same year, Gould's magnum opus *The Structure of Evolutionary Theory* was published and, 1 year later in 2003, *The Hedgehog, the Fox, and the Magister's Pox* appeared posthumously (Bonner 2002; Briggs 2002; David 2002; Segerstale 2002; Yoon 2002).

Work

Gould contributed to life sciences from a variety of different perspectives. His legacy includes philosophical ideas, as well as historical frameworks. Among the disciplines Gould impacted with his work are paleontology, evolutionary biology, and developmental biology, as well as philosophy of biology, history of biology, and history of science more in general. This section will discuss Gould's overall scientific contribution with a particular focus on his work on evo-devo, including his research on punctuated equilibria, recapitulation, heterochrony and neoteny, exaptation, sociobiology, and macroevolution.

Punctuated Equilibria

In 1972, with his colleague Niles Eldredge, Gould published an article in which they developed the theory of punctuated equilibria. The article appeared in the journal *Models in Paleobiology* with the title "Punctuated equilibria: an alternative to phyletic gradualism." In those years, it was commonly maintained that species evolved due to small gradual changes continuously accumulated over millions of years under the action of natural selection. Such a theory, dominant at the time, was referred to as phyletic gradualism. After analyzing fossil records, Gould and Eldredge claimed to have evidence to contrast the standard theory. In particular, they based their claim on the fact that fossil records showed many gaps in the transitions from one species to another. If species had evolved in a continuous and gradual fashion as most biologists held, then there would not be any gaps in the sequence of fossil records. However, the sequence of fossils available for investigation did not show a smooth gradual change. Up to that point, biologists had argued that such a gap in the fossil record reflected a problem of missing data and that the information missing was due to the fact that fossils are fragile and can therefore be eroded by geological processes. According to the standard view, most of the fossils do not survive in the paleontological records and, therefore, cannot be studied. In contrast, Gould and Eldredge argued that the gaps in the fossil records suggested that evolution proceeds at variable rates, with few isolated events such as geological catastrophes to interrupt long ages of evolutionary equilibria or stasis (Geary 2008). According to Gould's and Eldredge's theory of punctuated equilibria, the appearance of new species occurs relatively quickly, through a process that they called cladogenesis, or the formation of a new group of organisms by evolutionary divergence starting from an ancestral form. Gould's and Eldredge's theory initiated a long-lasting debate (Turner 1984).

Recapitulation, Heterochrony, and Neoteny

Among Gould's numerous books and articles, some of them turned out to be extremely influential – if not foundational – to evo-devo. With its historical analysis and theoretical contribution, Gould's 1977 book *Ontogeny and Phylogeny* has become a classic in the evo-devo literature. In this book, Gould discusses the concept of recapitulation as it was conceived at different times by different authors. Recapitulation generally refers to the idea that the development of a given embryo repeats or recapitulates the history of the group it belongs to. In the first part of the book, Gould reconstructs the history of this concept, analyzing its first appearance in antiquity and the various ways its meaning changed in the modern era. Then, Gould discusses its declinations in the nineteenth century, focusing in particular on von Baer's laws of development and how they compare to Haeckel's biogenetic law.

Karl Ernst von Baer (1792–1876) had claimed that embryos from different taxa looked similar to each other in the beginning of their development, although they

differ more and more as development progresses and they acquire the traits of the particular species they belong to (von Baer 1928). However, von Baer does not claim in his writings that developmental stages and evolutionary history are related at all. Such an approach is theoretically much different from Haeckel's law of recapitulation, which does establish that an embryo of a given species repeats, in its development, the adult stages of organisms belonging to various species within the same phylum. Haeckel's idea of recapitulation implies that there exists a relationship between development and evolution and – in his historical analysis – Gould builds upon such a sentiment.

According to Gould's historical interpretation, both von Baer and Haeckel offered valid insights and both their theories remained relevant during the twentieth century. In addition, Gould notes that although Haeckel was charged with fraud for his embryonic images, his theoretical contribution to evolutionary and developmental biology has been crucial. This is, for instance, the case of the concepts of heterochrony, heterotopy, and neoteny, which Haeckel himself proposed.

Heterochrony accounts for the change in the developmental timing, whereas heterotopy describes the changes in location that some morphological structures undergo during development (McNamara and McKinney 2005; see chapter ▶ "Heterochrony"). In his 1977 book, Gould shows that both heterochrony and heterotopy are phenomena that occur at a developmental level and such phenomena can influence the evolution of a given species. Two years later in 1979, in an article co-authored with Pere Alberch, George Oster, and David Wake, Gould introduced a quantitative method to describe the way heterochronic changes in development result in phyletic trends impacting evolution (see chapter ▶ "Pere Alberch (1954–1998)"). The authors formalized the ideas of size and shape into a framework to help explain and ultimately visualize evolutionary processes in a quantitative yet intuitive way. In *Ontogeny and Phylogeny* Gould developed – later formalized in the 1979 paper – a "clock model" to compare ancestors and descendants at the same developmental stage and show the changes in size and shape that occur at the very developmental stage being studied.

Similarly, the concept of neoteny plays a crucial role in evolutionary and developmental biology coming together into a unified discipline. Neoteny, or a delay in development, helps describe large morphological changes in a given species over millions of years. For instance, Gould discusses the case of the axolotl and the Mexican salamander. Although for long time the former had been believed to be a species per se, scientists had eventually found out that an axolotl is nothing else than a neotenous salamander (*Ambystoma mexicanum*) that retains its juvenile features, including gills, throughout its life and in particular beyond its reproductive age. Another example of neotenic animals is flightless insects, whose females retain their juvenile features and in particular flightlessness, a characteristic that turns out to be advantageous insofar as those neotenic females do not waste energy flying and that energy can instead be diverted toward fecundity (Turchetto 2012).

In his book, Gould reconstructs the history of all these concepts and discusses their theoretical implications in a way that opens an epistemological space that

evo-devo eventually fed into. Although *Ontogeny and Phylogeny* was not a huge editorial success due to its complexity and length, some of the concepts discussed in there have had a remarkable influence on evo-devo scholars (Love and Raff 2003).

A few years later, in an article that was eventually collected in the 1980 book *The Panda's Thumb*, Gould went back to discuss the concept of neoteny through the evolution of the Walt Disney character Mickey Mouse over the decades. Over the years, Disney designers have transformed Mickey Mouse who – Gould notes – has acquired more and more baby-like features to address parents' concerns that a mere animal-looking mouse would probably result scary to their kids. In his essay, Gould describes the evolution of Mickey Mouse as being *neotenic*, or displaying a tendency to regress more and more toward juvenile-looking forms as evolution progresses.

Spandrels, Developmental Constraints, and Exaptation

In 1979, 2 years after the publication of *Ontogeny and Phylogeny*, Gould contrasted again some aspects of the evolutionary theory that were commonly accepted within the scientific community, with a paper that he co-authored with Richard Lewontin titled "The Spandrels of San Marco and the Panglossian Paradigm: A Critique of the Adaptationist Programme." In their paper, Gould and Lewontin argued that the adaptationist paradigm of the time in evolutionary biology, or the intellectual attitude of biologists to explain traits and behaviors of organisms as *adaptations* to a particular environment failed to consider other possible factors in evolution, such as developmental constraints (Nielsen 2009). Gould and Lewontin used the example of spandrels, the spaces between arches in the church of San Marco in Venice, Italy, which were built as functional structures but were eventually decorated, thus appearing as decorative structures to most people. In Gould's and Lewontin's article, the spandrels worked as a metaphor to show that a complex biological character or trait may lead us to conclude that it was *designed* for a specific purpose, rather than it being simply the result of some other related processes. In contrast, the authors argued that the spandrel decorations in the church of San Marco could be better explained as the byproduct of the construction of the arches. Gould and Lewontin argued that, in architecture, spandrels are similar to some morphological features of organisms. As a consequence, Gould and Lewontin concluded that the way biologists often explained morphological features as adaptations was too simplistic and that some biological features can be explained as a result of developmental processes that constrain the evolution of the particular structure by putting biases on the production of phenotypic variability through the composition of a developmental system. To explain such a phenomenon, Gould introduced the idea of developmental constraint, or the constraints that in a given lineage limit the number of possible ways a feature can evolve into. Gould's idea of developmental constraint has played a crucial role in evo-devo as it provides an insightful explanation of how developmental processes impact evolution (Danieli et al. 2013).

On the same topic, in 1982 Gould published an article with Elisabeth Vrba on what they considered being a missing term in morphology. In the article, the authors introduced the concept of "exaptation" to describe a shift in the function of a trait or feature (see chapter ▶ "Developmental Exaptation"). To explain the phenomenon of exaptation, Gould and Vrba use the example of feathers, which in the first place evolved as a means of temperature regulation in some animals but have eventually been "co-opted" to serve a different function such as bird flight and display. In their interpretation, feathers have exapted, meaning they have adapted to serve functions different than the ones they had initially evolved for. The example of feathers has been used in current evo-devo literature, insofar as understanding how feathers develop in a single bird has helped show the evolutionary history of birds as having possibly evolved from reptiles (Prum and Brush 2003). In addition to filling in an epistemic gap, the concept of exaptation also helps avoid inaccurate interpretations of the evolutionary processes as being goal-oriented or teleological.

Macroevolution, Levels of Selection, and Evo-Devo

In 2002, just a few months before his death, Gould published *The Structure of Evolutionary Theory*, in which he discusses macroevolution, adaptation, and the levels of selection. In writing his book, Gould did not address the public but a much more restricted audience. In fact, *The Structure* is a quite technical work and, as such, it received much attention in the scientific community. This very long book is divided into two main parts: the first half of the book details the history of evolutionary though and focuses on natural selection, adaptation, and evolutionary changes; the second half of the book addresses the main critiques that have challenged the evolutionary theory, namely, the levels of selection, the mechanics of evolution, and the causes of evolution. In particular, Gould claims that evolution is based on hierarchical selection, meaning that evolution does not operate only on organisms, or genes, or species, but on more than one unit simultaneously. In stating that selection occurs at different levels of the hierarchy, Gould counters different arguments that were being widely discussed at his time, including Richard Dawkins's argument that selection applied to genes and that organisms were to be interpreted as gene-carriers. Far from accepting such a view, Gould argued for the richness and complexity of natural selection that, according to him, happens at different scales and on different units (Allmon 2008). Additionally, Gould clarifies that natural selection is not the only cause of evolutionary changes, but other factors such as developmental constraints play a role in the evolutionary game. For instance, Gould discusses the Cambrian explosion, or the period in which living forms displayed the widest diversity and most animal phyla appeared, in relation to the themes of evolutionary novelties as well as contingency (see chapter ▶ "Developmental Innovation and Phenotypic Novelty"). According to Gould, the route evolution underwent after the Cambrian explosion to preserve only a small selection of the numerous forms that existed then was not random, but contingent. Contingency, in Gould's interpretation, is the role played by accidental phenomena that influence a cascade of other consequent phenomena in evolution.

Legacy

With his views on evolution, Gould provoked different controversies and engaged different debates, including a long-lasting debate with Oxford Professor Richard Dawkins. Dawkins interpreted evolution as a competition between gene lineages where organisms were nothing but carriers, whereas Gould stressed the importance of chance and contingency in evolutionary processes. The debate between the two went on for decades and lasted beyond Gould's death, involving not only evolutionary biology, but their proponents' overall biological philosophies (Sterelny 2007).

As noted by Alan Love and Rudolph Raff, the origins of twentieth century evo-devo should not be traced back to Haeckel's recapitulation or experimental embryology much, but more to Gould's lifelong research articulated through the different themes herein discussed (Love and Raff 2003). Overall, Gould contributed to evo-devo with his entire work although some of his publications have played a more relevant role in unifying evolutionary and developmental biology. In addition to the concepts of heterochrony and neoteny, evo-devo scholars have stressed out the importance of the notion of *spandrels* in explaining developmental constraints from an evo-devo perspective (Müller 2013). All the themes Gould discussed in his work address, in one way or another, the core concepts of evo-devo. Gould's contributions to evolutionary biology as well as to the history of the discipline have been crucial to understanding the ways in which development and evolution interact with one another (Bowler 1984). In particular, Gould's work has been considered most relevant to subsequent evo-devo insofar as the notions of developmental constraints, heterochrony, neoteny, exaptation, evolutionary novelty, contingency, macroevolution, and many more helped build the significance of evo-devo (Thomas 2009).

Cross-References

- ▶ A Macroevolutionary Perspective on Developmental Constraints in Animals
- ▶ Convergence
- ▶ Developmental Exaptation
- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Gavin de Beer (1899–1972)
- ▶ Heterochrony
- ▶ Macroevolution
- ▶ Pere Alberch (1954–1998)

Selected Bibliography

Alberch P, Gould SJ, Oster GF, Wake DB (1979) Size and shape in ontogeny and phylogeny. Paleobiology 5:296–317

Gould SJ (1977) Ontogeny and phylogeny. Harvard University Press, Cambridge

Gould SJ (1989) Wonderful life: the burgess shale and the nature of history. W.W. Norton & Company, New York

Gould SJ (1992a) Ever since Darwin: reflections on natural history. W.W. Norton & Company, New York
Gould SJ (1992b) The panda's thumb: more reflections on natural history. W.W. Norton & Company, New York
Gould SJ (1996) The mismeasure of man. W.W. Norton & Company, New York
Gould SJ (2002) The structure of evolutionary theory. Harvard University Press, Cambridge
Gould SJ, Eldredge N (1972) Punctuated equilibria: an alternative to phyletic gradualism. In: Schopf TJM (ed) Models in paleobiology. Freeman, Cooper, and Co, San Francisco, pp 82–115
Gould SJ, Eldredge N (1977) Punctuated equilibria: the tempo and mode of evolution reconsidered. Paleobiology 3:115–151
Gould SJ, Lewontin R (1979) The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. Proc R Soc London Ser B 205:581–598
Gould SJ, Vrba E (1982) Exaptation – a missing term in the science of form. Paleobiology 8:4–15

References

Allen E et al (1975) Against 'sociobiology'. N Y Rev Books 22(Nov 13):182. 184–186
Allmon W (2008) The structure of Gould. In: Stephen Jay Gould: reflections on his view of life. Oxford University Press, Oxford
Bonner JT (2002) Stephen Jay Gould. J Biosci 27:450
Bowler PJ (1984) Evolution: the history of an idea. University of California Press, Berkeley/Los Angeles
Briggs D (2002) Obituary: Stephen Jay Gould (1941–2002). Nature 417:706
Danieli GA, Minelli A, Pievani T (eds) (2013) Stephen J. Gould: the scientific legacy. Springer, Milan
David JR (2002) Stephen Jay Gould (1941–2002). J Biosci 27:453–454
Geary DH (2008) The legacy of punctuated equilibrium. In: Stephen Jay Gould: reflections on his view of life. Oxford University Press, Oxford
Love AC, Raff RA (2003) Knowing your ancestors: themes in the history of evo-devo. Evol Dev 5:327–330
McNamara KJ, McKinney ML (2005) Heterochrony, disparity and macroevolution. In: Macroevolution: diversity, disparity, contingency essays in honor of Stephen Jay Gould. The Paleontological Society, Lawrence, pp 17–26
Müller G (2013) Beyond spandrels: Stephen J. Gould, EvoDevo, and the extended synthesis. In: Danieli GA, Minelli A, Pievani T (eds) Stephen J. Gould: the scientific legacy. Springer, Milan
Nielsen R (2009) Adaptationism-30 years after Gould and Lewontin. Evolution 63:2487–2490
Paleontologist, author Gould dies at 60. Harvard University Press Gazette, 20 May 2002
Prum RO, Brush AH (2003) Which came first, the feather or the bird? Sci Am 288:84–93
Segerstrale U (2002) The scientist of the underdog. J Biosci 27:460–463
Sterelny K (2007) Dawkins vs. Gould: survival of the fittest. Icon Books, Cambridge
Thomas RDK (2009) Gould, Stephen Jay (1941–2002). In: Ruse M, Travis J (eds) Evolution: the first four billion years. Belknap Press, Cambridge MA, pp 611–615
Turchetto M (ed) (2012) Ontogenesi e Filogenesi. Cavazzini, Andrea, De Cesare, Silvia, Pappalardo, Marco, and Turriziani Colonna, Federica (trans). Mimesis, Milano
Turner J (1984) Why we need evolution by jerks. New Sci 101(1396):34–35
Von Baer KE (1828) Über die Entwicklungsgeschichte der Thiere. Borntträger, Königsberg
Wilson EO (1975) Sociobiology: the new synthesis. Belknap, Cambridge
Yoon C (2002, May 21) Stephen Jay Gould, 60, is dead; enlivened evolutionary theory. New York Times

Pere Alberch (1954–1998)

Arantza Etxeberria and Laura Nuño de la Rosa

Contents

Abstract

Pere Alberch Vié (1954–1998) was an experimental embryologist, theoretical biologist, and evolutionary biologist of Catalan origins who studied and developed part of his career in the USA. With a focus on herpetology, his empirical studies combined conceptual research, theoretical models, and experiments in

A. Etxeberria
IAS-Research Center for Life, Mind, and Society, Department of Logic and Philosophy of Science, University of the Basque Country UPV/EHU, Donostia-San Sebastián, Spain

L. Nuño de la Rosa (✉)
Department of Logic and Theoretical Philosophy, Complutense University of Madrid, Madrid, Spain
e-mail: lauranun@ucm.es

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_35

order to integrate development and evolution. The 1980s were the most productive and innovative period of his career, when he was assistant professor and curator at the Museum of Comparative Zoology, Harvard University. In the 1990s, he continued his work as Director of the Museum of Natural History in Madrid, Spain. His contributions on topics such as heterochrony, developmental constraints, evolvability, possible variation, construction rules, the morphospace, or the "logic of monsters" have largely been conducive to shape the core concepts of evo-devo.

Keywords

Evo-devo · Form · Monsters · Developmental constraints · Morphospace · Morphogenetic process · Evolvability

Life

Pere Alberch Vié (1954–1998) was born in Badalona, Spain, on the 2nd of November 1954. He manifested an interest in natural history very early on and wrote his first two scientific papers on amphibians when he was only 19 years old.

In 1973 Alberch entered the University of Kansas, where 3 years later he completed a bachelor's degree with a double major in philosophy, and systematics and ecology. In 1976 he joined the University of California at Berkeley, to write his PhD in Zoology under the co-supervision of David Wake, an evolutionary biologist specialized in salamanders, and George Oster, a mathematical biologist and dynamical systems theorist.

In 1980 Alberch was hired by Harvard University as assistant professor and assistant curator of the Museum of Comparative Zoology. He published his better-known papers on evolution and development in this period and supervised the work of several students who were to become prominent researchers in evo-devo, such as Neil Shubin, Cliff Tabin, Ann Burke, and Chris Rose.

Fig. 1 Pere Alberch in 1990 giving a conference entitled "Beyond Neo-Darwinism: new trends in the study of Macroevolution," at the Juan March Foundation, Madrid. (Reproduced with permission from the Juan March foundation)

In 1989 Harvard declined to give him tenure, and Alberch moved to Madrid where he was hired as Research Professor at the Spanish Research Council (CSIC) and as Director of the National Museum of Natural Sciences. There he carried on with theoretical and empirical research, while he was also committed to a thorough renovation and modernization of the Museum. In 1995 Alberch fell seriously ill and had to quit the directorship of the Museum and slow down his research activities. After 3 years, he accepted a research position at the Institute Cavanilles for Biodiversity and Evolutionary Biology, Valencia. While still in Madrid, he died at the age of 43 on the 13th of March 1998.

Work

Most of Alberch's papers, published in the major academic journals of the field, are tightly integrated in a distinctive research framework for the study of how the ontogenetic generation of morphological variation influences evolution. His most relevant contributions were written between 1980 and 1989, but some significant ones appeared also in the 1990s and comprised various topics such as the relationship between science and art or museum curatorship and management (see papers collected in Rasskin-Gutman and De Renzi 2009; De Renzi 1999, 2009; Moya and Peretó 2010; Reiss et al. 2009; Wake 1998). Alberch's work is unique owing to his inventiveness in persuasively formulating and pursuing compelling new research paths in evolutionary biology and to his ability to illustrate theoretical claims, such as the role of developmental constraints, through original and audacious experiments. Within the framework of the integrative biology cultivated by David Wake's group, Alberch's contributions focused on the mechanistic approach to the generation of form, which he considered to have been largely neglected by the evolutionary biology of his time.

A Theory of Form

The role that morphology played in Alberch's work gives credence to the thesis that morphology had a central position in the origination of evo-devo as a discipline (Love and Raff 2003; Love 2003). Alberch conceived of the study of morphological variation, of its generation and of its evolutionary significance largely arose within Jacobian interplay of what is possible and what is actual, at the intersection between the forms enabled by morphogenetic processes and those extant ones adapted to the local contingencies of the environment (Alberch 1982).

In contrast with the linear genotype-phenotype map of random and continuous variation assumed by neo-Darwinian models of evolution, Alberch argued that the patterns of phenotypic variation are clustered around major "themes corresponding to taxa or classes of teratologies". When new morphological themes arise, the transitions among them are not random (Oster and Alberch 1982, p. 444), as morphological variants cluster in discrete groups of patterns. Of special interest are cases where patterns of variation cannot be explained by natural selection, such as the same recurrent phenotypic variants in widely unrelated species (Alberch

1983), or how, despite being strongly selected against, teratologies are generated in a regular way, following what he called a "logic of monsters" (Alberch 1989). He also explored how functional constraints related to the integration of the parts of the organism influence the appearance of similar convergent structures. Thus, after an invasion of new habitats in a highly diverse genus of salamanders (*Bolitoglossa*), convergent structures can be a response to adaptive requirements, but also a result of ontogenetic developmental correlations among parts (Alberch 1981; see chapter ▶ "Convergence"), the recurrence of the same phenotypic variants in widely unrelated species, and the "logic of monsters," insofar as, despite being strongly selected against, teratologies are generated in a recurrent way.

All these phenomena underpin Alberch's view of the morphospace as a discrete cluster of forms. Influenced by David Raup's theoretical morphology, Alberch observed that possible forms are not ubiquitous, because they do not fully occupy the space of conceivable forms. Unlike the standard view of population genetics, which assumes that phenotypic variations are generated by random small mutations later fixed by natural selection, the properties of the morphospace demand that morphological evolution be studied from a developmental perspective (Alberch 1980). Accordingly, evolutionary biology should explain not only the fixation of variant morphs in different populations, but also the developmental processes in which these novel variants generate or originate (Oster and Alberch 1982, p. 455). Patterns of variation appear as an order of forms inherently arising in development, their properties largely resulting from epigenetic interactions at the cellular level (Alberch 1983, 1985b).

Thus, Alberch vindicates an internalist approach to evolution and development: "I focus on the internal rules that control the appearance of morphological variation, on the mechanistic basis of such rules and on the evolutionary consequences of this internally determined order" (Alberch 1989, p. 28). The *internalist* program was a rather striking position and an extremely underdeveloped research line in the evolutionary biology of the time, opposed to the externalist or adaptationist program focused on natural selection.

Related to internalism is Alberch's vindication of a "theory of form" based on the global properties of the network of developmental interactions, independent of the adaptive role played by the resulting forms (Alberch 1989, p. 39). According to Alberch, neither genes nor environment specify form, generated by internal rules, but both mutations and environmental perturbations can change the outcome of development. Developmental systems are stable, endowed with the intrinsic, regulatory, and pattern-generating properties characteristic of complex dynamical systems.

Importantly, from this perspective, the notion of "biological function" is not restricted to the contribution of a character to adaptation by natural selection. Rather, in the internalist approach, functional constraints are those "imposed by functional interactions among different parts of the organism" (Alberch 1981, p. 84). Thus, new morphologies need to be integrated with the rest of the organism interacting with the environment (Alberch 1982b).

From Heterochrony to Morphogenetic Processes

Chronologically, Alberch's view on the relation between ontogeny and phylogeny progressed from a focus on the role of heterochrony, i.e., "the role of change in the relative timing of developmental events" (Hanken 2015), towards a mechanistic explanation of how developmental processes generate possible variations for evolution (the chapter on ▶ "Heterochrony").

Since the late 1970s, heterochrony has experienced a renaissance in evolutionary biology (Gould 1977; see Hanken 2015 and Wake 2015), and at the beginning of his career, Alberch himself considered that studies on heterochrony were crucial for the emergence of the field of evo-devo (Alberch 1995, p. 230). His interest in how ontogenesis influences morphological diversification started with a paper that elaborated Gould's clock model for describing how heterochronic changes in ontogeny are related to phyletic trends (Alberch et al. 1979; see chapter ▶ "Stephen Jay Gould (1941–2002)"). There Alberch and collaborators offered a dynamic and quantitative version of the clock model, characterizing the modifications in development that produce relative changes in size and shape and defining heterochrony in terms of shifts in developmental processes (onset, cessation, or rate of growth) rather than of end results. This approach was applied in subsequent empirical work on heterochrony in the salamander *Bolitoglossa occidentalis* (Alberch and Alberch 1981).

However, already as early as 1985 Alberch challenged what he then characterized as a "static," descriptive approach underlying heterochrony models to pursue a more dynamical, causal approach to development (Alberch 1985a; Oster et al. 1988; Alberch and Blanco 1996; see Nuño de la Rosa and Etxeberria 2012, p. 267). According to him, the static framework, inheritor of the traditional recapitulationist approach in comparative morphology, conceived of development as a sequence of discontinuous morphological stages conserved in evolution. In contrast, in the new dynamical approach, the changes between two related morphologies should "be searched for in the developmental rules of interaction or initial conditions" (Alberch 1985a, p. 51), instead of looking at the intermediate ontogenetic stages.

Following the tradition of experimental embryology, Alberch favored a "mechanistic" method in biology to explain how forms generate dynamically in development, as opposed to the standard view in evolutionary biology in which the origin of variation is taken for granted. This addressed the generation of morphological variation in ontogeny at the level of cellular dynamics, following an approach that had already come out in the early interactions with David Wake and George Oster. The aim was to capture how developmental "construction rules" emerge as dynamical systems mechanisms which remain stable during long periods of time, with a certain range of variation due to the alteration of developmental parameters. Construction rules arise from interactions among different "resources" at different organizational levels, from molecules up to tissues. Alberch thought that these rules "allow us to determine the relationships among different phenotypes, since the set of possible phenotypic transformations will be constrained by the generative potentialities of the morphogenetic rules involved in

the process" (Alberch 1982, p. 321). In order to investigate these morphogenetic rules, he carried out experiments to determine their material, physico-chemical properties and studied their formal properties through dynamical systems theory models.

A Cyclical View of Development

In an important conceptual contribution, Alberch argued that development is not the result of gene expression, but of entangled feedback processes going back from tissues into the genome itself. In his own words: "This depiction of genes and development as independent levels is incorrect in the sense that genes do not specify development, or even form, because gene action itself is intimately linked to developmental interactions" (Alberch 1991, p. 5). The distinction between a "hierarchical" and a "cyclical" scheme of development underlies Alberch's approach to evolution (Alberch 1991). The former portraits an extreme version of the neo-Darwinist view of genes as directly prescribing developmental processes that, in turn, specify morphology. This view reduces development and evolution to purely genetic problems, demoting development to a sequence of gene expression and evolution to a change in gene frequencies.

In several papers, Alberch emphasized the shortfalls associated to the concept of causality underlying this hierarchical scheme of development. First, such an open loop system would be extremely unstable against the random genetic and environmental perturbations of normal development. Second, the relation between genes and phenotypic traits is not a one-to-one correspondence (Alberch 1983). Rather, the effect of genes on morphology is mostly indirect: genes code for molecules which either regulate the expression of other genes or confer properties on cells (e.g., cell division rates, apoptosis, differentiation timing, or cytoskeletal properties), which then construct organs and structures in accordance with physico-chemical laws. Developmental interactions have properties that emerge from the dynamics of the system; they are not encoded in the genome (Alberch 1987, 1991; Oster and Alberch 1982; Oster et al. 1988). Moreover, due to the highly context-sensitive character of gene expression, similar genetic changes may yield different morphological effects, and the other way around. Therefore, in Alberch's view, phenotypic diversity is not so much the product of new genes as of permutations in context (i.e., the timing and location of expression) of existing genes. The evolutionary consequences of this asymmetry are obvious: there are qualitative differences between modes of evolution at the genetic and at the epigenetic levels, and therefore there is often no direct correspondence between genetic and morphological divergence (Alberch 1983).

In the alternative "cyclical" scheme of development embraced by Alberch, "gene expression is both the cause and the effect of a morphogenetic process" (Alberch 1991, p. 6). Developmental processes are divided in three interacting levels, including gene interactions, proteins and enzymes generating cell properties involved in morphogenesis, and tissue interactions (Alberch 1982a, p. 320).

Following Waddington's ideas, Alberch considered that these regulatory interactions specify the epigenetics according to which phenotypes are well-buffered systems with respect to both genetic and environmental perturbations during ontogeny (Alberch 1980; see chapter ▶ "Conrad Hal Waddington (1905–1975)").

A Dynamical Systems Theory of Developmental Evolution

Formally, Alberch appealed to the conceptual and mathematical tools of dynamical systems theory to study developmental processes "where a small set of simple rules of cellular and physico-chemical interaction can interact to generate a complex morphology" (Oster and Alberch 1982, p. 455). Construction rules are formally captured as developmental parameters, whereas genetic or environmental alterations of development are mathematically abstracted as parameter perturbations (Alberch 1982, p. 323). Thus, "morphological diversity is generated by perturbations in parameter values (such as rates of diffusion, mitotic rate, cell adhesion, etc.) while the structure of the interactions among the components remains constant" (Alberch 1989, p. 27).

In the framework of dynamical systems theory, the possible pathways of transformation among phenotypes are visualized using transformational diagrams (Alberch 1991). Each species or trait has a unique transformation diagram dependent on its position in the parameter space, and smooth perturbations of the parameters (resulting from genetic mutation or experimental manipulation) can result in a limited set of phenotypes. Alberch illustrated this idea with cases of teratologies, showing that even nonadaptive variations are discrete and constrained by developmental transformation rules generating the space of possible forms. In other words, even "monsters" have a logic. Thus, Alberch aimed at formalizing the stabilities and bifurcations of Waddington's epigenetic landscape with the language and mathematical tools of dynamical systems theory. In this framework, phenotypic stabilities are seen as emerging from dynamical attractors, regions in the parameter space where small perturbations do not disrupt the basic organization of development, whereas bifurcations correspond to developmental thresholds (e.g., critical cell number or inductive relationships) so that modifications that go beyond them may cause nonlinear effects. Thus, continuous changes of developmental parameters can result in phenotypic discontinuities. Both stability and the direction of variation depend on the formal properties of the developmental system. Transformational diagrams show the potential evolutionary transformations of phenotypes, predicting the most probable ones in the absence of external forces. They can be used as a "null hypothesis" of evolutionary transformations, because selection can only drive phenotypes along the internally specified directions. Thus, in Alberch's view, the dynamical properties of developmental systems limit possible variation in phenotypic space, but at the same time, provide potential directions to evolutionary change.

Unlike the neo-Darwinian view of evolution based on chance and contingency, Alberch believed that the study of variation was partly deterministic and predictable, since an understanding of developmental mechanisms "allows for predictions of what patterns of variation should be expected" (Alberch 1983, p. 915; see also Alberch 1982, p. 314; Etxeberria and Nuño de la Rosa 2009). "In evolution" – he argued – "selection may decide the winner of a given game but development non-randomly selects the players" (Alberch 1980, p. 665). Nonetheless, contrary to other internalist approaches such as Goodwin's process structuralism, Alberch did not conceive of evolution as an absolutely deterministic process, but as a relative one (Alberch 1981; on this debate, see chapter ▶ "Inherency"). In his view, the morphogenetic level emerges as a realm of determinism between two sources of uncertainty: the irreducible stochasticity of cell dynamics coming from development (Oster and Alberch 1982, p. 444) and the historical contingency resulting from the interaction between development and selection, given that the most probable forms from the point of view of development might or might not be those favoured by selection. As a result, randomness, determinism, and contingency coexist in biological processes, leading to a "world of opportunity within constraint" (Alberch 1986, p. 8).

An Experimental Approach to Development and Evolution

In addition to his innovative work, Alberch proved to be a bright experimental embryologist, determined to capture the mechanical and chemical aspects of morphogenesis (Oster et al. 1988). In a series of papers written together with Emily Gale, the influence of perturbations of developmental parameters on the generation of new forms was studied experimentally (Alberch and Gale 1983, 1985, 1986; Alberch 1986; Alberch et al. 1986). Alberch and Gale compared the results of treating the limb buds of a frog and a salamander with colchicine, a mitotic inhibitor. This treatment results in various abnormal morphologies such as limbs of smaller size and with some skeletal elements missing. However, these malformations exhibited a high degree of order, leading the authors to conclude that most of the patterns of diversity of digital morphology in amphibians could be explained as a reflection of developmental properties (Alberch and Gale 1985). In particular, Alberch used these experimental results to test the hypothesis that the digital pattern is affected by reduction in the number of mesenchymal cells in the limb bud. Changes in pattern formation took place when the size and the number of cells of the limb bud were reduced under a critical value, a result consistent with the mathematical models studied with Oster (Oster et al. 1988).

In Alberch's work, the formal and the experimental approaches were always seen as complementary. The theoretical consequences of the experimental manipulation of development for our understanding of evolution were, according to him, twofold. First, the experimentally generated patterns of variation can be compared with the patterns of natural variation, thus facilitating phylogenetic inferences and tracing possible evolutionary pathways. For example,

salamanders develop their limbs in a very different way from other tetrapods because the sequence of digit formation appears inverted (Alberch and Gale 1983). Digit reduction is a phenomenon that has taken place several times independently in amphibian evolution. Frogs usually lose their most internal digit (preaxial), whereas salamanders lose the most external one (postaxial). The result is a parallelism between experimentally generated patterns and the evolutionary trends towards digit reduction observed in the wild (Alberch and Gale 1985). Second, the variation generated in the laboratory is bounded, revealing that natural morphologies are limited by the system's morphogenetic properties. Only the interaction parameters, rather than the basic morphogenetic rules, seem to have changed during the evolutionary history of vertebrates (Shubin and Alberch 1986). Quantitative variations of these parameters may produce qualitative alterations such as changes in the branching and segmentation sequences, but since the rules of interaction remain the same, we are only able to explore their potentialities, mostly reiterating forms that have already been realized in evolution (Alberch 1991).

Developmental Constraints and Evolution

Alberch's mechanistic view of development underlies his experimental and theoretical elaboration of the concept of "developmental constraint," a notion that was being intensely discussed in the field in the 1980s. Alberch's work arouse the interest in discussing phenomena associated with this concept, and it constituted his most well-known contribution to evo-devo, especially because of the experiments conducted in collaboration with Emily Gale which became crucial exemplars of developmental constraints (Alberch 1982b, 1985b, 1986, 1989; Alberch and Gale 1983; Maynard Smith et al. 1985). While the notion of developmental constraint became very famous after Gould and Lewontin's *Spandrels* paper (1979), it was imported to biology from fields close to classical mechanics, where a constraint is understood as some limitation of degrees of freedom which at the same time drives or canalizes the system within a path that enables some novelty. In fact, this sense of a limitation of variation at one level combined with the emergence of possibilities at a higher level had been already noticed in a very influential paper by François Jacob (1977), which was discussed in Wake's lab (Wake 2015), and underlies Alberch's own use of the term.

As several scholars have recognized, since then the notion of constraint has struggled between these two seemingly contradictory senses of limiting factors of the variation available to natural selection and generative factors for organizing form (Amundson 1994; Brigandt 2015; Gould 1989; Schwenk 1995). Alberch's developmental constraints act on possible forms and not on natural selection (Amundson 1994). In his view, epigenetic interactions do not constrain natural selection, but how genetic mutations are expressed at the morphological level. As a consequence, constraints reflect the intrinsic abilities of developmental systems to generate "a biased subset of phenotypes upon which natural selection or population stochastic

factors can operate" (Alberch and Gale 1985, pp.19–20). They do not only limit the universe of possible novelties in evolution, but also "impose directionality in morphological transformations through phylogeny" (Alberch 1980, p. 654). Therefore, constraints trim adaptationist optimality thinking, but most importantly, they inspire the evolutionist's search of the source of innovations and possible directions of future evolutionary change.

Developmental Homology

Alberch's views of development and evolution entailed a deep reformulation of the classical notion of homology (see chapter ▶ "Developmental Homology"). In his view, homologies should be established on the basis of "the developmental processes which created them, rather than on their final geometric form" (Oster et al. 1988, p. 877). For example, the skeletal structure of the vertebrate limb was explained by a mechanistic model of embryonic branching and segmentation in initial chondrogenesis. According to this model, the loss of a digit may result from a failure of a branching bifurcation, and then, it is not sensible to ask "which" digit was lost, since it is the basic sequence what has been altered in evolution. Thus, from a developmental perspective, the units of comparison for homologies are no longer the morphological elements, but the morphogenetic processes generating them (Oster et al. 1988).

The replacement of typological thinking by population thinking was seen by Ernst Mayr as Darwin's greatest achievement, and every biological work suspicious of endorsing typology was censured as linked to essentialism and idealism, the big obstacles to evolutionism. In contrast, Alberch conceived of his own work as endorsing a form of typological thinking formulated in a purely mechanistic context, disconnected from a metaphysical commitment to immutable essences (Love 2003). In his own words: "The quest for a general set of principles of form is legitimate if we exchange the metaphysical concept of the Bauplan for a mechanistic one based on principles of morphogenesis and internal integration" (Shubin and Alberch 1986, p. 377). Thus, in Alberch's work on tetrapod limbs, type is not seen as an ideal entity, but as the result of a historically conserved developmental process which determines the range of possible variation upon which selection can act.

Evolvability

Alberch's understanding of developmental constraints as positive causal factors of evolution is particularly well illustrated in his work on evolvability (see chapter ▶ "Evolvability"). After Richard Dawkins (1989) coined the term, Alberch published an article on the differential capabilities that make developmental systems "better at evolving" (Alberch 1991, p. 9). From a developmental perspective, evolvability requires that developmental systems remain stable against

perturbations, but not so much as to be immune to absorb change and variation. Alberch argued that the general properties of developmental systems define their evolvability and allow evolutionary biologists to think of a new level of selection, "one that . . . does not act on the phenotype nor on the genotype, but rather on the emergent properties of developmental systems" (Alberch 1991, p. 10). Selection among pattern-generating systems would favor those that "exhibit the adequate balance between stability and potentiality to generate sufficient phenotypic variability" (Ibid.).

While the conservation of sets of interaction rules within ontogenetic types constrains the range of creativity of developmental systems, the truly creative mechanism that can produce really new forms is the transformation of the generative space by changing or removing some of these rules (see chapter ▶ "Developmental Innovation and Phenotypic Novelty"). Alberch saw the Cambrian explosion as one of the best examples of this form of creativity: "the invention of multicellularity, segmentation or the sequestration of the germ line appear . . . to have been key developmental events that have speeded up the evolutionary proliferation of lineages" (Alberch 1991, p. 9). He thought that the Cambrian was a period of experimentation in rules of cell-cell interaction, rules that exhibited different form-generating abilities as well as distinct stability properties (Alberch 1991). After this period–he concluded, no qualitatively new structural body plans seem to have appeared, and morphological variation looks as if reduced to variations within extant themes.

Legacy

The main threads of current evo-devo have advanced after ideas and research projects in which Alberch, along with many others, was involved during the last quarter of the twentieth century. In particular, the Dahlem Conference of 1982 organized by John Tyler Bonner (Bonner 1982), and in which Pere Alberch took part along with his mentors David Wake and George Oster, is considered a landmark in the history of the discipline (Love 2015). Although his earlier work focused on models of heterochrony, and his collective paper on the clock model remains as his most cited article, Alberch's name is especially associated with his efforts to clarify the notion of developmental constraints in evolution. It was at the Dahlem conference that he presented his views on constraints, which would become highly influential after the publication of that paper (Alberch 1982a) and particularly since the 1985 collective article headed by John Maynard Smith (Maynard Smith et al. 1985). Following Amundson's distinction between the notions of constraint used by adaptationist and developmentalist evolutionary biologists (Amundson 1994), Alberch's theoretical and experimental work on constraints has become the major illustration of the "constraints on form" versus the "constraints on adaptation."

Moreover, Alberch also appears as one of the core exponents of the positive notion of constraint. Schwenk (1995) and Brigandt (2015) have shown that the notion of developmental constraint was central in the field of evolution and development until the 1990s, while other positive concepts such as *evolutionary novelty* and *evolvability*

have become more prominent in contemporary evo-devo. As we saw in examining Alberch's early contributions to evolutionary novelties and evolvability, he was a forerunner of this shift of trend. Today Alberch is widely regarded as one of the founders of the evolvability research agenda, and particularly as the precursor of the developmental approach to evolvability (Pigliucci 2008). His 1991 paper contained some of the core conceptual elements that would later become central in evo-devo approaches to evolvability, including the concept of evolvability as a property depending on the G-P map, or the combination of robustness and flexibility as a basic property of evolvable developmental systems (Nuño de la Rosa 2017).

After the discovery of shared developmental genes across animal phyla in the late 1970s, the developmental genetics approach to evolution became the main trend of research in "molecular evo-devo" (Rasskin-Gutman 2009). Nonetheless, it is distinctive of authors in Alberch's tradition (including Gavin de Beer, Conrad H. Waddington, or Stephen J. Gould as precursors, and Gerd Müller, Stuart Newman, or Isaac Salazar-Ciudad thereafter) to focus on the morphogenetic level. In this sense, Alberch also emerges as one of the pioneers of a "morphological evo-devo" (Nuño de la Rosa and Etxeberria 2012; Olsson 2012; Rasskin-Gutman 2009), where the variational properties of developmental processes, rather than gene regulatory pathways, explain the bounded *patterns* of morphological variation (see chapters ► "Mechanisms of Pattern Formation, Morphogenesis, and Evolution," and ► "Inherency").

Alberch was also highly influential in the rescue of types and homology in evo-devo. While the general philosophical disapproval of typology and essentialism in biology resulted in difficulties in understanding the nature of variation at the morphological level, Alberch's insistence on the importance of studying the patterns of phenotypic variation was decisive for the evo-devo approaches interested in pattern formation. Moreover, Alberch's views on homology have played a significant role in the theoretical and philosophical discussions on the notions of type and homology. By grounding homological relationships in developmental processes, Alberch can be considered as one of the founders of the biological homology concept (Wagner 1989). His work on the homology of vertebrate limbs turned out to be the most cited case-study to illustrate this view (see, e.g., Wagner and Laubichler 2001; Rieppel 2006), and partly inspired the philosophical reinterpretation of homologies and body plans as "homeostatic property clusters" kinds. In this view, the essence of a natural kind is no longer identified with the properties that characterize that kind, but with the causal, developmental processes that account for the similarity of its members (Wagner 1996; Rieppel 2005; see chapter ► "Typology and Natural Kinds in Evo-Devo").

Current pleas for an ► "Evo-Devo's Contributions to the Extended Evolutionary Synthesis" vindicate the need of incorporating previously neglected disciplinary approaches into evolutionary theory. In this respect, Alberch's combination of conceptual, formal, and experimental approaches to pursue a new synthesis between development and evolution remains as one of the most salient incarnations of David Wake's bet for an integrative biology (Griesemer 2013, 2015; Wake 1998, 2015). Moreover, his work has been influential in other disciplinary fields such as cognitive sciences (Balari

and Lorenzo 2008; see chapter ▶ "Evo-Devo of Language and Cognition"), and the evolution of culture (see chapter ▶ "Evo-Devo and Culture").

Cross-References

- ▶ Conrad Hal Waddington (1905–1975)
- ▶ Convergence
- ▶ Developmental Homology
- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Evo-Devo and Culture
- ▶ Evo-Devo of Language and Cognition
- ▶ Evo-Devo's Contributions to the Extended Evolutionary Synthesis
- ▶ Evolvability
- ▶ Gavin de Beer (1899–1972)
- ▶ Heterochrony
- ▶ Inherency
- ▶ Mechanisms of Pattern Formation, Morphogenesis, and Evolution
- ▶ Stephen Jay Gould (1941–2002)
- ▶ Typology and Natural Kinds in Evo-Devo

Bibliography

Alberch P (1980) Ontogenesis and morphological diversification. Am Zool 20(4):653–667
Alberch P (1981) Convergence and parallelism in foot morphology in the neotropical salamander genus *Bolitoglossa*. I. Function. Evolution 35(1):84–100
Alberch P (1982a) Developmental constraints in evolutionary processes. In: Bonner JT (ed) Evolution and development. Dahlem Konferenzen/Springer, Berlin, pp 313–332
Alberch P (1982b) The generative and regulatory roles of development in evolution. In: Mossakowski D, Roth G (eds) Environmental adaptation and evolution: a theoretical and empirical approach. Gustav Fischer, Stuttgart, pp 19–35
Alberch P (1983) Morphological variation in the neotropical salamander genus *Bolitoglossa*. Evolution 37(5):906–919
Alberch P (1985a) Problems with the interpretation of developmental sequences. Syst Zool 34(1):46–58
Alberch P (1985b) Developmental constraints: why St. Bernards often have an extra digit and poodles never do. Am Nat 126(3):430–433
Alberch P (1986) Possible dogs. Nat Hist 95(12):4–8
Alberch P (1987) Evolution of a developmental process: irreversibility and redundancy in amphibian metamorphosis. In: Raff RA, Raff EC (eds) Development as an evolutionary process. Alan R. Liss, New York, pp 23–46
Alberch P (1989) The logic of monsters: evidence for internal constraint in development and evolution. Geobios 12:21–57
Alberch P (1991) From genes to phenotype: dynamical systems and evolvability. Genetica 84:5–11
Alberch P (1995) "Ontogeny and phylogeny" revisited: 18 years of heterochrony and developmental constraints. In: Arai R, Kato M, Doi Y (eds) Biodiversity and evolution. The National Science Museum Foundation, Tokyo, pp 229–249. (reprinted in Rasskin-Gutman and De Renzi, 2009)

Alberch P, Alberch J (1981) Heterochronic mechanisms of morphological diversification and evolutionary change in the neotropical salamander, *Bolitoglossa occidentalis* (Amphibia; Plethodontidae). J Morphol 167(2):249–264
Alberch P, Blanco MJ (1996) Evolutionary patterns in ontogenetic transformation: from laws to regularities. Int J Dev Biol 40:845–858
Alberch P, Gale E (1983) Size dependence during the development of the amphibian foot. Colchicine-induced digital loss and reduction. J Embryol Exp Morpholog 76:177–197
Alberch P, Gale E (1985) A developmental analysis of an evolutionary trend: digital reduction in amphibians. Evolution 39(1):8–23
Alberch P, Gale EA (1986) Pathways of cytodifferentiation during the metamorphosis of the epibranchial cartilage in the salamander Eurycea bislineata. Dev Biol 117(1):233–244
Alberch P, Gould SJ, Oster GF, Wake DB (1979) Size and shape in ontogeny and phylogeny. Paleobiology 5(3):296–317
Oster G, Alberch P (1982) Evolution and bifurcation of developmental programs. Evolution 36:444–459
Oster GF, Shubin N, Murray JD, Alberch P (1988) Evolution and morphogenetic rules: the shape of the vertebrate limb in ontogeny and phylogeny. Evolution 42:862–884
Shubin NH, Alberch P (1986) A morphogenetic approach to the origin and basic organization of the tetrapod limb. In: Hecht MK, Wallace B, Prance GT (eds) Evolutionary biology. Springer, Boston

References

Amundson R (1994) Two concepts of constraint: adaptationism and the challenge from developmental biology. Philos Sci 61(4):556–578
Balari S, Lorenzo G (2008) Pere Alberch's developmental morphospaces and the evolution of cognition. Biol Theory 3(4):297–304
Bonner JT (1982) Evolution and development. Dahlem Konferenzen/Springer, Berlin
Brigandt I (2015) From developmental constraint to evolvability: how concepts figure in explanation and disciplinary identity. In: Love AC (ed) Conceptual change in biology: scientific and philosophical perspectives on evolution and development. Springer, Dordrecht, pp 305–325
Dawkins R (1989) The evolution of evolvability. In: Langton C (ed.) Artificial life. Addison-Wesley, New York, pp 201–220
De Renzi M (1999) Evolution, development and complexity in Pere Alberch (1954–1998). J Evol Biol 12(3):624–626
De Renzi M (2009) Developmental and historical patterns at the cross-roads in the work of Pere Alberch. In: Rasskin-Gutman D, De Renzi M (eds) Pere Alberch: the creative trajectory of an evo-devo biologist. Publications Universitat de València, Valencia, pp 45–66
Etxeberria A, Nuño de la Rosa L (2009) A world of opportunity within constraint: Pere Alberch's early evo-devo. In: Rasskin-Gutman D, De Renzi M (eds) Pere Alberch. The creative trajectory of an evo-devo biologist. Publications Universitat de València, Valencia, pp 21–44
Gould SJ (1977) Ontogeny and phylogeny. Harvard University Press, Cambridge, MA
Gould SJ (1989) A developmental constraint in *Cerion*, with comments on the definition and interpretation of constraint in evolution. Evolution 43:516–539
Gould SJ, Lewontin R (1979) The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. Proc R Soc Lond B Biol Sci 205(1161):581–598
Griesemer J (2013) Integration of approaches in David Wake's model taxon research platform for evolutionary morphology. Stud Hist Phil Biol Biomed Sci 44(4):525–536
Griesemer J (2015) What salamander biologists have taught us about evo-devo. In: Love AC (ed) Conceptual change in biology. Boston studies in the philosophy and history of science. Springer, Dordrecht, pp 271–301

Hanken J (2015) Is heterochrony still an effective paradigm for contemporary studies of evo-devo? In: Love AC (ed) Scientific and philosophical perspectives on evolution and development. Springer, Dordrecht, pp 97–110
Jacob F (1977) Evolution and tinkering. Science New Ser 196(4295):1161–1166
Love AC (2003) Evolutionary morphology, innovation, and the synthesis of evolutionary and developmental biology. Biol Philos 18:309–345
Love AC (ed) (2015) Conceptual change in biology. Scientific and philosophical perspectives on evolution and development. Springer, Dordrecht
Love AC, Raff RA (2003) Knowing your ancestors: themes in the history of evo-devo. Evol Dev 5(4):327–330
Maynard Smith J, Burian R, Kauffman S, Alberch P, Campbell J, Goodwin B, Lande R, Raup D, Wolpert L (1985) Developmental constraints and evolution. Q Rev Biol 60:265–287
Moya A, Peretó J (2010) Pere Alberch (1954–1998) the passion for understanding evolution and development. Int Microbiol 1(2):159–160
Nuño de la Rosa L (2017) Computing the extended synthesis: mapping the dynamics and conceptual structure of the evolvability research front. J Exp Zool Part B Mol Dev Evol 328:395–411. https://doi.org/10.1002/jez.b.22741
Nuño de la Rosa L, Etxeberria A (2012) Patterns and processes in evo-devo: descriptions and explanations. In: de Regt H, Hartmann S, Okasha S (eds) EPSA philosophy of science. Amsterdam 2009. Springer, Dordrecht, pp 263–274
Olsson L (2012) Pere Alberch (1954–1998), pioneer of morphological evodevo. Acta Zool 93(2):245–246
Pigliucci M (2008) Is evolvability evolvable? Nat Rev Genet 9(1):75–82
Rasskin-Gutman D (2009) Molecular evo-devo: the path not taken by Pere Alberch. In: Rasskin-Gutman D, De Renzi M (eds) Pere Alberch, the creative trajectory of an evo-devo biologist. Universitat de Valencia, Valencia, pp 67–84
Rasskin-Gutman D, De Renzi M (eds) (2009) Pere Alberch, the creative trajectory of an evo-devo biologist. Universitat de Valencia, Valencia
Reiss J, Burke A, Archer C, De Renzi M, Dopazo H, Etxeberria A, Gale E, Hinchliffe R, Nuño de la Rosa L, Rose C, Rasskin-Gutman D, Müller GB (2009) Pere Alberch: originator of EvoDevo. Biol Theory 3(4):351–356
Rieppel O (2005) Modules, kinds, and homology. J Exp Zool 304B:18–27
Rieppel O (2006) 'Type' in morphology and phylogeny. J Morphol 267(5):528–535
Schwenk K (1995) A utilitarian approach to evolutionary constraint. Zoology 98:251–262
Wagner GP (1989) The biological homology concept. Annu Rev Ecol Syst 20(1):51–69
Wagner GP (1996) Homologues, natural kinds and the evolution of modularity. Integrative and Comparative Biol 36(1):36–43
Wagner GP, Laubichler M (2001) Character identification: the role of the organism. In: Wagner GP (ed) The character concept in evolutionary biology. Academic, pp 143–165
Wake D (1998) Pere Alberch (1954–1998) synthesizer of development and evolution. Nature 393:632
Wake D (2015) Homoplasy, a moving target. In: Love AC (ed) Conceptual change in biology. Boston studies in the philosophy and history of science. Springer, Dordrecht, pp 111–127

Part IV

Philosophy of Evo-Devo

Explanation in Evo-Devo

Marie I. Kaiser

Contents

Abstract

Evo-devo is a multidisciplinary field that investigates the interplay between evolutionary and developmental processes and brings together different kinds of explanatory strategies. This chapter examines the structure of paradigmatic explanations in evo-devo (e.g., the explanation of the origin of an evolutionary novelty) and raises philosophical questions about explanation in evo-devo. Much research in evo-devo is concerned with studying the developmental mechanisms that constrain and facilitate phenotypic evolution, which suggests that a distinctive feature of evo-devo is that it constructs mechanistic explanations. In this chapter, I discuss three major challenges for thinking about evo-devo as providing mechanistic explanations. First, an explanation of an evolutionary novelty consists of several mechanistic explanations, rather than being a single one. Second, developmental mechanisms are ontogenetically and phylogenetically dynamic and thus difficult to individuate. Third, an explanation of an evolutionary novelty

M. I. Kaiser (✉)
Department of Philosophy, Bielefeld University, Bielefeld, Germany
e-mail: kaiser.m@uni-bielefeld.de

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_90

also has a non-mechanistic, historical part. Finally, I discuss whether evo-devo's emphasis on mechanistic explanation promotes explanatory integration or results in a reductionist view of evolution.

Keywords

Mechanistic explanation · Historical explanation · Explanatory integration · Reductive explanation

Introduction: Philosophical Debate About Scientific Explanation

Developing explanations that demonstrate how and why natural phenomena occur is a major goal of science, including the field of evolutionary developmental biology (evo-devo). Traditionally, philosophers of science have focused on explicating the nature of scientific explanation by proposing different philosophical accounts of explanation. These accounts seek to specify a feature (or a set of features) that all scientific explanations have in common and that distinguishes them from both mere descriptions or predictions and non-scientific explanations (e.g., explanations given in everyday contexts).

An influential philosophical account of explanation is Hempel and Oppenheim's (1948) *deductive-nomological model* of explanation, according to which a scientific explanation is a sound deductive argument. A sentence describing the phenomenon to be explained (the *explanandum*) is logically deduced from at least one general law statement and certain statements of antecedent conditions (the *explanans*). The basic idea behind this account is that a phenomenon is explained by showing that, given the particular circumstances and the laws in question, the phenomenon was to be expected. For instance, assuming that the generalization that all genes are composed of nucleic acids is a biological law, it follows from this law and from the circumstance that x_1 is a gene that x_1 is composed of nucleic acids.

Other philosophers have challenged the deductive-nomological model and its assumptions that explanations are characterized by their logical structure and must refer to laws. Proponents of the *causal-mechanical model* of explanation hold that scientific explanations show how and why natural phenomena occur by tracing the causes, causal processes, or causal mechanisms that lead to or compose the phenomenon. What distinguishes scientific explanations from mere descriptions is that explanations situate phenomena within the causal structure of the world. Different philosophers diverge in their views about what it means to trace the causes of a phenomenon. Woodward's (2003) *interventionist account* of causal explanation is among the accounts most relevant to the biological sciences. It emphasizes that explanations must provide answers to what-if-things-had-been-different questions that can be used to manipulate and control the phenomenon to be explained. According to this account, causal explanations must exhibit patterns of counterfactual dependency – they must describe factors that, if they were changed by interventions, would regularly lead to changes in the phenomenon to be explained. For

example, there is a counterfactual dependency between the stimulation of a muscle fiber and the contraction of this muscle fiber such that if a muscle fiber were stimulated by an incoming action potential, the muscle fiber would contract. Waters (2007) takes up the basic idea of interventionism and argues further that causal explanations in the biological sciences must describe actual difference makers. A second major causal-mechanical account of explanation that is relevant to biology is the *mechanistic account*. The new mechanists (e.g., Machamer et al. 2000; Craver 2007; Craver and Darden 2013; Glennan 2017) stress that many explanations in the life sciences explain a phenomenon by describing the causal mechanism that underlies or produces it.

In recent decades, philosophical attention has shifted largely from the general nature of scientific explanation toward explanatory practices in specific scientific fields. Rather than debating universal features that all scientific explanations have (or should have) in common, philosophers now focus more on actual cases of explanations and analyze the explanatory strategies that occur in scientific practice. They identify, for instance, different *types of explanations* that can be found in different scientific contexts (e.g., topological, mechanistic, reductive, functional, etiological, or mathematical explanations). They also examine the "functional roles" (Woody 2015) that explanations play in scientific practice. These philosophical analyses are often characterized by pluralist and pragmatist perspectives. Scientific pluralists emphasize the diversity of scientific approaches such as the various types of explanations that can be found in the biological sciences (Kellert et al. 2006). Pragmatists draw our attention to the fact that explanations are answers to questions (or solutions of problems) where the adequacy of an explanation depends on both the specific question that it addresses and the purpose or goal that is supposed to be achieved in a particular scientific context (van Fraassen 1980, Chap. 5). Philosophers who adopt pluralist and pragmatist stances do not deny that describing laws, revealing counterfactual dependencies (e.g., by identifying difference makers), or describing causal mechanisms is essential to scientific explanations. They rather emphasize that scientific explanations do not consist in only one of these things but differ from context to context, depending on the scientific question that they seek to answer and the functional roles they are supposed to play. In particular, not all biological explanations seem to explain a phenomenon by describing its underlying causal mechanism. For example, it is argued that biological phenomena, such as a species' optimal number of offspring or the long-term stability of human-microbe associations, are explained structurally in virtue of the mathematical properties of the system rather than in terms of the mechanisms that underlie the phenomena (Huneman 2018). Furthermore, only a few (if any) biological explanations seem to appeal to generalizations that are natural laws in a strict sense (i.e., that do not have exceptions and that are not historically contingent; see chapter ▶ "Generalization in Evo-Devo"). Mendel's law of independent assortment, for example, explains why the two pea traits "yellow seeds" and "round seeds" are inherited and combined independently from each other. Despite its name, Mendel's law is not a natural law but a generalization with major exceptions because it does not hold for genes that are close together on a chromosome.

With this general background in view, the remainder of the chapter focuses on biological explanations that are developed in the field of evo-devo. My approach aligns broadly with pluralist and pragmatist perspectives. Evo-devo is a multidisciplinary field that brings together explanatory strategies of different kinds. The types of explanation given can vary depending on the phenomenon under study, the research question that is being asked, and the purpose of research. Despite this diversity, however, the explanatory strategies in evo-devo also share some general features that raise interesting philosophical questions about mechanisms, integration, and reduction. The starting point of my analysis is the observation that, since evo-devo investigates the interplay between evolutionary and developmental processes (e.g., how development constrains and facilitates phenotypic evolution), it provides explanations that differ from population-level explanations in standard evolutionary biology. Explanations in evo-devo often seem to be *mechanistic explanations* because they explain phenotypic evolution by describing the molecular or cellular mechanisms that figure in the development of a phenotypic trait (see chapter ▶ "Mechanisms in Evo-Devo"). On the other hand, "[e]vo-devo is partly built around denying the correctness of one-sided explanatory strategies" (Hamilton 2009, 213). Accordingly, explanations in evo-devo not only refer to developmental mechanisms but also describe historical processes, such as the process of how a phenotypic trait or a developmental system changes over time (Calcott 2009). The integrative character of these explanations seems to pose a challenge for thinking about evo-devo as providing mechanistic explanations alone because the historical part of the explanation does not seem to be mechanistic. Other authors, however, argue that it is the mechanistic framework in particular that provides a "true integration of developmental and evolutionary processes" (Laubichler 2009, 38).

This chapter proceeds as follows. In section "What Evo-Devo Seeks to Explain," I clarify which kinds of phenomena evo-devo seeks to explain. Section "The Concept of a Mechanistic Explanation" introduces the philosophical concept of a mechanistic explanation. In section "Evo-Devo and Mechanistic Explanation," I apply this concept to the explanations developed in evo-devo and highlight some challenges for thinking about evo-devo as providing mechanistic explanations. In section "Mechanistic Explanation, Integration, and Reduction," I discuss how far mechanistic explanations furnish explanatory integration and whether or not they result in a reductionist view of evolution.

What Evo-Devo Seeks to Explain

Evo-devo studies the interrelations between development and evolution on multiple levels. It not only brings together developmental biology and evolutionary biology but also integrates a variety of biological disciplines, from paleontology to quantitative genetics and from morphology to ecology (Moczek et al. 2015; Brigandt 2015; see also chapter ▶ "Interdisciplinarity in Evo-Devo"). This integration is necessary because evo-devo investigates and seeks to explain a wide range of phenomena. Two main axes of research can be distinguished (see also Müller 2007; Love 2015). First,

evo-devo studies how development evolves, such as how developmental systems originated and how developmental processes are modified through time. Second, evo-devo also investigates how development constrains and facilitates evolution, such as how features of development influence the rate and direction of phenotypic variation and how development contributes to phenotypic novelty. Sometimes a third set of research questions is mentioned, which concerns the role that the environment plays in evolutionary developmental interactions. This third set of research questions considers the two main research axes in particular ways, such as by focusing on how phenotypic plasticity evolves or how ecologically responsive features of development share evolutionary trajectories (see chapters ▶ "Eco-Evo-Devo" and ▶ "Developmental Plasticity and Evolution").

One way to specify the phenomena evo-devo seeks to explain is by contrast with the phenomena explained by standard evolutionary biology. Both fields study phenotypic change over time, but standard evolutionary biology typically regards it as a quantitative phenomenon that takes place on the level of populations. This focuses investigation on how variation in phenotypic traits is correlated with allele frequencies in populations and the processes that drive change in populations over time (such as natural selection, drift, and mutation). Evo-devo, in contrast, often treats phenotypic change at the level of individuals or lineages and directs our attention to how changes in developmental mechanisms give rise to both quantitative and qualitative changes in phenotypic traits and how this shapes (and is shaped) by evolutionary processes.

A major type of phenomenon that evo-devo seeks to explain is the origin of evolutionary novelties, such as how fish fins evolved into tetrapod limbs (see chapters ▶ "Developmental Innovation and Phenotypic Novelty" and ▶ "Evo-Devo of the Fin-to-Limb Transition"). Here the question is not which selective factors played a role in the modification and propagation of limbs in a population. Rather, many evo-devo researchers ask how a developmental mechanism that produces fins could have changed through time to become a developmental mechanism that produces limbs. One approach to explaining the origin of an evolutionary novelty involves describing a continuous trajectory of phenotypic change in a lineage of organisms in connection with how the developmental mechanisms that produce specific phenotypic traits likely changed at each stage (Calcott 2009). This evo-devo approach explains phenotypic evolution on the level of individual organisms by integrative descriptions of evolutionary processes and developmental mechanisms. Another approach to explaining the origin of evolutionary novelty involves specifying the details and governing principles of the genotype-phenotype map to understand how new possibilities arise. This distinct approach (sometimes labeled devo-evo) explains phenotypic evolution by tracing how the possible trajectories from genotype to phenotype can be modified and in what ways, as well as how easy or difficult it is to make particular modifications (see chapter ▶ "Devo-Evo of Cell Types"). Both approaches differ from explanations in standard evolutionary theory that invoke population-level processes, such as natural selection, to account for evolutionary changes in the distribution of phenotypes.

The Concept of a Mechanistic Explanation

Proponents of the new mechanistic philosophy emphasize that much research in the life sciences is devoted to discovering mechanisms and developing mechanistic explanations (see chapter ▶ "Mechanisms in Evo-Devo"). To mechanistically explain a phenomenon is to describe the mechanism that underlies or produces the phenomenon (Machamer et al. 2000; Craver 2007; Craver and Darden 2013; Glennan 2017). Despite different interpretations among the new mechanists, there is a broad consensus on a minimal characterization of mechanisms that is applicable to biology and other scientific fields:

> A mechanism for a phenomenon consists of entities and activities organized in such a way that they are responsible for the phenomenon. (Illari and Williamson 2012, 120)

This characterization highlights four features: (1) a mechanism is decomposable into components of two kinds, *entities and activities*, (2) how the components are *organized* is crucial to the working of a mechanism, (3) a mechanism is individuated with respect to a specific *phenomenon*, and (4) a mechanism bears a characteristic relation to its phenomenon, namely, according to the above characterization, a mechanism is *responsible for* a phenomenon.

Explaining a phenomenon by describing the mechanism that underlies or produces it means describing how the entities and activities that compose a mechanism work together to bring about the phenomenon in question. Consider the mechanism for anterior/posterior patterning in vertebrate limb development. It consists of material objects ("entities"), such as the Zone Polarizing Activity (ZPA), which is a gradient of the protein Sonic hedgehog (Shh), the Apical Ectodermal Ridge (AER), and the proteins FGF4 and FGF8. These entities engage in activities, such as inducing, expressing, producing, and maintaining, which bring about particular kinds of effects. For example, the AER induces cell outgrowth in the nascent limb bud that leads to its enlargement.

Entities and activities are spatially, temporally, and hierarchically organized in a specific way, which is why mechanisms differ from mere aggregates, mere spatial arrangements, and mere temporal sequences. For instance, the Shh proteins are spatially distributed in the limb bud in a characteristic way. The activities of entities are temporally ordered in a specific way; for example, Shh induces the AER, which produces FGF4 and FGF8, which in turn maintain the expression of Shh. Hierarchical ordering is found in the ZPA, which consists of a gradient of Shh proteins. Mechanistic explanations describe which entities and which activities are organized in which ways to bring about the phenomenon to be explained.

Mechanisms consist of all and of only those entities and activities that are relevant to a specific phenomenon. In other words, the components and boundaries of mechanisms are individuated with regard to a specific phenomenon. This is why mechanisms are said to be mechanisms *for* phenomena. The mechanism for anterior/posterior patterning in vertebrate limb development typically excludes ATPases producing ATP because they are not specifically relevant to the formation of the

anterior/posterior axis in limbs. Spelling out the relevance condition is controversial (Kaiser 2018). One idea, "constitutive relevance" (Craver 2007, 139), is that an entity engaged in an activity is a component of a mechanism for a phenomenon only if the components and the phenomenon are mutually manipulable: changing a component (e.g., knocking out the Shh gene) changes the phenomenon (e.g., syndactyly or loss of digits) and changing the phenomenon requires changes to one or more of the components.

A mechanism can be "responsible for" a phenomenon in many ways, such as regulating, maintaining states, or exhibiting behaviors (Illari and Williamson 2012, 123–125), with different degrees of regularity (Machamer et al. 2000). Because mechanisms can be related to phenomena in different ways, it is important to distinguish etiological mechanisms from constitutive mechanisms (Kaiser and Krickel 2017). The former cause or produce a phenomenon, and therefore etiological mechanistic explanations describe the antecedent causes that lead up to the phenomenon in question. By contrast, constitutive mechanisms constitute or compose a phenomenon, and therefore constitutive mechanistic explanations describe the entities and activities that constitute the phenomenon to be explained, similar to explanations of the behavior of a whole in terms of its parts (Kaiser 2015, Chap. 6).

Evo-Devo and Mechanistic Explanation

Evo-devo not only studies evolutionary processes but also takes into account the developmental mechanisms that produce phenotypes of individual organisms. It is called a "mechanistic science" (Wagner et al. 2000, 819) because it "represents a causal mechanistic approach towards the understanding of phenotypic change in evolution" (Müller 2007, 945). However, the relationship between mechanistic explanations, as described in section "The Concept of a Mechanistic Explanation," and this "causal mechanistic approach" is not so clear. Evo-devo does not simply explain evolutionary phenomena in terms of developmental mechanisms, but it also integrates explanatory strategies from different fields in more complex ways (Love 2008). The resulting "mechanistic" explanations refer to developmental mechanisms *and* evolutionary processes, and it is unclear whether and how these correspond to mechanistic explanations as discussed by proponents of the new mechanistic philosophy.

Importantly, the central question is not whether evo-devo provides *only* mechanistic explanations. The phenomena that evo-devo investigates and seeks to explain are diverse and suggest multiple explanatory strategies (recall section "What Evo-Devo Seeks to Explain"). However, exploring the question of whether paradigmatic examples of evo-devo explanations can be characterized as mechanistic explanations (sensu section "The Concept of a Mechanistic Explanation") reveals three major challenges (sections "Several Mechanistic Explanations," "Difficulties with Individuating Mechanisms," and "The Historical Part of Lineage Explanations"). One such paradigmatic example of explanation is the origin of evolutionary novelties such as the origin of tetrapod limbs (see chapter

▶ "Developmental Innovation and Phenotypic Novelty"). These explanations constitute a central explanatory focus of evo-devo, in part because they lie outside the explanatory capacities of standard evolutionary biology (Wagner et al. 2000, 822). They require understanding how developmental mechanisms generate trait variability on the trajectory from genotype to phenotype in individual organisms.

Reconsidering the example from above, how does evo-devo attempt to explain the origin of tetrapod limbs from fish fins? Calcott characterizes one approach as "lineage explanations" (2009, 52), which have two dimensions. First, they describe a continuous trajectory of phenotypic change in a lineage of organisms (continuity dimension). Second, they demonstrate how developmental mechanisms bring about each of the phenotypic traits in the continuous trajectory (production dimension). On this approach, the origin of tetrapod limbs is explained by describing a continuous sequence of phenotypic traits that starts from fish fins and ends with tetrapod limbs with there being only small changes between the successive traits and their underlying mechanisms at any one juncture. Furthermore, each phenotypic trait in the continuous sequence is given a mechanistic explanation that shows how a developmental mechanism brings about this trait in an individual organism. So, an evo-devo explanation of the origin of tetrapod limbs demonstrates how organisms that develop fins could have continuously changed and transformed into organisms that develop limbs: "how something that works like *this* could have turned into something that works like *that*" (Calcott 2009, 56; emphasis in the original). They provide an understanding of how a phenotypic trait is produced and how it could have changed.

Several Mechanistic Explanations

A lineage explanation of the origin of an evolutionary novelty is a mechanistic explanation because it describes the developmental mechanism that produces a trait and shows how the components of this mechanism could have continuously changed into a mechanism that produces a different trait. However, this approach does not yield a single mechanistic explanation but rather several different mechanistic explanations – one mechanistic explanation for each trait in the continuous sequence of changing traits. Each mechanistic explanation describes how the underlying developmental mechanism brings about a specific phenotypic trait that some organisms in the lineage exhibit. For each trait, we have an account of which entities engage in which activities and how they are spatially, temporally, and hierarchically organized so that they generate the phenotypic trait in question (Fig. 1).

A lineage explanation for the origin of vertebrate limbs contains one mechanistic explanation for each phenotypic trait in the continuous sequence of traits. In our example, it describes the mechanism of how fish develop fins (M_1), the mechanism of how vertebrates develop limbs (M_4), and the mechanisms of how each intermediate phenotypic trait is developed, so how the extinct *Eusthenopteron* develops fins with lobes (M_2) and how the extinct *Tiktaalik roseae* develops relatively strong and robust pelvic fins (M_3). (A four-step sequence is an idealization still; obviously, there would be more intermediate steps in the sequence.) Although current scientific

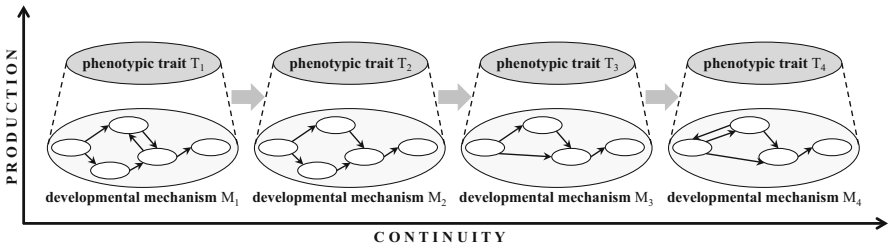


Fig. 1 A schematic representation of a lineage explanation for the origin of evolutionary novelties. (Redrawn from Craver (2007, 7) and Calcott (2009, 58, 60))

research might lack information about intervening steps in a sequence because not all of the developmental mechanisms are known, evo-devo biologists aim to provide a complete lineage explanation of the origin of vertebrate limbs that contains a description of all relevant mechanisms.

The entities and activities in a mechanism that are relevant to bringing about a phenotypic trait and how they are organized vary from trait to trait. This is why the white circles and arrows in Fig. 1, representing the entities, activities, and their organization, vary from mechanism to mechanism. In the fin case, the developing fin bud has an elongated structure, the apical fold (AF), which is missing in the limb bud, and the limb skeleton is composed of endochondral bones (endoskeleton), whereas the fin skeleton consists almost entirely of fin rays (exoskeleton), without a robust endoskeleton. The developmental mechanisms, however, share many entities and activities (e.g., *Hox* genes that are expressed, gradients of the protein Shh that induces the expression of other genes, etc.), which gives rise to continuity in the sequence of developmental mechanisms and makes plausible that organisms with one phenotypic trait (e.g., T_3) have evolved from organisms with the preceding phenotypic trait (e.g., T_2).

In sum, that lineage explanations consist of several different mechanistic explanations is an important aspect of their structure, though it does not present a serious obstacle for claiming that evo-devo provides mechanistic explanations.

Difficulties with Individuating Mechanisms

The entities and activities that compose a developmental mechanism and how they are organized within it change dramatically during the time of its operation (i.e., from its starting conditions to its finishing conditions). Entities come into existence, transform, and sometimes go out of existence again. How entities are spatially located and how they are spatially related to each other can vary extremely. Likewise, in different developmental stages, entities engage in different activities, and the temporal ordering of continuing activities changes. This "dynamic constitution and organization" (Love 2018, 343) of developmental mechanisms makes it challenging to individuate them or draw the boundaries of a particular mechanism by

telling apart those entities and activities that belong to it from those that do not (Kaiser 2018, 124–126).

This holds especially for developmental mechanisms that are described in lineage explanations since they span evolutionary time as well. Lineage explanations describe developmental mechanisms that *evolve*. They not only describe (changing) mechanisms for how a phenotypic trait originates developmentally in an individual organism, but they also describe how those dynamic mechanisms have undergone radical evolutionary shifts in their components and how they are organized. It is even more difficult to individuate developmental mechanisms that evolve because one has to decide when the dynamic organized components of a mechanism have changed enough to be characterized as a new developmental mechanism that is distinct from its precursor (see chapter ▶ "Mechanisms in Evo-Devo"). Identifying the ontogenetic and phylogenetic boundaries of developmental mechanisms is a serious challenge for evo-devo biologists, but it does not provide us with an argument against the claim that, at least sometimes, evo-devo biologists do succeed in individuating developmental mechanisms and providing mechanistic explanations.

The Historical Part of Lineage Explanations

Lineage explanations describe not only developmental mechanisms but also evolutionary processes that are historical in character. Explaining the origin of an evolutionary novelty requires describing how the different developmental mechanisms bring about the different phenotypic traits (production dimension), as well as how these mechanisms and their components could have gradually changed (continuity dimension). As "genuine explanations of evolutionary change" (Calcott 2009, 72), lineage explanations must also describe the historical process of how developmental mechanisms of individual organisms are continuously modified to give rise to a specific sequence of phenotypic traits. If the mechanistic explanations in the sequence of change are predicated of individual organisms, then the historical process of how a mechanism can change to become a different mechanism is not itself a mechanistic explanation. Instead, it is a historical explanation of how the components of a mechanism can change so that the mechanism transforms into a new mechanism (and so on) until the mechanism that brings about the evolutionary novelty has evolved. However, if we predicate the mechanism of the lineage rather than individual organisms, then this interpretation can be avoided (Nuño de la Rosa and Villegas 2019; see chapter ▶ "Dispositional Properties in Evo-Devo").

Another conception of mechanism could avoid this interpretation. One might argue that even though the historical part of the lineage explanation does not describe the working of a developmental mechanism, it still describes the working of the mechanism of natural selection. This would make lineage explanations in evo-devo mechanistic in a different sense. Some argue that the study of ultimate causes in evolutionary biology results in mechanistic explanations because natural selection can be characterized as a mechanism (Barros 2008). However, there are difficulties with this conception since there are good reasons to worry that natural selection is

not mechanistic in the appropriate sense (Millstein and Skipper 2005). Glennan has argued that historical explanations can describe "ephemeral mechanisms" (2017, 129), which are non-stable and produce singular events (and thus are non-regular). Whether the corresponding weakening of the regularity and the stability requirement on mechanisms should be embraced remains an open question.

There is another reason for focusing on mechanistic explanations in evo-devo without appealing to natural selection as a mechanism. First, although it is plausible (in principle) to speak of natural selection as an evolutionary mechanism, this is not how the notion of mechanism is primarily used in evo-devo. Practitioners typically invoke "causal mechanism," "mechanistic relationships," or "mechanistic approach" to refer to developmental mechanisms. If the aim is to characterize lineage explanations in evo-devo as "purely" mechanistic, then an alternative interpretation, such as predicating mechanisms of lineages, is preferable to avoid the misleading impression that they describe developmental mechanisms only. Second and more importantly, lineage explanations and other evo-devo approaches to the origin of evolutionary novelties simply do not refer to natural selection. They focus on how developmental mechanisms, including mapping relations between genotype and phenotype, could have changed to produce novel traits; they do not refer to populations and specify population processes that could have modified these phenotypic traits.

To conclude, lineage explanations consist of two parts. The mechanistic part of the explanation describes the developmental mechanisms that underlie each phenotypic trait in the sequence of traits leading to the evolutionarily novel trait. The historical, non-mechanistic part of the explanation describes the continuous changes between the different developmental mechanisms and how this led to the evolution of the developmental mechanism that brings about the evolutionarily novel trait. Hence, evo-devo provides mechanistic explanations but they are not fully mechanistic.

Mechanistic Explanation, Integration, and Reduction

Does evo-devo's emphasis on developmental mechanisms and mechanistic explanation indicate an underlying commitment to a reductionist approach? Or do these explanatory approaches also suggest forms of integration? Consider the relation between mechanistic explanation and explanatory integration first. Evo-devo is said to provide not only an "extended, more inclusive explanatory framework" (Müller 2007, 500; see chapter ► "Evo-Devo's Contributions to the Extended Evolutionary Synthesis") but also an approach that is *integrative* in at least two ways. First, it is a multidisciplinary field that brings together a variety of disciplines (Moczek et al. 2015; Brigandt 2015). Accounting for evolutionary novelty requires integrating insights from evolutionary genetics, developmental biology, paleontology, phylogeny, morphology, and other scientific fields (Love 2008). Second, evo-devo brings together different explanatory strategies in one explanation. It integrates the analysis of historical processes with that of extant developmental systems, bringing together form-related and function-related causes into a single

explanatory model (Hamilton 2009, 215; see chapter ▶ "Form and Function in Evo-Devo"). Laubichler argues for "a true integration" (2009, 38) of evolution and development through the molecular analysis of developmental mechanisms and gene regulatory networks in combination with the comparison of these mechanisms across taxa. This provides an explanatory integration in terms of a mechanistic understanding of both the development and evolution of phenotypic traits (Laubichler 2009, 36–39).

Mechanistic explanations often combine several levels of organization and thus provide some sort of explanatory integration (Brigandt 2010; see chapters ▶ "Mechanisms in Evo-Devo" and ▶ "Levels of Organization in Evo-Devo"). However, if adopting a "mechanistic framework" means that only mechanistic explanations are legitimate, then this could impede forms of explanatory integration that bring together different approaches for both development and evolution. The preceding section indicated that paradigmatic explanations in evo-devo, such as lineage explanations, involve the integration of multiple parts and these may not always be construed as mechanistic. Successful explanatory integration in evo-devo need not result in *purely* mechanistic explanations, and the more salient aspect is evo-devo's efforts to integrate different explanatory strategies in a single explanation to account for the origin of evolutionary novelties and other phenomena.

One might worry that evo-devo's focus on mechanistic explanations runs contrary to promoting plurality and integration because it is based on a *reductionist* approach. By seeking to explain phenotypic evolution in terms of molecular developmental mechanisms and gene regulatory networks, mainstream evo-devo appears to privilege the molecular gene in its explanatory framework and neglect to investigate other potentially relevant causes (Hamilton 2009, 215). Explanations of phenotypic evolution that refer only to the actions of and interactions between genes are a kind of reductive explanation that can be characterized as a "fundamental-level explanation" (Kaiser 2015, 202) because it assumes that the level of genes is explanatorily fundamental and that any successful explanation must refer to genes. The criticism that evo-devo's emphasis on mechanistic explanations results in gene-centrism and misleading reductive explanations may be true for several evo-devo research programs. However, mechanistic explanations need not be gene-centric, fundamental-level explanations. Evo-devo's actual explanatory practice shows that there are also research programs that take into account epigenetic causes and the influence of environmental factors on development and evolution (Müller 2007; see chapter ▶ "Evo-Devo's Contributions to the Extended Evolutionary Synthesis"). Whether the resulting mechanistic explanations would still be reductive explanations (e.g., because they refer to lower-level and internal factors; Kaiser 2015) is an important question, but clearly, they would not be "naively reductionist" (Hamilton 2009, 216).

In conclusion, evo-devo's emphasis on developmental mechanisms and mechanistic explanation can furnish explanatory integration because it integrates evolution and development and because mechanistic explanations often bridge several levels of organization. If this emphasis on developmental mechanisms, however, comes along with gene-centrism and the search for fundamental-level explanations, evo-devo is practiced in a reductionistic fashion.

Cross-References

- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Developmental Plasticity and Evolution
- ▶ Devo-Evo of Cell Types
- ▶ Dispositional Properties in Evo-Devo
- ▶ Eco-Evo-Devo
- ▶ Evo-Devo's Contributions to the Extended Evolutionary Synthesis
- ▶ Evo-Devo of the Fin-to-Limb Transition
- ▶ Form and Function in Evo-Devo
- ▶ Generalization in Evo-Devo
- ▶ Interdisciplinarity in Evo-Devo
- ▶ Levels of Organization in Evo-Devo
- ▶ Mechanisms in Evo-Devo

References

Barros DB (2008) Natural selection as a mechanism. Philos Sci 75:306–322
Brigandt I (2010) Beyond reduction and pluralism: toward an epistemology of explanatory integration in biology. Erkenntnis 73:295–311
Brigandt I (2015) Evolutionary developmental biology and the limits of philosophical accounts of mechanistic explanation. In: Braillard PA, Malaterre C (eds) Explanation in biology: an enquiry into the diversity of explanatory patterns in the life sciences. Springer, Dordrecht, pp 135–173
Calcott B (2009) Lineage explanations: explaining how biological mechanisms change. Br J Philos Sci 60:51–78
Craver C (2007) Explaining the brain. Clarendon Press, Oxford
Craver C, Darden L (2013) In search of mechanisms. University of Chicago Press, Chicago
Glennan S (2017) The new mechanical philosophy. Oxford University Press, Oxford
Hamilton AL (2009) Toward a mechanistic evo devo. In: Laubichler MD, Matenschein J (eds) Form and function in developmental evolution. Cambridge University Press, Cambridge, pp 213–224
Hempel CG, Oppenheim P (1948) Studies in the logic of explanation. Philos Sci 15:135–175
Huneman P (2018) Outlines of a theory of structural explanations. Philos Stud 175:665–702
Illari P, Williamson J (2012) What is a mechanism? Thinking about mechanisms *across* the sciences. Eur J Philos Sci 2:119–135
Kaiser MI (2015) Reductive explanation in the biological sciences. Springer, Cham
Kaiser MI (2018) The components and boundaries of mechanisms. In: Glennan S, Illari P (eds) Routledge handbook of mechanisms and mechanical philosophy. Routledge, New York, pp 116–130
Kaiser MI, Krickel B (2017) The metaphysics of constitutive mechanistic phenomena. Br J Philos Sci 68(3):745–779
Kellert SH, Longino HE, Waters CK (2006) Scientific pluralism. University of Minnesota Press, Minneapolis
Laubichler MD (2009) Form and function in Evo Devo: historical and conceptual reflection. In: Laubichler MD, Maienschein J (eds) Form and function in developmental evolution. Cambridge University Press, Cambridge, pp 10–46
Love AC (2008) Explaining evolutionary innovations and novelties: criteria of explanatory adequacy and epistemological prerequisites. Philos Sci 75:874–886

Love AC (2015) Evolutionary developmental biology: philosophical issues. In: Heams T, Huneman P, Lecointre L, Silberstein M (eds) Handbook of evolutionary thinking in the sciences. Springer, Berlin, pp 265–283

Love AC (2018) Developmental mechanisms. In: Glennan S, Illari P (eds) Routledge handbook of mechanisms and mechanical philosophy. Routledge, New York, pp 332–347

Machamer P, Darden L, Craver CF (2000) Thinking about mechanisms. Philos Sci 67:1–25

Millstein RL, Skipper RA (2005) Thinking about evolutionary mechanisms: natural selection. Stud Hist Philos Biol Biomed Sci 36(2):327–347

Moczek AP, Sears KE, Stollewerk A, Wittkopp PJ, Diggle P, Dworkin I, Ledon-Rettig C, Matus DQ, Roth S, Abouheif E, Brown FD, Chiu C-H, Cohen CS, De Tomaso AW, Gilbert SF, Hall B, Love AC, Lyons DC, Sanger TJ, Smith J, Specht C, Vallejo-Marin M, Extavour CG (2015) The significance and scope of evolutionary developmental biology: a vision for the 21st century. Evol Dev 17(3):198–219

Müller GB (2007) Evo-devo: extending the evolutionary synthesis. Nat Rev Genet 8:943–949

Nuño de la Rosa L, Villegas C (2019) Chances and propensities in evo-devo. Br J Philos Sci. https://doi.org/10.1093/bjps/axz048

van Fraassen BC (1980) The scientific image. Oxford University Press, Oxford

Wagner GP, Chiu C-H, Laubichler M (2000) Developmental evolution as a mechanistic science: the inference from developmental mechanisms to evolutionary processes. Am Zool 40:819–831

Waters CK (2007) Causes that make a difference. J Philos 104:551–579

Woodward J (2003) Making things happen: a theory of causal explanation. Oxford University Press, New York

Woody AI (2015) Re-orienting discussions of scientific explanation: a functional perspective. Stud Hist Philos Sci 52:79–87

Generalization in Evo-Devo

Jani Raerinne

Contents

Abstract

How general are our findings concerning the evolution and development of life forms? I will discuss this question of generality and the related question concerning the existence of lawlike generalizations with specific reference to evolutionary developmental biology (evo-devo). On the one hand, evo-devo suggests that the evolution and development of life forms is more contingent than we have previously presumed, thus showing that we have accidental and non-generalizable results concerning evolutionary and developmental phenomena. On the other hand, evo-devo reveals the existence of generality-maintaining mechanisms, thus showing that certain evolutionary and developmental generalizations have stability and necessity. These two findings suggest a picture of evolution as being both stable and contingent, repeatable and unique, and generalizable and non-generalizable. This picture of evolution was already present during the Modern Synthesis. At the same time, evo-devo has provided us with new insights into what are generality-destroying, generality-maintaining, and generality-creating mechanisms behind the evolution and development of life forms.

J. Raerinne (✉)
Helsinki Collegium for Advanced Studies, University of Helsinki, Helsinki, Finland
e-mail: jani.raerinne@helsinki.fi

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_92

Keywords

Constraints · Contingency · Natural kinds · Generalizations · Laws

Introduction

How general are our findings concerning the evolution and development of extant and extinct life forms? What kinds of generalizations can be identified in evolutionary developmental biology (evo-devo)? This topic is related to many central epistemic, ontological, and methodological issues in both evolutionary and developmental studies. As a consequence, answering these questions is pertinent to ongoing scientific inquiry found in evo-devo.

A classic question surrounding generality in evolution is whether there is a direction to evolution or whether evolutionary outcomes can be predicted. Can we discern what evolutionary trajectories will be followed by traits? Can we predict when and where new traits or novelties will arise? Or, are we limited to only explain the origin of a novelty post facto? Are evolutionary processes capable of producing general and stable patterns, or do they yield idiosyncratic and unique results? If evolution is unique and non-repeatable, as evidence at least partly suggests, how and with what kind of evidence do we test phylogenetic hypotheses concerning the ancestral relationships between taxa, especially since extant and extinct life forms exhibit a considerable diversity of traits and body plans? Do evolutionary or developmental laws exist? What role do natural selection or various kinds of constraints have in creating, destroying, or maintaining generalities around which classical biologists formulated many bold "laws"? Can anything general be predicted about the evolution of possible life forms elsewhere based on our evolutionary and developmental understanding?

Answering all, or even many, of the above questions is beyond the scope of this chapter. The focus here will be on the question of how general results and findings concerning the evolution and development of life forms are and how evo-devo has helped answer this question vis-à-vis its predecessors.

In the "The Significance of Exceptions," the traditional idea of biological generalizations being riddled with exceptions is discussed. Then, in "Accidental Products of Evolutionary History," the picture of evolution being a contingent and historical process is portrayed as already (at least partly) included in the Modern Synthesis. The implication of these sections is that we lack universally true and stable biological generalizations and laws concerning evolutionary and developmental phenomena, since biological generalizations are both riddled with exceptions and accidental products of evolutionary history. However, in "From Contingency to Constraints and Generative Entrenchment," the implications of evo-devo on traditional ideas about stable generalizations and accidental outcomes are discussed before treating the "Disappearance and Return of Laws and Natural Kinds" in biology. The final section concludes.

The Significance of Exceptions

Biologists have always sought generalizations that would allow predicting, explaining, and understanding phenomena. A common pattern is that a bold generalization is initially formulated based on empirical findings and data. Subsequent research, however, reveals an increasing number of exceptions to the original generalization.

Classical evolutionary biologists, such as Mayr (1942) and Rensch (1960), devoted considerable time to finding generalities and elucidating generalizations for both evolutionary and developmental phenomena while trying to explain these as effects of natural selection. Many generalizations were dubbed as laws, at least initially. For instance, Cope's law – a trend toward increased body size in many taxa through life's history – was explained as a gradual pattern arising from selection for larger body sizes, which were thought to confer adaptive advantages on individuals (e.g., via reduced predation). Other examples of classical evolutionary and developmental generalizations include the biogenetic law, according to which ontogeny recapitulates phylogeny; Dollo's law of phylogenetic irreversibility, which includes the idea that evolution cannot be reversed and that complex structures lost in evolution cannot be regained; Mendel's laws, which include both segregation (during gamete formation, alleles dissociate so that each gamete carries only one allele for each genetic locus) and independent assortment (allele dissociation occurs independently at each genetic locus); the law of the generalist, according to which unspecialized species tend to avoid extinction longer than specialized species; Williston's law (or the law of anisomerism), a phylogenetic trend in which serial, repetitive, similar, or unspecialized traits or parts in organisms evolve toward fewer numbers and more specialized functions; Mayr's law (allopatric speciation), which holds that new species evolve when a population is separated by geographic isolation from its parent population; and the central dogma, according to which DNA is transcribed to RNA and RNA is translated to proteins unidirectionally.

Subsequent to the formulation of each of these classic "laws," biologists have spent considerable time appraising the validity of these laws, questioning previous explanations given for them, and identifying exceptions to the purported generalizations. For example, Mendel's laws have many exceptions (Crow 1979). Linkage is an exception to independent assortment because two (or more) alleles at different loci exhibit correlations in assortment due to proximity of location on a chromosome. Dollo's law is considered by some to be relatively established, though there is plenty of discussion about how to define it, what would count as an exception, and what are proper methods for its testing (Gould 1970). There are clear exceptions to this law as well, such as the re-evolution of mandibular teeth in a frog genus after the disappearance of the trait in the lineage (Wiens 2011).

Cope's law does not apply to flying animals in general; and, even in lineages that do exhibit this trend (e.g., equines), there is considerable stasis and reversals (i.e., decreases in body size) in lineages (MacFadden 1986). Some exceptions to Mayr's law include polyploidy and sympatric speciation. Retroviruses provide a well-known exception to the classical central dogma of molecular genetics. Overall,

similar considerations apply to other generalizations. They are riddled with exceptions, which seem to question the lawlike status of generalizations.

Some authors have suggested that the exception-riddled nature of generalizations is not damaging to their lawlike and general status, since the generalizations hold when ceteris is paribus, i.e., "when some other unknown interfering conditions remain the same or absent" (e.g., Carrier 1995). That is, when biological generalizations are qualified with "other things being equal" clauses, we have exceptionless generalizations because the exceptions are only apparent rather than genuine. Other things are not equal when generalizations are extended beyond their intended domains of application. For example, generalizations are not expected to apply to cases in which unknown interfering factors do not remain the same or are not absent. Moreover, in biology other things are rarely equal. Hence, according to these authors and contrary to appearances, biological generalizations are exceptionless in their relevant domains of application.

Let us presuppose that the ceteris paribus strategy is valid. There is another line of argumentation showing that even if generalizations were exceptionless and thus true (with or without ceteris paribus clauses), they might lack necessity, because the generalizations could be accidental rather than lawlike as generalizations, that is, *true but only accidentally so.*

The issue is thus not only whether evolutionary or developmental generalizations are true. This is only a necessary condition for lawlike and truly general generalizations. A generalization might be true due to prevailing conditions only, such as "all pure lumps of gold have a mass less than 1,000 kilograms." Nothing guarantees that this generalization will hold in the future if some of the conditions change. The generalization "all pure lumps of uranium-235 have a mass less than 1,000 kilograms" is true as well. The difference is that the latter continues to be true even if various background conditions were changed. Nuclear physical facts guarantee the holding of the latter generalization in various background conditions, since 1,000 kilograms exceeds the critical mass of pure uranium-235. Another way to express the difference is that accidentally or contingently true, but non-lawlike generalizations, such as the gold generalization, lack the stability (Mitchell 1997, 2000) we associate with lawlike generalizations. Lawlike generalizations have necessity or stability that guarantees their holding even if background conditions changed in various different ways.

Accidental Products of Evolutionary History

Beatty (1995) has argued that evolution is a contingent process that can lead to different outcomes despite the same or similar starting points and even with the same or similar selection pressures (see also Gould 1989). That is, given the same or similar selection pressures, the same or similar adaptations do not necessarily follow. This implies that our evolutionary and developmental generalizations are contingent, accidental, and unique outcomes since their evolution can be or could have been

easily switched to another track or be disturbed by minimal changes in their initial or past historical conditions.

Some classical reasons why our generalizations concerning evolutionary and developmental phenomena are contingent are that, in addition to natural selection, stochastic and random forces, such as mutations, founder effect, and genetic drift, affect the outcomes of evolution. Moreover, even natural selection typically has multiple trait variants from which to choose, which are similar in their fitness, but differ in their realization (cf. the argument from the multiple realizability of biological properties to the non-existence of biological laws in Rosenberg 1985: 59–65). That is, even natural selection leads to contingency in biology.

Had there been no mitosis or some equivalently fit or fitter alternative to mitosis in the past, then meiosis would not have evolved, since meiosis evolved from mitosis. Consequently, Mendel's laws would also not have evolved on this planet, since the validity of these generalizations depends on the operation of meiosis and mitosis. In this manner, the generalizations behind both Mendel's "laws" are contingent and accidental products of history. In fact, non-Mendelian mechanisms of inheritance occur on our planet. If the future environment changes so that non-Mendelian mechanisms become as fit or fitter than Mendelian ones, then they could become as omnipresent as Mendelian mechanisms are presently.

Similar considerations apply to other previously discussed generalizations. Evolutionary and developmental generalizations are accidental products of evolutionary history, even if they are true and lack exceptions. The generalizations lack the stability traditionally associated with laws. Different regularities and generalizations could have evolved and held on this planet had different initial or past historical conditions prevailed. Similarly, a slight change in our current conditions would amount to changes in the holding of our current developmental and evolutionary generalizations.

From Contingency to Constraints and Generative Entrenchment

Some central findings of evo-devo suggest that generalizations concerning evolutionary and developmental phenomena are more contingent and accidental than already suggested.

Developmental plasticity and, more generally, eco-evo-devo suggest that the way phenotypes of organisms are determined does not solely, and in some cases perhaps not even mainly, depend on their genotypes, but on the environmental conditions (see chapters ▶ "Developmental Plasticity and Evolution" and ▶ "Eco-Evo-Devo"). For instance, the final developmental form of an insect species may depend on its ecological environment, such as the presence of predator species (cf. Abouheif et al. 2014). Environmentally induced phenotypic variation, such as polyphenisms, facilitates the adaptations of phenotypes and allows for rapid changes in phenotypes vis-à-vis environmental changes. In other words, ontogeny as a process is more complex and contingent on environmental factors, including the syn-ecological or community context of a species, than what was previously presumed.

The idea of genetic material being the only way to carry inheritance information from parents to offspring is being questioned by findings about epigenetic factors, such as DNA methylation and histone modifications. Epigenetic factors, heritable or not, are often essential for normal development. Again, the implication is that our generalizations concerning developmental phenomena are contingent, since non-genetic factors, such as epigenetic and environmental or ecological factors, not only broaden our understanding of heredity and development but also provide new sources of variation for ontogeny.

These findings concerning ontogeny could have major phylogenetic consequences. As phenotypic plasticity, polyphenism, and epigenetic factors provide new sources of variation for development and selection to act on, variation in these factors may lead to evolutionary novelties, reproductive isolation, and speciation events (see chapter ▶ "Developmental Innovation and Phenotypic Novelty"). In other words, our generalizations concerning developmental and evolutionary phenomena may be true, but they are contingently true. Perhaps even more so than suggested by the Modern Synthesis, there are many other contingent factors and sources of variation than genetic mutations and drift upon which the truth of these generalizations depend.

Simultaneously, evo-devo has revealed that there are also *generality-maintaining mechanisms* in evolution and development (see chapter ▶ "Mechanisms in Evo-Devo"). These findings suggest that our generalizations concerning evolutionary and developmental phenomena might be more stable, necessary, and less prone to exceptions than formerly presumed. Relevant phenomena include generative entrenchment and constraints (see chapter ▶ "A Macroevolutionary Perspective on Developmental Constraints in Animals"). The idea of generative entrenchment is simple: even though, for instance, a trait or a gene was originally a highly contingent result of an evolutionary history, it can become a functional necessity that is essential and cannot be changed. A constraint is here defined as any factor that limits evolutionary change.

Although on this planet it is a true generalization that hereditary information – with the possible exception of epigenetic factors – is carried by *nucleic acids*, this is a conditional and contingent fact of our planet's and life's history. Had the past conditions or other initial background conditions been different, then other materials could have evolved to do the same thing. Hence, the ubiquity of the genetic code is an accident whose evolution could have been disturbed or switched to another track. Note that contingency and accident do not imply that the code is arbitrary or without adaptive advantages.

Yet throughout the history of life, this code has become so generatively entrenched as to be nearly impossible to change because so many other things depend on it. In other words, the code and the way it is realized represent a functional necessity. The same is true of many basic biological mechanisms, the presence and functioning of many ancestral or primitive characters or traits: Mendel's laws and the mechanisms of mitosis and meiosis, diplobiontic life cycle, cell respiration, the Krebs cycle, and the use of ATP in metabolic processes, eukaryoticity, homeobox genes (see below), the mechanism of photosynthesis, bilateral symmetry, dorsal-

ventral polarity, initiator and terminator codons, DNA ligase and DNA methylation, morphogenesis and organogenesis in general, and the specific mechanisms of apoptosis in ontogeny. It is not just genetic factors that might become generatively entrenched, but also epigenetic factors.

Generatively entrenched traits (Wimsatt 2001) are functional necessities for organisms' development, survival, and reproduction, which resist evolutionary change, even if we suppose that evolution proceeds via forces other than selection, such as mutation and drift. For a trait to become generatively entrenched, it does not matter how or under what evolutionary forces it has evolved or will evolve. The number of other traits that come to depend on the functioning or development of the trait in question is what matters (see chapter ▶ "Concept of Burden in Evo-Devo"). A change in a deeply generatively entrenched trait has serious, deleterious effects on other traits in the development or functioning of an organism. Therefore, deeply generatively entrenched traits are preserved and resist evolutionary change, regardless of whether the trait was a consequence of drift, mutation, or selection.

Generative entrenchment thus provides us with exception- and contingency buffering and stability- and generality-maintaining mechanisms, thereby allowing for the discovery of repeatable, projectable, and stable generalizations concerning evolutionary and developmental phenomena. Generative entrenchment, however, also restricts the emergence of evolutionary novelties by forcing evolution to be locked into certain directions, since it is more likely that conserved traits are preserved rather than changed – the functioning of so many other things depend on their preservation. This again implies that evolution might not be as contingent in its results, nor so easily switched to another track, as was suggested earlier. Our generalizations concerning evolutionary and developmental phenomena might be associated with stability and necessity after all.

When a generatively entrenched trait is successfully changed, however, this can produce novel traits and even new forms. The same generality-begetting mechanisms can produce novel generalizations in the context of macroevolution.

Regulatory genes, such as homeobox genes, switch other genes on or off during the development of an organism. Changes in regulatory genes (or changes in their regulatory networks) are also quite likely responsible for major evolutionary and developmental changes. Different organismal traits, shapes, and forms follow not from differences between genotypes per se, but from how and when different genes are (in)activated during ontogeny. Many of these regulatory genes are not only conserved, but almost all animals use the same or similar regulatory genes, such as homeobox genes, during development. There is thus a puzzle. On the one hand, many regulatory genes are *highly conserved and generatively entrenched* within diverse taxa that, on the other hand, *differ* greatly in development. One way to both preserve regulatory genes and allow diverse phenotypes to develop is through the duplication of conserved genes, which has happened in the case of many different regulatory genes.

Wimsatt's (2001) notion of generative entrenchment is deliberately broader and somewhat different than that of traditional constraints (on constraints, see

Richardson and Chipman 2003 and Shanahan 2008). Many kinds of constraints are discussed in the literature, such as physical, chemical, phylogenetic, architectural, and developmental. Generative entrenchment as a notion encompasses the meaning of many of these. A *functional* necessity begets stability to the development and evolution of the system in which it operates regardless of the nature or origin of the constraint. Moreover, generative entrenchment not only restricts variation and produces inertia to developmental and evolutionary change – functioning similarly to traditional constraints – but can work as an engine for evolutionary and developmental change and function as a source of novelties as well (see Nuño de la Rosa and Villegas 2019 for a similar function of constraints). The importance of generative entrenchment is thus that it is capable of explaining the stability and generality of micro and macro evolutionary trends while at the same time allowing for evolutionary changes or origins of novelties when generative entrenched traits are successfully changed (cf. homeobox genes, above).

Disappearance and Return of Laws and Natural Kinds

Traditional philosophers of biology, such as Rosenberg (1985), were interested in the issue of whether there exists lawlike biological generalizations. The putative lack of laws would not only imply that biology is inferior and perhaps reducible to the physical sciences; it would also indicate that there exist no autonomous and distinctive biological explanations and predictions. Laws were deemed necessary for scientific explanations and prediction (see Hempel 1965).

For many philosophers, laws were expressed as true, empirically testable, and universally quantified statements that included predicate terms making reference to natural kinds or classes (see again Hempel 1965). Natural kinds or classes refer to such terms as "mass," "gold," "regulatory gene," or "predator," which make sure that laws make no reference to particulars. Why no reference to particulars? Because laws needed to be distinguished from accidentally true, i.e., non-lawlike, generalizations that typically refer to particulars or hold because of particular background conditions but not outside of them (e.g., "all coins in my pocket today are fifty cents").

In addition to exceptions and contingency, another issue concerning the lawlikeness in biology was that species and many other taxa, such as Mammalia, and perhaps many central causal developmental factors, such as the *pax-6* and *hox* genes that evolutionary and developmental biologists focused on, were not natural kinds or classes, but something else, such as individuals (Ghiselin 1974; Mayr 1976), i.e., particulars. Thus, no laws were to be found concerning developmental or evolutionary phenomena, according to some philosophers, since biological generalizations were riddled with references made to particulars rather than being expressed as universally true generalizations concerning nature's classes (see Lange 1995 for further discussion and references).

Philosophers have more recently questioned the notion of lawlikeness as an important property of generalizations that scientists, including biologists, should

be and in fact are looking for to ground their predictions and explanations of nature. For instance, according to Woodward (2001) the central property of causal generalizations is their invariance under manipulations rather than their lawlikeness (see also Waters 1998; Raerinne 2013). Under this framework, the issue of whether generalizations refer to natural kinds is obsolete. What matters is whether generalizations give us accurate recipes for successful manipulations of natural phenomena. This aligns with the ideas of some central defenders of the Modern Synthesis as well, who rejected typological or kind and class thinking (cf. Sober 1980 and Lewens 2009 for discussion and references).

Despite this, some issues that were discussed in the traditional philosophical context of laws are being reintroduced to evo-devo literature, such as the existence of natural kinds in evo-devo (Rieppel 2005a, b; see chapter ► "Typology and Natural Kinds in Evo-Devo"). Examples of kinds are, for instance, body plans and developmental types that distinct taxa share. Natural kindness of other concepts in evo-devo has also been discussed, such as "modularity." The existence and stability of kinds and that distinct taxa share kinds pose no problems for evo-devo. Generative entrenchment and constraints can be used as explanations (but see Wagner 1996 for an alternative explanation concerning modularity as a kind).

Typological themes in evo-devo have already been discussed by others. For instance, Lewens (2009) views typological thinking in evo-devo as a complementary explanatory strategy to the population thinking of the Modern Synthesis. Nevertheless, there are aspects of it that have received less attention. What is it that makes kinds explanatory? It seems that certain authors think that kinds are explanatory due to their generality and unifying power. This was also the basic premise of a covering law account of scientific explanation by Hempel (1965). The more diverse and distinct the set of phenomena a law covers as its instances, the more general and unifying the law, and the better the explanation or prediction by the law. If this is so, then typologists might be understood as arguing for the return of laws and law-based accounts of scientific explanation in evo-devo.

Natural kinds are postulated not only as explanantia, but as explananda or targets of explanations as well. For instance, "modularity" and "developmental types" are both used in explanations and as targets of explanations. In general, not much discussion exists on different explanatory, heuristic, evidential, or classificatory roles of natural kinds in evo-devo (but see Love 2009 for discussion and references) and by virtue of which properties the kinds function in these roles (e.g., causality vs. unification; fruitfulness vs. carving nature at its joints; and so on). In evo-devo, natural kinds seem to have diverse, if not elusive, functions and roles.

Conclusion

How has evo-devo changed our understanding of evolutionary and developmental phenomena and especially what has it contributed to the question of how general our evolutionary and developmental findings are? The same general picture of evolution currently reigns as during the days of Modern Synthesis. Rensch summarized the

central findings and tenets of the Modern Synthesis as follows: "Now these new results [of evolutionary research] allow two kinds of conclusions, which seem to be very contradictory. On the one hand, evolution may be looked at as an undirected unique historical process; on the other hand, it seems to be determined by a great number of laws and rules" (Rensch 1960: 95).

Our understanding of the causes and mechanisms of why evolutionary and developmental phenomena appear to be both stable and contingent, repeatable and unique, and generalizable and non-generalizable have changed, however. Evo-devo has provided us with new insights about the sources of variation in the development and evolution of life forms, such as developmental plasticity and epigenetics (see chapter ▶ "Evo-Devo's Contributions to the Extended Evolutionary Synthesis"). Evo-devo has contributed a better understanding of how evolutionary novelties could arise through changes in highly conserved traits of organisms. Simultaneously, it has provided us with insights of what constraints on variation and causes of stability and generality in evolution and development are, such as generative entrenchment.

It is an open question to what extent evo-devo implies changes in epistemic practices. Many authors believe that natural kinds have important and even central roles in evo-devo. But it is still unclear what diverse and distinct roles kinds have in evo-devo and what it is about kinds that furnishes them with such roles. Most of the discussion on kinds in evo-devo has focused on ontic issues (e.g., homeostatic property clusters or traditional essences), whereas the diverse epistemic roles of kinds deserve equal attention.

Cross-References

▶ A Macroevolutionary Perspective on Developmental Constraints in Animals
▶ Concept of Burden in Evo-Devo
▶ Developmental Innovation and Phenotypic Novelty
▶ Developmental Plasticity and Evolution
▶ Eco-Evo-Devo
▶ Evo-Devo's Contributions to the Extended Evolutionary Synthesis
▶ Mechanisms in Evo-Devo
▶ Typology and Natural Kinds in Evo-Devo

References

Abouheif E, Favé M-J, Ibarrán-Viniegra A, Lesoway M, Rafiqi A, Rajakuma R (2014) Eco-evo-devo: the time has come. In: Landry C, Aubin-Horth N (eds) Ecological genomics: ecology and the evolution of genes and genomes. Springer, Dordrecht, pp 107–125

Beatty J (1995) The evolutionary contingency thesis. In: Wolters G, Lennox J (eds) Concepts, theories, and rationality in the biological sciences. Universitätsverlag Konstanz, Konstanz, pp 45–81

Carrier M (1995) Evolutionary change and lawlikeness. Beatty of biological generalizations. In: Wolters G, Lennox J (eds) Concepts, theories, and rationality in the biological sciences. Universitätsverlag Konstanz, Konstanz, pp 83–97
Crow J (1979) Genes that violate Mendel's rules. Sci Am 240:134–146
Ghiselin M (1974) A radical solution to the species problem. Syst Zool 23:536–544
Gould S (1970) Dollo on Dollo's law: irreversibility and the status of evolutionary laws. J Hist Biol 3:189–212
Gould S (1989) Wonderful life. Norton, London
Hempel C (1965) Aspects of scientific explanation. Free Press, New York
Lange M (1995) Are there natural laws concerning particular biological species? J Philos 92:430–451
Lewens T (2009) Evo-devo and "typological thinking": an exculpation. J Exp Zool (Mol Dev Evol) 312B:789–796
Love A (2009) Typology reconfigured: from the metaphysics of essentialism to the epistemology of representation. Acta Biotheor 57:51–75
MacFadden B (1986) Fossil horses from 'eohippus' (Hyracotherium) to Equus: scaling, Cope's law, and the evolution of body size. Paleobiology 12:355–369
Mayr E (1942) Systematics and the origin of species. Columbia University Press, New York
Mayr E (1976) Is the species a class or an individual? Syst Zool 25:192
Mitchell S (1997) Pragmatic laws. Philos Sci 64:S468–S479
Mitchell S (2000) Dimensions of scientific law. Philos Sci 67:242–265
Nuño de la Rosa L, Villegas C (2019) Chances and propensities in evo-devo. Brit J Phil Sci. https://doi.org/10.1093/bjps/axz048
Raerinne J (2013) Stability and lawlikeness. Biol Philos 28:838–851
Rensch B (1960) The laws of evolution. In: Tax S (ed) Evolution after Darwin, vol 1. University of Chicago, Chicago, pp 95–116
Richardson M, Chipman A (2003) Developmental constraints in a comparative framework: a test case using variations in phalanx number during Amniote evolution. J Exp Zool (Mol Dev Evol) 296B:8–22
Rieppel O (2005a) Modules, kinds, and homology. J Exp Zool (Mol Dev Evol) 304B:18–27
Rieppel O (2005b) Type' in morphology and phylogeny. J Morphol 267:525–535
Rosenberg A (1985) The structure of biological science. Cambridge University Press, Cambridge
Shanahan T (2008) Why don't zebras have machine guns? Adaptation, selection, and constraints in evolutionary theory. Stud Hist Phil Biol Biomed Sci 39:135–146
Sober E (1980) Evolution, population thinking, and essentialism. Phil Sci 34(47):350–383
Wagner G (1996) Homologues, natural kinds and the evolution of modularity. Amer Zool 36:36–43
Waters C (1998) Causal regularities in the biological world of contingent distributions. Biol Philos 13:5–36
Wiens J (2011) Re-evolution of lost mandibular teeth in frogs after more than 200 million years, and re-evaluating Dollo's law. Evolution 65:1238–1296
Wimsatt W (2001) Generative entrenchment and the developmental approach to evolutionary processes. In: Oyama S, Griffiths P, Gray R (eds) Cycles of contingency. MIT, Cambridge, pp 219–237
Woodward J (2001) Law and explanation in biology: invariance is the kind of stability that matters. Philos Sci 68:1–20

Mechanisms in Evo-Devo

Jan Baedke

Contents

Abstract

The concept of mechanism is central in evolutionary developmental biology (evo-devo). Researchers in this field seek to identify and characterize mechanisms that link individual development with evolutionary change. However, while the centrality of mechanisms for evo-devo is widely accepted, there is a lack of clarity about how mechanisms should be conceptualized in evo-devo to fulfill different explanatory interests and fit with methodological practices. This chapter begins by describing the different ways that philosophers of science understand the concept of mechanism as used by biologists. Next, I survey challenges to these philosophical views that arise when applied to developmental and evolutionary mechanisms, as well as to those mechanisms that link the two. The final section shows how some of these challenges can be addressed via complementary conceptions of mechanisms and mechanistic explanation, especially those that understand mechanisms by means of scales rather than parthood.

J. Baedke (✉)
Department of Philosophy I, Ruhr University Bochum, Bochum, Germany
e-mail: Jan.Baedke@rub.de

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_94

Keywords

Developmental mechanism · Evo-devo · Evolutionary mechanism · Levels of organization · Parthood · Scales

Introduction

In biology, describing a mechanism is often equated with explaining how something works. A mechanistic explanation offers a model in which certain processes, typically located on lower levels of organization, explain behaviors or other processes manifested at higher levels (see chapter ► "Levels of Organization in Evo-Devo"). For example, genetic processes mechanistically explain cell differentiation, cellular processes explain tissue formation, and tissue interactions explain organogenesis. Mechanisms and mechanistic explanation are of particular importance in evolutionary developmental biology (evo-devo) and appear commonly in terminology: gene regulatory mechanisms, mechanisms of pattern formation, mechanisms of cellular differentiation, mechanisms of environmental responsiveness and plasticity, and mechanisms imposing constraints or introducing novelties in evolution. Usually, these are described as "developmental" mechanisms because they are considered to cause, bias, or constrain phenotypic outcomes during ontogeny. Thus, they affect the amount and kinds of variation upon which natural selection can act.

Both biologists and philosophers of science have recognized the crucial role of developmental mechanisms for evo-devo research. For example, Winther (2015) describes research in evo-devo as being guided by three styles: mathematical modeling, historical narratives, and mechanism. This latter style consists of decomposing a functional system into parts or sub-systems in order to investigate how it works. In addition, some have described evo-devo as a paradigmatic mechanistic science, which has as its main objective the identification of those developmental mechanisms that bring about evolutionary change in the phenotypes of organisms (Hall 2012). This centrality of mechanistic explanation is thought to arise from a methodological approach that bridges many levels of biological organization (see chapter ► "Explanation in Evo-Devo").

While researchers appeal repeatedly to mechanistic explanations and use the language of mechanisms, there is a lack of clarity about the nature of developmental mechanisms and mechanistic explanation in evo-devo. For example, what does it mean to say that developmental mechanisms make organisms evolve and facilitate the origin of novel features? If we assume that evo-devo aims to account for how developmental mechanisms evolve and, at the same time, how they causally affect evolutionary trajectories, which conceptual frameworks support these aims? How can a concept of mechanism help to integrate phylogenetic and ontogenetic processes? Addressing these types of questions is crucial both to understand how evo-devo researchers conceptualize and investigate living systems and ascertain what distinguishes their explanations from those offered by other biologists,

especially other evolutionary biologists, such as population geneticists (see chapter ▶ "Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics").

Within the traditional population genetics framework of the Modern Synthesis, developmental mechanisms were thought to have little explanatory value. According to Ernst Mayr's proximate-ultimate distinction, one should not conflate descriptions of developmental mechanisms that answer *how* systems work with evolutionary explanations that answer *why* a trait evolved (see chapter ▶ "Proximate Versus Ultimate Causation and Evo-Devo"). In contrast, however, developmental mechanisms are usually understood to play a central role in evolutionary explanations in the field of evo-devo. As a consequence, it becomes important to understand how these mechanisms can (or should) be conceptualized to serve this unusual role (at least according to Mayr).

A good starting point for this endeavor is a set of conceptual frameworks developed by philosophers of biology to reconstruct what molecular and cell biologists mean when they talk about mechanisms and mechanistic explanation (Craver 2007; Bechtel and Abrahamsen 2010). It has been argued that such frameworks face a number of challenges when applied to developmental and evolutionary mechanisms, as well as to mechanisms in evo-devo (Mc Manus 2012; Brigandt 2015). This chapter discusses these challenges, as well as alternative conceptual frameworks for mechanisms, especially those that understand mechanistic relations based on scales. These alternative conceptions of mechanism can play important explanatory roles in evo-devo that complement mechanistic explanations based on parthood relations.

The Concept of Mechanism

Over the last two decades, philosophers of science have described mechanistic explanation in terms of the construction of models of mechanisms that link *parts of systems* with behaviors of the *whole system* (Machamer et al. 2000; Craver 2007; Illari and Williamson 2012). Many of these so-called new mechanists understand mechanistic models to be hierarchically organized (Craver and Bechtel 2007; Craver 2015) and include details about a system's parts and organization to varying degrees (Levy and Bechtel 2013). In this framework, mechanistic models help scientists to comprehend relationships between *different compositional levels of organization* (e.g., those between genes and phenotypes or cells and tissues) and function as heuristics for guiding new experimental interventions. The former is facilitated by the fact that mechanistic models typically trace relations between causal capacities of lower-level parts of a system and their organization and the capacities of a higher-level system as a whole. These inter-level relations are often depicted in pictorial diagrams and schemata.

Based on specific cases of mechanistic models in molecular and cell biology, many new mechanists have characterized mechanistic explanation as operating in a two-step procedure, similar to the strategy of functional analysis (Machamer et al. 2000; Craver 2007; Menzies 2012). First, we decompose the analyzed capacity of a given system (e.g., a cell's behavior to synthesize proteins) into simpler ones

(e.g., transcription or translation). Second, we show how these capacities are realized by concrete entities or component parts (e.g., DNA, mRNAs, and ribosomes). This prominent view of mechanistic explanation via decomposition and localization has been criticized in at least three ways. First, it has been argued that those new mechanists who defend this view should not understand it as rooted in the longer philosophical tradition of mechanicism, which conceptualized living systems as decomposable machines. Nicholson (2013) argues that conceptualizing living systems as decomposable machines is problematic when one tries to investigate properties of living systems that machines do not share, such as self-maintenance and reproduction or intrinsic purposiveness (see chapter ▶ "Teleology in Evo-Devo"). As a consequence, Nicholson encourages new mechanists to more clearly distinguish their discussion of mechanisms and mechanistic explanation in biology from that of mechanisms understood as machines.

Second, the methodological strategy of decomposition and localization pays little attention to the temporal organization and dynamics of mechanisms. As a consequence, it ignores the epistemic tools available to predict and explain temporal organization, such as dynamical models (Bechtel and Abrahamsen 2010; Brigandt 2015). Therefore, mechanistic explanation should include not only decomposition and localization but also "recomposition." After decomposing a system and identifying causal contributions of parts or sub-systems, dynamical models help to demonstrate how these contributions operate together to bring about a whole system's behavior. Bechtel and Abrahamsen (2010) have called this modeling process "dynamic mechanistic explanation."

Finally, the precise nature of functional explanations offered by decomposition and localization has been analyzed. Some new mechanists hold that these explanations are not causal because they primarily address questions about composition (Craver 2007, 2015; Craver and Bechtel 2007). For example, answering "What synthesizes a protein?" or "What makes a cell contain a protein?" is different from "How is a protein synthesized?" Whereas the former two questions focus on those molecular entities and their interactions that constitute the cell or one of its sub-systems, the latter question is an inquiry about the causal history or process leading to the synthesis of proteins. The former *mechanistic explanation* relates causal *capacities* or *properties* of entities composing different levels of organization, while the latter *causal explanation* refers to relations of interaction between *events* or *processes* located on the same level of organization (Ylikoski 2013). This distinction is based on the idea that parthood is not a causal but a constitutive relation, and therefore parthood is a *synchronous* relation that does not "occur" over time (Craver and Bechtel 2007). For example, to be a cell that has the causal capacity to synthesize proteins *is to be* specifically constituted (at a time) by entities like DNA and mRNA. Although a mechanism's parts (e.g., mRNA) are usually organized spatio*temporally* and thus may show certain dynamics at every level, inter-level functional relations between the properties of parts and those of the system are conceptualized synchronically.

Thus, according to some new mechanists, mechanistic explanations approach multilevel complexity by dividing it into functional relations between parts and wholes located at different levels of organization. These inter-level parthood

relations link entities in a synchronous manner within a mechanism and account for their causal capacities. Recently, some philosophers have applied this framework to analyze mechanisms and mechanistic explanations in developmental and evolutionary biology, as well as in evo-devo. However, these applications have provoked controversies about the challenges and limits of this framework.

Mechanisms in Development and Evolution

In evo-devo, and in biology more generally, at least two interpretations of the concept of mechanism are common. The concept appears in explanations that invoke hierarchically organized structures and trace relations *across* levels of organization as well as in explanations that describe causal processes occurring *on a single* level of organization (i.e., causal explanation). For example, while Brian K. Hall (2012: 184) states that "studies in evo-devo highlight the mechanisms that link genes (the genotype) with structures (the phenotype)" across levels of organization, Jaeger and Sharpe (2014: 57, 74) define a mechanism as a "causal explanation of how something works" and a developmental mechanism as a "regulatory process" seemingly operating on a single level.

Such a pluralist framework that applies different concepts of mechanism makes it possible to offer complementary accounts of complex sequences and multilevel phenomena. However, it can also lead to conceptual ambiguities about the nature of mechanisms and mechanistic explanation, which could have negative consequences, especially given that mechanisms are often considered to play a key role in evo-devo's attempts to integrate development and evolution. For example, as Moczek et al. (2015) emphasize, a major objective of evo-devo is to integrate principles of *developmental mechanisms* producing variation with those of *evolutionary mechanisms* selecting variation:

> As evolutionary biology looks to the 21st century, one of its challenges will be to better describe the way that the variation generating and biasing mechanisms of development interact with the variation sorting mechanism of natural selection to produce organismal diversity and adaptation. Evo-devo research helps meet this challenge by providing powerful empirical and conceptual avenues that allow us to integrate the principles of phenotype construction with those of phenotype selection, across all levels of biological organization. (Moczek et al. 2015: 201)

In order to make good on this promise and provide the conceptual resources that facilitate explanatory integration, one has to be clear about the meaning of the concepts of developmental mechanism and evolutionary mechanism. This is not as easy as it seems, at least not based on the conceptual frameworks offered by some new mechanists.

Two features of developmental mechanisms have been interpreted as challenges to the conceptual framework prominent among new mechanists when it comes to describing mechanisms in evo-devo. First, *organization* plays a different role in developmental mechanistic explanations (Mc Manus 2012). These explanations

usually treat organization, such as the dorsal-ventral spatial axis of an embryo, not only as a part of the *explanans* (i.e., the items which do the explaining of a phenomenon) but also as an element of the *explanandum* (i.e., the phenomenon-to-be-explained). This derives from the fact that some types of organization, such as tissue-level organization, are not assumed to be present initially in development, which contrasts with what many new mechanists seem to assume. These authors do not offer an account of the origination of levels of organization depicted in their multilevel diagrams of mechanisms (Baedke forthcoming). Instead, levels and other forms of organization (e.g., spatial axes), including their origins, are addressed specifically by developmental biologists in their mechanistic explanations. In this sense, developmental mechanisms require a different explanatory strategy in order to account for the origins of organization. Second, Mc Manus (2012) has argued that the concept of *part* in developmental mechanisms differs from the one used by many new mechanists. Glennan (1996), for example, defines parts as discrete objects that are empirically robust, experimentally isolatable and preferably spatially localizable. This common view of parts (see, e.g., Craver 2007) seems to differ somewhat from practices in developmental biology where diffuse entities, such as morphogenetic fields, are considered to be parts of a mechanism.

In addition to questions about whether the new mechanists' framework is applicable, Love (2018) discusses another conceptual problem related to developmental mechanisms. These mechanisms include both molecular genetic mechanisms (e.g., signaling or gene regulatory networks) that are highly conserved across phylogenetically disparate taxa and cellular-physical mechanisms based on physical principles (e.g., cell migration). Sometimes a trade-off emerges when one seeks to integrate the two types. Both explanations based on molecular genetic mechanisms alone and explanations based on cellular-physical mechanisms alone can yield high degrees of generality (see chapter ▶ "Generalization in Evo-Devo"). However, because cellular-physical mechanisms are not conserved phylogenetically, the type of generality they yield differs and does not correlate with that of molecular genetic mechanisms. When one attempts to integrate the two types of mechanisms in order to establish a more complete explanation, the result can be a less general explanation. Love notes that many developmental biologists react to this trade-off by preferring the non-integrated generality of highly conserved molecular genetic mechanisms since this fits with the justification of using model organisms. This orientation can foster a preference for explanations primarily in terms of molecular genetic mechanisms rather than cellular-physical mechanisms. Love argues that a better philosophical understanding of the relative significance of explanatory values like generality, specificity, and completeness is necessary in these contexts.

Some evo-devo biologists seem to understand the concept of evolutionary mechanism in a wide sense, including not only classical evolutionary processes, like natural selection and drift, but also phenotypic plasticity and developmental constraints, which can affect evolutionary trajectories. However, despite frequent appeals to natural selection as a mechanism, Skipper and Millstein (2005) argue that this is not accurate given the assumptions of the new mechanists. Should natural selection be seen as a system that is organized and behaves more or less regularly

(like a motor) or as a fragile process of mechanistically explainable events (like World War II)? If the former, then what would count as the "system"? If the latter, why does it lead to robust outcomes, such as convergence, rather than merely contingent historical events? Moreover, it is unclear whether natural selection is decomposable into distinct parts. For example, it seems strained to say that the environment or populations are parts or sets of parts in a mechanism and that they show stable enough properties for individuation.

These concerns about whether the views of new mechanists are apt for describing mechanisms in development and evolution suggest exploring different ways to understand mechanisms of developmental evolution, such as pattern formation or cellular differentiation, that might affect evolutionary pathways within a lineage. A reconceptualization seems necessary to foster an integration of developmental and evolutionary explanations.

Mechanisms in Evo-Devo

Assuming Mayr's proximate-ultimate distinction, one should not invoke developmental mechanisms when seeking to answer *why* evolution has produced a particular trait. This view contrasts with studies in evo-devo that address how developmental mechanisms bias and constrain evolutionary trajectories, how evolutionary novelties arise through developmental plasticity, and how biological systems generate heritable, adaptive phenotypic variation in a lineage, i.e., evolvability (see chapters ▶ "A Macroevolutionary Perspective on Developmental Constraints in Animals" ▶ "Developmental Innovation and Phenotypic Novelty," and ▶ "Evolvability").

Against this background, Brigandt (2015) highlights that some mechanistic models in evo-devo, rather than representing a certain mechanistic structure, deal with the dispositional properties of organismal systems that allow for modifications and reactions to perturbations (see chapter ▶ "Dispositional Properties in Evo-Devo"). In order to mechanistically explain this dynamic potentiality of organisms, Brigandt argues that these explanations necessitate a revised and broadened philosophical conception of mechanisms and mechanistic explanation. Besides a structural analysis of a system and its basic interactions, quantitative mathematical models are necessary to explain and predict system dynamics as well as how qualitative properties emerge (see chapter ▶ "Modeling and Simulation in Evo-Devo"). This perspective is especially important to understand developmental mechanisms that generate a wide range of qualitative changes through underlying quantitative changes in their component parts and activities. In this context, mathematical models can be used to quantitatively describe the temporal dynamics of parts (e.g., changes in gene expression rates, reaction rates of enzymes, or concentrations of metabolites).

Brigandt shows that these changes have explanatory relevance for qualitative phenotypic properties of major evolutionary significance, including robustness or canalization, phenotypic plasticity, and modularity (see chapters ▶ "Canalization: A Central but Controversial Concept in Evo-Devo," ▶ "Developmental Plasticity and Evolution,"). For example, computational models help to account for how the

robustness of metabolic networks in *E. coli* depends on a threshold set by the rates of individual enzymatic reactions, above which overall metabolic flux is largely unaffected. Quantitatively changing these rates so that they fall below the threshold leads to a drastic qualitative change in the metabolic network. In addition, plastic and qualitatively distinct alternative morphologies that develop from one genome (i.e., polyphenisms) often depend on quantitative changes in developmental mechanisms. For instance, the two morphs in *Daphnia* that affect how efficiently they can be swallowed by a predator (i.e., with or without a helmet-like extension of their head) develop depending on the concentration of chemicals in the water that indicate a predator's presence.

Other discussions of how developmental mechanisms evolve and affect evolutionary trajectories more directly challenge Mayr's claim that one should not mix descriptions of developmental mechanisms with evolutionary explanations. Calcott (2009, 2013) has argued that Mayr's distinction does not consider "lineage explanations," which are claimed to occur frequently in evo-devo. Lineage explanations differ from developmental explanations that answer "How do *individuals* work *at a time*?" and evolutionary explanations that answer "How do *populations* change *over time*?" Instead, lineage explanations answer "How do *individuals* change *over time*?" and address this question by providing a *series of mechanistic models*, which traces differences between the developmental mechanisms of individuals that produce the relevant morphological structures at different times. Similar to assumptions made by new mechanists, each mechanistic model offers constitutive relations between parts and wholes at a particular time slice. From one time slice to the next, these relations undergo small modifications. Calcott exemplifies this idea by the transformation of the word "scale" into "plume" through gradual changes in its component characters:

$$\text{scale} > \text{scal}p > \text{sca}m\text{p} > \text{s}t\text{amp} > \text{st}u\text{mp} > \text{s}l\text{ump} > p\text{lump} > \text{plum}e.$$

Each word in this series is *decomposable* into the parts (characters) that constitute it, and these parts can be changed separately, one at a time, in a modular fashion. In every time slice, the parts (by virtue of their capacities and organization) account for the *function* of making up a particular word. Calcott believes that developmental mechanisms have sometimes changed in this way to bring about novelties like eyes and feathers in evolution.

However, there is at least one type of mechanistic explanation in evo-devo that cannot be captured by lineage explanations: developmental mechanisms whose inter-level relations are "vertically dynamic" (Baedke and Mc Manus 2018). Calcott (2013) claims that developmental mechanisms usually address the question "How do individuals work *at a time*?" However, given that developmental explanations involve causal relations occurring over time, it is necessary to more explicitly include temporality in an account of mechanisms. Phenomena like morphogenesis operate at a different time scale compared to evolutionary phenomena like adaptation and therefore they show that time scale in mechanistic models is required. The "vertical" relations between higher and lower levels traced by developmental mechanisms are not exhausted by the *synchronic, constitutive relations* between parts and

wholes, as some new mechanists suggest (e.g., Craver and Bechtel 2007). In contrast, developmental explanations trace changing relationships between causal capacities of (component parts of) a system at different levels of organization at different time intervals during ontogeny. For example, knockout or knockdown methodologies relate causal capacities of a system's parts (e.g., genes or epigenetic signatures) at earlier phases of development with capacities of the whole system (e.g., a particular trait) at a later time in development, often in adult organisms. For example, modifications of epigenetic marks in pluripotent stem cells can be related to differences in the phenotype of a cell, tissue, or the whole organism. Such studies of trait development ask which modifications in a particular gene's activity *early* in differentiation affect the fully differentiated phenotype *later* in development.

Following Ylikoski (2013), mechanistic explanations tracing these particular developmental relations can be labeled as *hybrid explanations* (Fig. 1). They combine causal and constitutive elements by relating causal capacities of entities – the usual relata of constitutive mechanistic relations – located on different levels of biological organization over time, i.e., diachronically instead of synchronically (see also Love and Hüttemann 2011 on the temporality of parthood relations).

Explicitly representing time in mechanistic models makes it possible to pursue evo-devo explanations that integrate developmental (hybrid) mechanisms and evolutionary mechanisms; each mechanistic model of a lineage offers an explanation of *vertical dynamics* for a life history. Take, for example, the evolutionary transition to multicellularity. One attempt to explain this transition on developmental grounds is to study the changes in location and context of lower-level developmental units that can promote the shift to multicellularity over ontogenetic time. For example, in the Volvocine algae *Gonium*, the co-option and recruitment of existing genes involved in cell cycle regulation promote such a transition (Olson and Nedelcu 2016). Their relocation in *Gonium* leads to the effect that cells no longer break apart from division clusters in mitosis. They stay attached to each other, which diachronically (during development) leads to the production of (higher-level) multicellular daughter colonies. In this way, developmental hybrid explanations that trace such changes in vertical relations during ontogeny may contribute to a better understanding of major

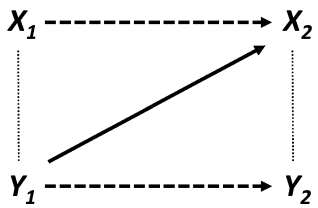


Fig. 1 Hybrid explanation. Depicted are two biological processes located on different levels of organization (dotted horizontal arrows). Mechanistic explanation relates each state of a process (e.g., when entity *Y* has a particular property at state *1*) constitutively (i.e., synchronously) to another state (e.g., *X* in state *1*) in the opposite process (dotted vertical line). In contrast, hybrid explanation relates a state of each process at a certain time in a diachronic, successive manner (diagonal arrow)

evolutionary transitions, such as the evolution of multicellularity (i.e., a new level of organization; see Baedke forthcoming).

This discussion indicates that the explicit representation of time in mechanistic models is necessary to have adequate conceptualizations of mechanism that facilitate the integration of both evolution and development. Inter-level dynamics are important for the mechanisms that evolutionary developmental biologists study. Although lineage explanations comparing the effects of similar developmental mechanisms across different taxa are found in evo-devo, there also are experimental studies on developmental threshold phenomena and processual, diachronic shifts during development that bear on evolution. These forms of explanation commonly found in evo-devo require conceptualizing mechanistic explanation differently.

Scales and Process Ontology

In order to integrate mechanisms of vertical dynamics during development with mechanisms of evolutionary transition, additional concepts beyond parthood are needed to describe hierarchically structured living systems. Another way to locate causal processes (genetic, cellular, or organismic) at particular levels of organization in a mechanism and to distinguish these processes from one another is to use the concept of *scale*, especially *scale of time* or *rate* (Green and Batterman 2017). The rate of a process can refer to its frequency or the time the process takes to overcome perturbations (i.e., relaxation time).

Time scale is recognized by some new mechanists as an additional criterion to locate and characterize entities at different mechanistic levels, but it is usually understood as a secondary (and sometimes accidental) consequence of parthood relations (Craver 2015). This means rates are interpreted as properties of parts and wholes. In contrast, others have argued that time scales offer an understanding of mechanistic hierarchies that is independent of (and complementary to) parthood hierarchies. Both draw on different ontological assumptions – the former on process ontology and the latter on substance ontology (Baedke and Mc Manus 2018). Process ontology has recently been explored in detail by a number of authors in philosophy of biology (Nicholson and Dupré 2018), as well as in evo-devo (Jaeger and Monk 2015; Nuño de la Rosa and Etxeberria 2012). From this perspective, nature is not a construction of discrete substances with distinct properties but rather is comprised of numerous interconnected processes with general patterns of activity that can be characterized through the time scales or rates at which they occur.

In the history of embryology and evo-devo, many authors have made use of this connection between time scales and processual views in order to develop hierarchical mechanistic models. For example, temporal epistemologies and rate-based views of the developing embryo can be found in twentieth-century gradient theories of morphogenesis and pattern formation. The idea of ordering living systems hierarchically by means of rates or time scales became prominent among organicists such as Conrad H. Waddington (see chapter ▶ "Conrad Hal Waddington (1905–1975)"), as well as in the work of his student Brian Goodwin, who used the rate criterion to

demarcate the levels of genetic, epigenetic, and metabolic causal processes in cells, and describe their dynamic interactions. He located different cellular variables at the same level if they shared a similar relaxation time.

Today, similar ideas can be seen in evo-devo studies on how the rate with which genes are expressed in gene regulatory networks makes a difference for the characteristics of developing phenotypes. Often these mechanistic models consist of multiple processes running at different rates. Changes in these rates can affect one another. For example, the higher-level mechanism of body segmentation in vertebrates is influenced by two lower-level rates: the rate of genetic oscillations for the formation of each new somite through changes in gene expression levels in the tissue and the rate at which tissue shape changes during development (Soroldoni et al. 2014; see chapter ▶ "The Evolution and Development of Segmented Body Plans"). The gradually increasing rate of tissue shortening modulates the gene expression waves across the tissue and thus the rhythm of segmentation in the embryo. Dynamical systems theory provides a tool to mathematically integrate such rate-based mechanisms using data from high-throughput concentration analyses of mRNAs, proteins, and metabolites combined with other information about the system, such as how gene expression varies across space (Jaeger and Sharpe 2014).

A related concept in evo-devo that incorporates a time scale perspective into the framework of hierarchical mechanistic models is heterochrony (see chapter ▶ "Heterochrony"). Whereas heterotopy (the relocation of gene expression and developmental modules) is popular in parthood-based mechanistic models, heterochrony instead refers to changes in the rate and timing of developmental mechanisms that lead to changes in morphological structures. For example, changes in the timing of gene expression can lead to paedomorphosis (retention of juvenile traits in adult life) or influence speciation in populations, as seems to be the case in cicadas species that reach adulthood at different ages. Based on such findings, Minelli (2015) suggests that the temporal organization of development should be understood as a "temporal phenotype" that can evolve like a morphological phenotype. Overall, these concepts distinguish different processes by means of the rate at which they occur in mechanistic models and thereby complement models of mechanisms understood as composed of parts and wholes in evo-devo.

Conclusions and Outlook

Predominant philosophical conceptions of mechanism and mechanistic explanation have recently faced challenges when applied to mechanisms in development and evolution. In particular, some evo-devo mechanisms do not fit into the framework of mechanistic concepts of parthood and decomposition or the idea of static functional relations between levels of organization. This is because evo-devo researchers seek not only to decompose wholes into parts but also to recompose wholes to explain their dynamics, as well as describe relations across levels of organization in developmental mechanisms that are vertically dynamic.

In order to develop adequate conceptual frameworks for mechanisms that suit the explanatory interests and methodological practices of evo-devo, future studies should incorporate temporal patterns and processual perspectives in their analyses of developmental mechanisms, such as by investigating the roles that scales (in addition to parthood relations) play in hierarchically ordering biological complexity. More generally, parthood- and scale-based conceptions of mechanism should be seen as distinct but complementary explanatory strategies. The former orders and relates things and their properties, whereas the latter emphasizes process rates. One is well suited for identifying a particular system, its relevant components, and their relations, while the other conveys an understanding of a system's temporal organization across different levels. Future studies of the conceptual elements of evo-devo's mechanistic framework will be critical to identify and characterize those mechanisms that link individual development with evolutionary change.

Cross-References

- ▶ A Macroevolutionary Perspective on Developmental Constraints in Animals
- ▶ Canalization: A Central but Controversial Concept in Evo-Devo
- ▶ Conrad Hal Waddington (1905–1975)
- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Developmental Plasticity and Evolution
- ▶ Dispositional Properties in Evo-Devo
- ▶ Explanation in Evo-Devo
- ▶ Evolvability
- ▶ Generalization in Evo-Devo
- ▶ Heterochrony
- ▶ Levels of Organization in Evo-Devo
- ▶ Modeling and Simulation in Evo-Devo
- ▶ Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics
- ▶ Proximate Versus Ultimate Causation and Evo-Devo
- ▶ Teleology in Evo-Devo
- ▶ The Evolution and Development of Segmented Body Plans

Acknowledgments I would like to thank Laura Nuño de la Rosa and Gerd B. Müller for the opportunity to participate in this project. I am grateful for comments received on earlier versions of this chapter by Laura Nuño de la Rosa and Alan C. Love. In addition, I gratefully acknowledge financial support from the German Research Foundation (DFG; Project No. BA 5808/1–1).

References

Baedke J (forthcoming) The origin of new levels of organization. In: Brooks D, Di Frisco J, Wimsatt W (eds) Levels of organization in the biological sciences. MIT Press, Cambridge

Baedke J, Mc Manus SF (2018) From seconds to eons: time scales, hierarchies, and processes in evo-devo. Stud Hist Phil Biol Biomed Sci 72:38–48

Bechtel W, Abrahamsen A (2010) Dynamic mechanistic explanation: computational modeling of circadian rhythms as an exemplar for cognitive science. Stud Hist Philos Sci A 41:321–333
Brigandt I (2015) Evolutionary developmental biology and the limits of philosophical accounts of mechanistic explanation. In: Braillard PL, Malaterre C (eds) Explanation in biology. Springer, Dordrecht, pp 135–173
Calcott B (2009) Lineage explanations: explaining how biological mechanisms change. Br J Philos Sci 60:51–78
Calcott B (2013) Why how and why aren't enough: more problems with Mayr's proximate-ultimate distinction. Biol Philos 28:767–780
Craver CF (2007) Explaining the brain. Oxford University Press, Oxford
Craver CF (2015) Levels. In: Metzinger T, Windt JM (eds) Open MIND. MIND Group, Frankfurt, pp 1–26
Craver CF, Bechtel W (2007) Top-down causation without top-down causes. Biol Philos 22:547–563
Glennan S (1996) Mechanisms and the nature of causation. Erkenntnis 44:49–71
Green S, Batterman R (2017) Biology meets physics: reductionism and multi-scale modeling of morphogenesis. Stud Hist Phil Biol Biomed Sci 61:20–34
Hall BK (2012) Evolutionary developmental biology (evo-devo): past, present, and future. Evo Edu Outreach 5:184–193
Illari P, Williamson J (2012) What is a mechanism? Thinking about mechanisms across the sciences. Eur J Philos 2:119–135
Jaeger J, Monk N (2015) Everything flows. EMBO Rep 16:1064–1067
Jaeger J, Sharpe J (2014) On the concept of mechanism in development. In: Minelli A, Pradeu T (eds) Towards a theory of development. Oxford University Press, Oxford, pp 56–78
Levy A, Bechtel W (2013) Abstraction and the organization of mechanisms. Philos Sci 80:241–261
Love AC (2018) Developmental mechanisms. In: Glennan S, Illari P (eds) The Routledge handbook of the philosophy of mechanisms and mechanical philosophy. Routledge, New York, pp 332–347
Love AC, Hüttemann A (2011) Comparing part-whole reductive explanations in biology and physics. In: Dieks D, Gonzalez WJ, Hartmann S, Uebel T, Weber M (eds) Explanation, prediction, and confirmation. Springer, Dordrecht, pp 183–202
Machamer P, Darden L, Craver CF (2000) Thinking about mechanisms. Philos Sci 67:1–25
Mc Manus FG (2012) Development and mechanistic explanation. Stud Hist Phil Biol Biomed Sci 43:532–541
Menzies P (2012) The causal structure of mechanisms. Stud Hist Phil Biol Biomed Sci 43:796–805
Minelli A (2015) Biological systematics in the evo-devo era. Eur J Taxon 125:1–23
Moczek AP, Sears KE, Stollewerk A, Wittkopp PJ, Diggle P, Dworkin I, Ledon-Rettig C, Matus DQ, Roth S, Abouheif E et al (2015) The significance and scope of evolutionary developmental biology: a vision for the 21st century. Evol Dev 17:198–219
Nicholson DJ (2013) Organisms ≠ machines. Stud Hist Phil Biol Biomed Sci 44:669–678
Nicholson DJ, Dupré J (eds) (2018) Everything flows: towards a processual philosophy of biology. Oxford University Press, Oxford
Nuño de la Rosa L, Etxeberria A (2012) Pattern and process in evo-devo: descriptions and explanations. In: de Regt S, Henk W, Hartmann S, Okasha S (eds) EPSA philosophy of science: Amsterdam 2009, vol 1. Springer, Dordrecht, pp 263–274
Olson BJSC, Nedelcu AM (2016) Co-option during the evolution of multicellular and developmental complexity in the Volvocine green algae. Curr Opin Genet Dev 39:107–115
Skipper RA, Millstein RL (2005) Thinking about evolutionary mechanisms: natural selection. Stud Hist Phil Biol Biomed Sci 36:327–347
Soroldoni D, Jörg DJ, Morelli LG et al (2014) Genetic oscillations. A Doppler effect in embryonic pattern formation. Science 345:222–225
Winther RG (2015) Evo-devo as a trading zone. In: Love A (ed) Conceptual change in biology. Springer, Dordrecht, pp 459–482
Ylikoski P (2013) Causal and constitutive explanation compared. Erkenntnis 78:277–297

Modeling and Simulation in Evo-Devo

Brett Calcott and Arnon Levy

Contents

Abstract

Modeling and simulation are increasingly important as tools in many scientific disciplines. Our aim in this chapter is to outline some key aspects of modeling, illustrating our discussion with examples from evo-devo and emphasizing features of particular relevance to the discipline. We begin by characterizing modeling as a distinctive two-step theoretical strategy in science. Then we highlight the role of the modeler's aims in assessing a model, distinguish two types of progress in a modeling tradition, and outline the influential idea of trade-offs in modeling. Last, we focus on modeling as a way to clarify the link between evolution and development.

Keywords

Modeling · Simulation · Scientific strategies

B. Calcott (✉)
Department of Philosophy, University of Sydney, Sydney, NSW, Australia
e-mail: brett.calcott@gmail.com

A. Levy
The Hebrew University of Jerusalem, Jerusalem, Israel
e-mail: arnon.levy@mail.huji.ac.il

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_95

Introduction

In this chapter, we sketch some key aspects of modeling, illustrating them with examples from evo-devo and connecting them to central aims in the field, such as the explaining the generation of variation. We begin by circumscribing the kinds of "models" of interest in the chapter, for the terms "model" and "modeling" are used broadly. In some uses, all theoretical activity in science is a kind of modeling; in others, the notion of a model encompasses diagrams or applies to "model" organisms. For this chapter, however, the models we focus upon are those that demand specialized technical skills to construct; skills particular to building and analyzing models, rather than any particular scientific discipline. Thus, the examples we use are mathematical models, such as sets of coupled differential equations, or computer simulations written in a programming language such as C or Python or R.

Modeling as a Distinctive Strategy

We view modeling as a distinctive two-step strategy for investigating and understanding a complex natural phenomenon:

- Step 1: the modeler(s) construct and study a simplified analogue of the phenomenon. This is the model.
- Step 2: insights drawn from investigating the model are used to say something about the original phenomenon (the target).

These two steps provide a framework that helps organize the topics we cover below and can be illustrated with a concrete example. Consider one modeling approach used in evo-devo where Boolean equations capture gene regulatory interactions and their changes over time (see chapter ▶ "Modeling Evolution of Developmental Gene Regulatory Networks"). In these models, gene expression is either on or off, and controlled by a Boolean function that responds to whether other genes are on or off (Kauffman 1969). Typically, time is divided into discrete steps, so gene **A** might be switched on at time $t+1$ when gene **B** is on and gene **C** is off at the previous time-step, t. In this case, **B** is an excitatory regulatory connection and **C** is inhibitory. This modeling approach allows networks of gene regulation to be represented with sets of interrelated Boolean equations. Incremental changes to these equations, such as changing the logic functions or the genes referenced in the equations, capture the evolution of the regulatory networks.

In step 1, the role of simplification is crucial. Models are inevitably partial (or abstract) representations of the target: they include a selection of the target's features and rarely aim for completeness. And models are often highly idealized, making assumptions that are known not to hold of the target. Thus, modeling simplifies the target, often replacing it with something that is false. This may make the model more readily comprehensible, easier to analyze, or simply render the problem tractable, given mathematical or computational constraints. The Boolean

models mentioned above are abstract: focusing on gene regulation clearly omits many other features of regulation and development process. The Boolean models also are idealized: we know that genes are not simply on or off, but can be transcribed at a variety of different rates. Yet such assumptions clearly make building, analyzing, and exploring the model far more tractable. We will come back to this important issue in the sections about "aims" and "trade-offs."

Importantly, in Step 1, the model has a standing of its own – it can be, and often is, studied independently of its bearing on the target. This might require some formal analytic proofs if the model is a set of equations, or perhaps the statistical analysis of data produced by many runs of a simulation. This first step typically represents a significant proportion of what we consider "modeling." For example, Boolean Networks are often studied with little mention of actual biology; instead, the goal has been to more deeply understand the model itself (see, e.g., Aracena et al. 2009). We return to this issue in the section about "progress" in modeling.

In Step 2, we tie the insights drawn from the model back to the *target(s)* – one or more systems in the natural world that the model represents and that we aim to understand with the model's aid. Targets can range from specific events and processes with known empirical significance to far more general phenomena with less direct connections to familiar empirical cases. For example, Peter et al. (2012) construct a Boolean gene regulatory model with over 50 genes based on years of meticulous studies of the initial stages of sea urchin development. They use the model to explore the outcome of manipulating particular genes and compare these outcomes to experimental manipulations. The target in this case is highly specific: the regulatory system of a particular animal. In contrast, Payne and Wagner (2015) construct a Boolean model of gene regulation that contains only three genes. They use it to "systematically explore the relationship between circuit form and function" by generating nearly 17 million distinct three-gene circuits with different Boolean equations. In cases such as this, the models are not tracking a particular target, but are being used to explore the consequences of certain assumptions in a more generic domain (see Wimsatt 1987; Kokko 2007 for interesting general discussions). We return to this idea in the section on "exploring possibilities."

Above we described this two-step approach as "distinctive." By this we mean that the explicit focus on the model and its analysis contrasts with approaches to theorizing where scientists construct theories about the natural world with no intermediate analysis of a model. Classic examples of *direct* analysis are Darwin's theory of evolution by natural selection or Mendeleev's periodic table (see Godfrey-Smith 2007; Weisberg 2007). Neither used mathematical models in their theorizing. In contrast, the strategy of modeling operates *indirectly.* By this, we mean that we can break it into two steps outlined above. These steps need not be distinct, but it is helpful to think of them as separate steps for analytical purposes.

We can find both direct and indirect approaches in evo-devo. Consider two books: Kirschner and Gerhart's *Plausibility of Life*, a book-length treatment of their theory of facilitated variation (Kirschner and Gerhart 2006) and Andreas Wagner's *The Origins of Evolutionary Innovations* (Wagner 2011). Both books pursue the same central question: how is new variation generated? This topic falls under the rubric of *evolvability* – a

central theme in evo-devo (Hendrikse et al. 2007; see chapter ▶ "Evolvability"). Yet the way the authors address this question is different in each book. Kirschner and Gerhart lay out several general principles such as "weak signaling" and "exploratory processes" that capture aspects of developmental systems affecting their ability to produce new variation. These principles are distilled from a careful analysis of actual examples and supported by arguments that draw specific causal details of developmental systems. Wagner's book also contains a good deal of detail about developmental systems. But for theorizing, Wagner derives his key results from highly idealized mathematical models and simulations of these systems. Wagner's approach is indirect as his work relies on studying and theorizing about the models themselves. This does not mean that Kirschner and Gerhart's ideas cannot serve as inspiration for constructing formal models (see Parter et al. 2008). Rather, it shows that their own approach to theorizing does not revolve around the use of formal models as an intermediary step.

The Aims of the Modeler

We characterized modeling as a two-step process. A modeler constructs and explores a model and then makes claims about the target based on properties of the model. What licenses this inference from model to target? This question has generated much discussion in philosophy (see Weisberg 2013 for an overview), but a key idea is that models must resemble their targets in some key respects.

The mere claim that a model resembles its target is not enough, however. Since a model omits, abstracts, and idealizes, we need to know which kinds of model-world resemblances matter and to what extent: they may be alike in terms of input-output profile and/or in dynamics and/or in qualitative versus quantitative details, and in various other respects. It is tempting to think we can answer this question by looking at the model and the target and judging the objective similarities and differences between them: treating genes as Boolean switches is a good approximation, or it is not. But this is too simple. The merits of a model are context-sensitive, and they depend on the aims of those using it. Ronald Giere captures this point by characterizing modeling in the following generic way:

> *Scientists (S) use a model (X) to represent some aspect of the world (W), for specific purpose (P).* (Giere 2004)

Thus, what makes the resemblance between a model and target sufficiently good depends (at least partially) on what the modeler's goals are when constructing and using it. For instance, a model might supply accurate predictions while not capturing important causal factors. If the modeler's goal was understanding the causal structure of the target, then such a model may count as a failure, its predictive prowess notwithstanding.

Attending to the modeler's goals is important because one's goals may vary (and there may be more than one goal) even when similar abstractions are made in the model. The contrasting uses of Boolean models above by Peter et al. and Payne and

Wagner supply one example of this. Both models use a simple Boolean representation of gene expression and regulation, but their aims are very different. Peter et al. use their model to predict outcomes in one particular system, while Payne and Wagner use their model to make a very general point about the relationship between regulatory architecture and its function.

These goals can vary even when scientists use the same model. Consider a sophisticated model of the evolution of tooth development built by Salazar-Ciudad and Jernvall (2002). The model has several layers: it captures the interaction of gene products and their regulation, the physical forces governing cell growth and division, and the diffusion of signaling molecules between cells. It can produce a wide variety of three-dimensional shapes comparable to actual tooth shape by comparing the number and location of cusps: the pointy outcrops on a tooth's surface. This model figures in several papers; here, we contrast just two of them (see chapter ▶ "Computational Modeling at the Cell and Tissue Level in Evo-Devo"). The first, "A computational model of teeth and the developmental origins of morphological variation" (Salazar-Ciudad and Jernvall 2010), focuses directly on the evolution of tooth morphology, showing that by "systematically tinkering" with their model, it could produce a wide variation of shapes, comparable to those found in an actual population of seals. The second paper, "Adaptive dynamics under development-based genotype–phenotype maps" (Salazar-Ciudad and Marín-Riera 2013), uses the same model but, as its title suggests, the focus is on something far more general. Here, the model is used to obtain generic insights into the interaction between natural selection and development. These two papers have different aims, and thus we need to test the adequacy of the model with different criteria in each case. For example, the more general claims made in the second paper rely on a key assumption: that the tooth model represents development in general. The aims of the modeler thus form an important part of any modeling endeavor, and of evaluating its success (see Wimsatt 1987 for examples of the rich diversity of aims).

Two Kinds of Progress in Modeling

Like the living world, modeling projects change, diversify, and often go extinct over time. Paying attention to the trajectory of a modeling project is informative because it highlights a common worry regarding models: they oversimplify the empirical realities of the systems under study. When we look at a productive modeling project over time, we can often see two types of progress, each related to one of the two steps of modeling described above (Levy 2011).

Internal progress occurs when improvements are made to the model itself – a more elegant, tractable mode of representation is discovered, complexities and details get added, a new analytical technique provides deeper or more widely applicable insight. (whether and to what extent this will count as progress will also depend on the inquiry's goals). In contrast, *external progress* involves improvements regarding how well the model captures real-world phenomena, increasing one's

understanding or predictive capacity regarding the target. This mode of progress requires validation of results vis-à-vis the world, and a closer tracking of targets.

Both modes of progress can be important and valuable – internal progress increases one's understanding of the model, perhaps allowing one to explore more possibilities or provide additional confidence in the results. External progress, in contrast, enhances one's understanding *of the world* through the model. However, it is important not to confuse the two modes, and some criticisms of modeling projects (such as their degree of realism, or concerns about the legitimacy of idealizations) can be seen as making such a charge.

Consider, for example, reaction-diffusion models originating with Turing (1952). These models portray basic pattern generation processes via a simplified scenario in which a pair of diffusing morphogens interact with each other and with embryonic cells. (Interestingly, Turing was entirely self-conscious of his modelling approach, noting early in his paper that "this model will be a simplification and an idealization, and consequently a falsification" (p. 37).) Subsequent work saw a steady course of development of these models for the next 40 or 50 years (Kondo and Miura 2010). It would have been easy to view this as progress in explaining actual pattern formation. But, in fact, only more recently have we seen empirical, external progress (Vanag and Epstein 2009). It now appears somewhat more reasonable to conclude that reaction-diffusion models have advanced our understanding of *actual* pattern formation processes. We have made external progress regarding pattern formation.

This example illustrates several points concerning the practice of modeling. First, the two-step process has definite utility, as it can free the modeler from the need to keep close tabs on the empirical applicability of her work, thus allowing her to make headway on more in-principle aspects. At the same time, it is important not to confuse this kind of internal progress – primarily at the mathematical level alone and involving an increased understanding of the model itself – with progress on understanding the model's target.

Trade-Offs and Model-Model Relationships

Following a seminal paper by ecologist Richard Levins (1966), many see modeling as a balancing act in which one is attempting to satisfy multiple, partly conflicting goals. Levins highlighted three such goals: generality, precision and realism. His principal idea was that these three desiderata often trade off against each other: to be realistic, a model has to either sacrifice generality, applying faithfully to a small set of targets and/or sacrifice precision, because a complex realistic model is often not amenable to exact analysis. Likewise, if one aims for generality – especially if one also aims to attain exact results in one's modeling – this requires idealization and approximation, thus resulting in an unrealistic model. Other potential trade-offs also exist, such as between explanatory power and predictive accuracy. The extent to which a given accuracy or insight counts toward a model's success will depend on the goals with which it is put forward. Sometimes a qualitative, yet explanatorily valuable model is preferable to an accurate yet less explanatory one (or vice versa).

This conflict between different modeling goals is relevant in evo-devo models as these trade-offs must be made regarding both evolution and development – processes that operate across very different timescales. In most traditional evolutionary models, the representation of the relationship between genotype and phenotype (or fitness) is highly simplified. Yet this is often coupled with a model of evolutionary change that is meant to accurately track information about changes in genetic variation. In contrast, a model that incorporates phenotypic or developmental detail might adopt a very simple view of evolutionary change. Niklas (1994), for example, explores the rise of disparity in plant shape using a model of plants that combines facts about their development, morphology, and fitness. But the same model tracks evolution using a simple hill-climbing adaptive walk, thus ignoring many important details about evolution change, such as mutation rates. Our point is that it is possible to make different trade-offs regarding different parts of models, so a model may be realistic with respect to development, but highly abstract where evolution is concerned.

We can address the conflict between these desiderata in various ways. Levins himself suggests building multiple models of the same phenomenon, each embodying a different trade-off, to give it a more complete treatment. Another approach is to clarify the limitations of one's assumptions and methods. Developing this understanding is itself a modeling endeavor, typically proceeding by contrasting different modeling approaches. For example, Jong and Ropers (2006) compare several qualitative approaches to gene regulation including the Boolean regulatory models mentioned above. They conclude that understanding the connectivity of interactions in networks, rather than tracking the precise details with high accuracy, may explain how a network behaves. Depending on the aims of the modeler, analyses like this may justify when and why certain trade-offs can be made.

Exploring and Contrasting Possibilities in Evo-Devo

We have described models as standalone systems, one step removed from the real world. Most times, such systems do not represent any actual phenomenon in the world. Seen in this light, models are an excellent arena in which to explore a space of possibilities: How could a particular trait evolve? What would occur if such-and-such a constraint were present? How would an organism of *this* sort respond to *that* kind of selection pressure?

One modeling approach used to explore possibilities relevant to evo-devo is the construction of a *developmental morphospace* (see chapter ▶ "Mechanisms of Pattern Formation, Morphogenesis, and Evolution"). This is a multidimensional space where each of the dimensions captures some aspect controlling the generation of a particular morphology. Raup's shell morphospace (Raup 1966) is a well-known example. Raup used a mathematical model with just four parameters to generate a huge variety of different shell shapes. Many of the resulting regions of the resulting four-dimensional morphospace held shell shapes that could be categorized into taxonomic groups. But there were also shapes that no shell has ever taken. It was these unrealized possibilities that many found interesting. Were these shapes absent because they were selected against or was there some developmental constraint

preventing them from being generated at all? Reflecting on this model played a central role in one early cross-disciplinary effort to link evolution and development using *developmental constraints* (Smith et al. 1985; see chapter ▶ "A Macroevolutionary Perspective on Developmental Constraints in Animals").

Using models to generate and explore these possibilities provides a rigorous approach to thinking about what kinds of phenotypic variations are possible and the connections between them. A morphospace is not the only example – above we referenced a model that generated a space of gene regulatory circuits. Such modeling endeavors are not without problems, however. Because they are highly abstract and idealized, they often draw on subtle assumptions about the way a model represents the world. For example, visualizing a morphospace often prompts ideas that draw on the distance between different shell shapes or the relative size of regions of shell shapes. But distance and volume in morphospace may reflect the model's mathematical assumptions more than reality (see Mitteroecker and Huttegger 2009). Again, this highlights the two steps in modeling: regardless of how rigorous the model itself is, we must be careful when interpreting how models map to the world.

Importantly, this ability to contrast different possible scenarios places modelers in an ideal position to pursue a central aim of evo-devo: to clarify when and how development makes a difference to evolution. One way to do this is by contrasting the performance of several related models, and then identifying what factor *makes the difference* between them. For example, Jiménez et al. (2015) explore six different regulatory mechanisms that can produce the same patterning in multicellular development. They show that, despite producing the same pattern, these mechanisms vary in their propensity to generate new patterns under mutational changes. The difference-maker, in this case, is the developmental mechanism generating the particular pattern. By showing the contrasting evolutionary effects produced by the different possible mechanisms, such modeling demonstrates why development is essential to understanding the trajectory of evolutionary change.

Summary

Modeling is a particular scientific strategy, with distinctive advantages and pitfalls. In this chapter we highlighted some core features of this strategy, including the role of idealization and abstraction, its "two-step" structure, and the importance of the goals of a modeler. We also exposed some possible problems associated with modeling, such as confusing internal and external progress. Last, we emphasized the importance of modeling for evo-devo, of how it can highlight the role that developmental processes play in understanding evolutionary change.

Cross-References

▶ A Macroevolutionary Perspective on Developmental Constraints in Animals
▶ Computational Modeling at the Cell and Tissue Level in Evo-Devo

▶ Evolvability
▶ Mechanisms of Pattern Formation, Morphogenesis, and Evolution
▶ Modeling Evolution of Developmental Gene Regulatory Networks

References

Aracena JE, Moreira GA, Salinas L (2009) On the robustness of update schedules in Boolean networks. Biosystems 97(1):1–8

de Jong H, Ropers D (2006) Qualitative approaches to the analysis of genetic regulatory networks. In: Szallasi Z, Stelling J, Periwal V (eds) System modeling in cellular biology: from concepts to nuts and bolts. The MIT Press, Cambridge

Giere RN (2004) How models are used to represent reality. Philos Sci 71:742–752. https://doi.org/10.1086/425063

Godfrey-Smith P (2007) The strategy of model-based science. Biol Philos 21:725–740. https://doi.org/10.1007/s10539-006-9054-6

Hendrikse JL, Parsons TE, Hallgrímsson B (2007) Evolvability as the proper focus of evolutionary developmental biology. Evol Dev 9:393–401. https://doi.org/10.1111/j.1525-142X.2007.00176.x

Jiménez A, Cotterell J, Munteanu A, Sharpe J (2015) Dynamics of gene circuits shapes evolvability. PNAS 112:2103–2108. https://doi.org/10.1073/pnas.1411065112

Kauffman SA (1969) Metabolic stability and epigenesis in randomly constructed genetic nets. J Theor Biol 22:437–467

Kirschner MW, Gerhart JC (2006) The plausibility of life: resolving Darwin's Dilemma. Yale University Press, New Haven

Kokko H (2007) Modelling for field biologists and other interesting people, 1st edn. Cambridge University Press, Cambridge

Kondo S, Miura T (2010) Reaction-diffusion model as a framework for understanding biological pattern formation. Science 329:1616–1620. https://doi.org/10.1126/science.1179047

Levins R (1966) The strategy of model building in population biology. Am Sci 54(4):421–431

Levy A (2011) Game theory, indirect modeling, and the origin of morality. J Philos 108:171–187

Mitteroecker P, Huttegger SM (2009) The concept of morphospaces in evolutionary and developmental biology: mathematics and metaphors. Biol Theory 4:54–67

Niklas KJ (1994) Morphological evolution through complex domains of fitness. Proc Natl Acad Sci 91:6772–6779. https://doi.org/10.1073/pnas.91.15.6772

Parter M, Kashtan N, Alon U (2008) Facilitated variation: how evolution learns from past environments to generalize to new environments. PLoS Comput Biol 4:e1000206. https://doi.org/10.1371/journal.pcbi.1000206

Payne JL, Wagner A (2015) Function does not follow form in gene regulatory circuits. Sci Rep 5:13015. https://doi.org/10.1038/srep13015

Peter IS, Faure E, Davidson EH (2012) Predictive computation of genomic logic processing functions in embryonic development. PNAS 109:16434–16442. https://doi.org/10.1073/pnas.1207852109

Raup DM (1966) Geometric analysis of shell coiling: general problems. J Paleontol 40:1178–1190

Salazar-Ciudad I, Jernvall J (2002) A gene network model accounting for development and evolution of mammalian teeth. PNAS 99:8116–8120. https://doi.org/10.1073/pnas.132069499

Salazar-Ciudad I, Jernvall J (2010) A computational model of teeth and the developmental origins of morphological variation. Nature 464:583–586. https://doi.org/10.1038/nature08838

Salazar-Ciudad I, Marín-Riera M (2013) Adaptive dynamics under development-based genotype-phenotype maps. Nature 497:361–364. https://doi.org/10.1038/nature12142

Smith JM, Burian R, Kauffman S et al (1985) Developmental constraints and evolution: a perspective from the mountain lake conference on development and evolution. Q Rev Biol 60:265–287

Turing AM (1952) The chemical basis of morphogenesis. Philos Trans R Soc Lond B 237:37–72. https://doi.org/10.1098/rstb.1952.0012

Vanag VK, Epstein IR (2009) Pattern formation mechanisms in reaction-diffusion systems. Int J Dev Biol 53(5–6):673–681

Wagner A (2011) The origins of evolutionary innovations: a theory of transformative change in living systems, 1st edn. Oxford University Press, Oxford/New York

Weisberg M (2007) Who is a modeler? Br J Philos Sci 58:207–233. https://doi.org/10.1093/bjps/axm011

Weisberg M (2013) Simulation and similarity: using models to understand the world. Oxford University Press, Oxford/New York

Wimsatt WC (1987) False models as means to truer theories. In: Neutral models in biology. Oxford University Press, New York, pp 23–55

Interdisciplinarity in Evo-Devo

Alan C. Love

Contents

Abstract

Evolutionary developmental biology (evo-devo) is an inherently multidisciplinary area of contemporary biology. Although there are different perspectives on what evo-devo is and different formulations of the meaning of interdisciplinarity, I concentrate on the question of why evo-devo investigations exhibit such a diverse mixture of disciplinary contributors. One prominent answer is the complexity of the evolutionary and developmental phenomena under scrutiny, both with respect to their spatial levels of organization and the temporal scales on which causal interactions across these levels occur. This complexity in nature is translated into structured problem agendas for biologists that drive interdisciplinarity through organizing diverse research questions and coordinating distinct evaluative standards in relation to heterogeneous methods (e.g., what counts as relevant data or a good explanation). These problem agendas had been somewhat neglected in evolutionary biology and themselves change over time. I briefly illustrate some of these features with a well-known case study – the fin-limb transition – before concluding with several epistemological and sociological implications of interdisciplinarity in evo-devo.

A. C. Love (✉)
Department of Philosophy, Minnesota Center for Philosophy of Science, University of Minnesota, Minneapolis, MN, USA
e-mail: aclove@umn.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_65

Keywords

Complexity · Evolutionary novelty · Integration · Multidisciplinary · Problem agenda

What Is Evo-Devo? What Is Interdisciplinarity?

Evolutionary developmental biology (evo-devo) is a loose conglomerate of disciplinary approaches in the life sciences organized around two main axes (Hall 1999; Raff 2000; Wagner et al. 2000; Müller 2007): (a) the evolution of development – inquiry into the pattern and processes of how ontogeny varies and changes over time; and (b) the developmental basis of evolution – inquiry into the causal impact of ontogenetic processes on evolutionary trajectories, both in terms of constraint and facilitation. Distinguishing these two main axes is significant for two reasons. First, the former has been more prominent than the latter. Fewer researchers have devoted attention to understanding how properties of developmental processes affect evolution and integrating this understanding into standard models found in evolutionary theory (see chapters ▶ "Developmental Evolutionary Biology (Devo-Evo)" and ▶ "Micro-Evo-Devo"). Second, different researchers from different disciplinary backgrounds, all of whom use an assortment of methods and approaches, see themselves as working within evo-devo, sometimes to the exclusion of one another. One fault line in these divergent conceptions can be captured by distinguishing between narrow and wide characterizations of evo-devo.

A narrow characterization of evo-devo is typically focused on the comparative developmental genetics of metazoans (De Robertis 2008). Conserved genetic regulatory networks (GRNs) and signaling pathways underlying developmental processes ("the genetic toolkit") are studied to characterize evolutionary change in terms of alterations of gene regulation (Carroll 2008). For example, the discovery of highly conserved GRNs where the homology of structure was debated or highly suspect, such as the role of *distalless* in appendage outgrowth (Panganiban et al. 1997), led to the formulation of the concept of "deep homology," which implied that new morphological traits evolved by repeated modifications of the shared metazoan genetic toolkit to transform developmental processes in distinctive ways (Shubin et al. 2009). Although much of this empirical research has been prosecuted using model organisms from mainstream developmental biology, a phylogenetic context is crucial for drawing the relevant evolutionary inferences (see chapter ▶ "Evo-Devo and Phylogenetics"). In some cases, this is done within a clade (e.g., drosophilids; Kopp 2011); in others, a wider range of taxa is in view (e.g., appendage outgrowth and *distalless*). Additionally, paleontology plays a crucial role in establishing ancestral character states and the range of phenotypic variation extant at different phylogenetic junctures (Raff 2007; see chapter ▶ "Methods and Practices in Paleo-Evo-Devo").

The narrow depiction of evo-devo is problematic, in part because it accents the evolution of development over the developmental basis of evolution. It is misleading

to say that, "evo-devo began in the pre-genomic era when genetic studies in *Drosophila* and gene cloning in *Xenopus* revealed that the Hox genes that control the anterior-posterior (A-P) axis were unexpectedly conserved" (De Robertis 2008, 186). However, the selective exclusion of historical factors points in the direction of a wider conception that both predates the rise of comparative developmental genetics and continues into the present (Love 2015; see chapter ▶ "Developmental Evolutionary Biology (Devo-Evo)"). Broader depictions of evo-devo include comparative developmental genetics but also draw attention to comparative embryology and morphology, experimental investigations of epigenetic dynamics at different levels of organization (including physical forces), and computational or simulation oriented inquiry (Hall 1999; Wagner et al. 2000; Müller 2007; see chapters ▶ "Modeling and Simulation in Evo-Devo" and ▶ "Computational Modeling at the Cell and Tissue Level in Evo-Devo"). A major challenge is to integrate these different approaches, either through combinations of multiple models or in terms of an overarching theoretical perspective (Love et al. 2017). These broader depictions enlarge what counts as the evolution of development (e.g., physical forces are not often treated in comparative developmental genetics), while also accenting the causal impact of ontogenetic processes on evolutionary trajectories (chapter ▶ "Developmental Evolutionary Biology (Devo-Evo)").

Both the narrow and the wide conception of evo-devo appeal to a similar motivation in addressing a gap in evolutionary theory with respect to understanding the origin and evolution of form or structure (Kirschner and Gerhart 2005; Müller 2007; Carroll 2008; see chapter ▶ "Form and Function in Evo-Devo"). This motivation leads to a common commitment that the developmental processes which mechanistically produce phenotypic variation must be incorporated into evolutionary theorizing. However, there remains disagreement about which configurations of disciplinary approaches are necessary to account for these developmental processes that underlie variation. Those advocating for a wider conception hold that evo-devo "necessarily includes many more factors than the evolution of gene regulation alone, notably the dynamics of epigenetic interactions, the chemicophysical properties of growing cell and tissue masses, and the influences of environmental parameters" (Müller 2007, 944; see chapters ▶ "Eco-Evo-Devo" and ▶ "Evo-Devo's Contributions to the Extended Evolutionary Synthesis"). Although rapprochement between these developmental-form advocates has not yet occurred (Love 2017), the key thing to notice is that both the narrow and the wide conceptions are multidisciplinary, despite divergence in their constellations of disciplinary approaches.

Is there a difference between multidisciplinary and interdisciplinary? What is interdisciplinarity? Etymologically, interdisciplinarity literally means "between disciplines" where disciplines are standardized fields of study in contemporary research (Repko 2008). The intuitive presumption is that something distinctive and unavailable to standard areas of study emerges out of or from interdisciplinarity, such as a more adequate or general explanation (see chapters ▶ "Explanation in Evo-Devo" and ▶ "Generalization in Evo-Devo"). Some have argued that "multidisciplinary" refers to research that brings disciplines together for a particular purpose while retaining their distinctness, whereas "interdisciplinary" in some way

more permanently integrates multiple disciplines to produce a new discipline (Collins 2002). The latter is one way that evo-devo has been characterized (e.g., Raff 2000), and the growth of institutional infrastructure, such as professional societies, new research journals, dedicated funding, textbooks, and specified job openings, captures a sense in which contemporary evo-devo is interdisciplinary (Moczek et al. 2015). However, other strands of research in evo-devo seem more transient, with disciplines organized around the solution of specific research questions, such as the origin of novelties in the fin-limb transition (Brigandt 2010; see below, section "Interdisciplinarity and the Fin-Limb Transition"). The institutional structure of contemporary science might be unaffected even though morphologists from a department of ecology and evolution, paleontologists from a department of earth science, and developmental biologists from a department of genetics, cell, and development collaborate to address research questions about the evolution of development.

Fortunately, there is no need to adjudicate between these conceptions or be fussy about terminology. As with the wide and narrow depictions of evo-devo, both of which involve multiple participating fields of study, multidisciplinary and interdisciplinary elements are clearly present in contemporary evo-devo. This aligns with inquiry into interdisciplinarity, which has identified the presence and articulation of a broad or complex problem as a prerequisite (Repko 2008). Consensus has coalesced around a characterization of interdisciplinary research as: "a mode of research by teams or individuals that integrates information, data, techniques, tools, perspective, concepts, and/or theories from two or more disciplines or bodies of specialized knowledge to advance fundamental understanding or to solve problems whose solutions are beyond the scope of a single discipline or area of research practice" (National Academy of Sciences 2005, 39). The evolution of development and the developmental basis of evolution fit squarely within this charge.

Complexity and the Phenomena of Evo-Devo

That evo-devo is interdisciplinary is beyond dispute. Why it should be interdisciplinary is another question. One prominent answer is the complexity of the evolutionary and developmental phenomena under scrutiny, both with respect to their spatial levels of organization and the temporal scales on which causal interactions across these levels occur. Consider again the common commitment among different conceptions of evo-devo: "In order to achieve a modification in adult form, evolution must modify the embryological processes responsible for that form. Therefore an understanding of evolution requires an understanding of development" (Amundson 2005, 176). What is required to understand development? When researchers emphasize the need to understand mechanistically how phenotypic variation is produced, one part of this is comprehending the dynamics of embryological processes at multiple levels of organization (see chapter ▶ "Levels of Organization in Evo-Devo"). Embryogenesis begins in many metazoans with a single cell from which multicellular aggregates (tissues) are formed; these tissues, in

turn, can compose a functioning organ or anatomical feature (e.g., a limb). Differential gene expression leads to changes in cell fate that facilitate different kinds of tissues, organs, and anatomy. Sometimes the movement of tissues or mechanical pressure from anatomy induces a change in gene expression. An understanding of development requires dissecting the variety of causal mechanisms operating during ontogeny (see chapters ▶ "Mechanisms in Evo-Devo" and ▶ "Mechanisms of Pattern Formation, Morphogenesis, and Evolution").

This variety of mechanisms in development that yields hierarchies of developmental structure and process can be described as complex, and the complexity can be distinguished into two types: compositional and organizational (see chapter ▶ "Complexity in Evo-Devo"). Compositional complexity refers to the material constitution of characters (part-whole relationships), both in terms of the number and types of components. Larger numbers of cells and a higher number of cell types that comprise a character mark increases in complexity. Organizational or procedural complexity refers to the causal relations that obtain between components. One aspect is the degree of aggregativity, which refers to how much causes act in a linear fashion, with increasing nonaggregativity (or nonlinearity) indicating a higher degree of complexity (Wimsatt 1997). Another aspect is the number and kind of structural arrangements exhibited by components and component types that are relevant to yielding particular causal outcomes. Higher numbers and kinds of structural arrangements are correspondingly more complex.

Thus far we have considered compositional complexity (or scalar hierarchies) and organizational complexity (or procedural hierarchies) in developmental time, within a single generation. However, investigation of the evolution of development and the developmental basis of evolution demands that we also consider evolutionary time across generations (Calcott 2009; see also chapter ▶ "Proximate Versus Ultimate Causation and Evo-Devo"). This expansion of temporal scales indicates unambiguously that complexity is a multifaceted attribute of the phenomena of interest studied by evo-devo researchers (see chapter ▶ "Evolution of Complexity"). To understand the origin of neural crest cell migration (the evolution of development), GRNs (organizational complexity) involved in the folding of the neural tube (compositional complexity) need to be detailed both in an ancestral and derived lineage (two distinct developmental times) so that we can identify how changes in gene expression in the lineage (evolutionary time) yielded the capacity for the detachment and migration of neural crest cells. Aspects of compositional complexity, such as how neural crest cells compose different tissues, also are subject to transformations of organizational complexity through evolutionary time. To understand how the genotype-phenotype map facilitates evolvability of a trait (the developmental basis of evolution; see chapter ▶ "Evolvability"), properties such as pleiotropy (organizational complexity) and considerations of how they relate to distinct developmental modules (compositional complexity) need to be theoretically articulated in ancestral and derived taxon representatives (different developmental times) so that we can identify through selection experiments or simulation modeling what happens across generations (evolutionary time). Again, aspects of complexity, such as the degree of pleiotropy in the genotype-phenotype map, are subject to evolutionary transformations.

One further element of complexity for evo-devo phenomena is relevant for interdisciplinarity: taxonomic scope. The properties and processes at diverse spatial and temporal scales are instantiated in a diversity of taxa across the tree of life. Compositional relationships differ across taxa, such as diploblasts versus triploblasts. Temporal scale relationships also differ across taxa, such as in variation related to generation time or complexity in life history trajectories. Given that some of the disciplinary structure in biology is taxon-specific (e.g., entomology or herpetology), interdisciplinarity in evo-devo involves more than coordinating approaches from cell biology, development, paleontology, and systematics to address both compositional and organizational complexity. The aim of formulating explanatory accounts of the evolution of development or developmental basis of evolution that hold generally across different parts of the tree of life requires recognizing the contribution of taxonomic scope to developmental and evolutionary phenomena.

Biologists recognize that this complex reality – wherever and however it is distributed taxonomically – undergirds the rationale for interdisciplinarity: "Because the mechanisms of each trait of interest are manifested at lower levels of biological organization and the significance of a trait is only apparent at higher levels, understanding a given trait usually requires the simultaneous use of molecular, cellular, organismal, population and ecological approaches" (Feder and Mitchell-Olds 2003, 649). The multifaceted complexity of development that needs to be comprehended if one is to reasonably incorporate how phenotypic variation is generated and what possibilities for variation are available (i.e., variability) through evolutionary time requires interdisciplinarity in evo-devo (see chapter ▶ "Variational Approaches to Evolvability: Short- and Long-Term Perspectives"). The different spatial levels of organization and the distinct temporal scales on which interactions across these levels occur in different taxa are the province of many life science disciplines (as well as physics, chemistry, and engineering), including systematics to characterize ancestral and derived states for variation in particular biological traits (see chapter ▶ "Evo-Devo and Phylogenetics"). Adequate explanations of different dimensions of the evolution of development or the developmental basis of evolution will not emerge from a single disciplinary approach. However, the fact that the phenomena of interest in evo-devo research exhibit a multifaceted complexity and demand interdisciplinarity does not yet illuminate *how* researchers coordinate their diverse methods and approaches across disciplines or achieve a synthesis or integration of their results, especially given that evaluative standards for what counts as data or a good explanation can vary across fields of study.

Structured Problem Agendas for Interdisciplinary Integration

One of the most philosophically conspicuous features of interdisciplinary investigation (in its different manifestations) is that it does not arise in response to the aims of theory construction and confirmation. The request originates from complex problem domains that elude scientific explanations arising from specific disciplinary approaches, as would be expected for complex phenomena such as the evolution

of development and the developmental basis of evolution that had been neglected in the discipline of evolutionary biology. "A common argument favoring interdisciplinary research refers to the nature of problems that science is supposed to help solve . . . their solutions require the combined effort of many traditional disciplines. . . . Scientists need to integrate all these facts to solve the problem" (Hansson 1999, 339). These complex problem domains pick out suites of research questions that can be described as *problem agendas* since they represent lists of things to be done by scientists (Love 2008). Although these suites of questions are addressed in part through the application of existing, well-confirmed theories, it is the complicated interrelations among large numbers of questions within a problem agenda that are responsible for spurring the call to interdisciplinary research. Thus, the complex phenomena of development and evolution in the world are partitioned into problem agendas (e.g., the nature of evolvability or the origin of novelties) that themselves are complex features of scientific reasoning.

A pressing concern is whether problem agendas have features or structure that facilitates the coordination of disciplinary approaches (where a contribution is made) and their evaluation (the standards for assessing the contributions). Coordination can be understood in terms of the structural depth of a complex problem domain. The interrelated suites of research questions that compose a problem agenda exhibit organizational architecture (Brigandt and Love 2012). Two dimensions of this structure are epistemic heterogeneity and nested hierarchies. Heterogeneity is represented by different kinds of research questions, including both empirical (e.g., At what phylogenetic juncture did neural crest cell migration emerge?) and theoretical (e.g., What general properties of the genotype-phenotype map facilitate evolvability?). Nested hierarchies can be found in the way questions contain subquestions (as part to whole, such as questions about how mesoderm originated being one part of understanding how germ layers originated), and in how one question depends on answers to other questions (in relations of dependency, such as how binding sites for transcription factors emerged evolutionarily to answer questions about the origin of novelty through altered regulation and heterotopy).

Consider the problem agenda of the origin of evolutionary novelties (Brigandt and Love 2012; see chapter ▶ "Developmental Innovation and Phenotypic Novelty"). The heterogeneity aspect of problem agenda structure can be illustrated by different types of questions. Empirical questions ("What regulatory genes or mechanical movements control segment formation?") are answered differently than theoretical questions ("How is epistasis represented in a mathematical model of the genotype-phenotype map?" – see chapter ▶ "Epistasis"); pattern questions ("What is the phylogenetic juncture for understanding the origins of the vertebrate head?") are answered differently from process questions ("Do changes in *cis*-regulatory binding sites yield different kinds of evolutionary origins compared to changes in protein-protein interaction, such as heterochrony versus heterotopy?"). Questions about the cellular level of organization differ from questions about the anatomical level of organization; questions about the origin of features early in ontogeny (e.g., mesodermal specification) differ from questions about the origin of features that occur later in ontogeny (e.g., ossification).

The hierarchy aspect of a problem agenda picks out systematic relations among component research questions. Questions that are more abstract ("How is variation generated?" or "How can complex traits overcome developmental constraints?") exist higher in the problem structure hierarchy than others ("How is gene regulatory network variation generated?" – see chapter ▶ "Modeling Evolution of Developmental Gene Regulatory Networks" – or "how can post-cranial skeletal traits overcome developmental constraints due to pleiotropy?" – see chapter ▶ "Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics"). Questions that are more general ("How do novelties originate in chordates?") can be seen as higher in the problem agenda hierarchy than others (how do novelties originate in mammals?). Questions at different levels of generality and abstraction have potentially important connections with one another. Answering a more general question about how novelties originate in animals might require answering a more specific question about how variation is generated: "evolutionary change in animal form cannot be explained except in terms of change in gene regulatory network architecture" (Davidson and Erwin 2006, 29).

Heterogeneity and hierarchy (among other dimensions) operate concurrently to yield rich structure that can both coordinate disciplinary approaches, including how they should be integrated, and contribute to their evaluation. The latter is especially important since there is not agreement about solutions to general questions, such as how novelties originate in animals: "epigenetic mechanisms, rather than genetic changes, are the major sources of morphological novelty in evolution" (Newman et al. 2006, 290). For example, the hierarchical structure of research questions points to distinct investigative approaches. More concrete questions involve distinct biological processes ("How is GRN variation generated?" vs. "How is epigenetic variation generated?"), which are the province of different fields of study (comparative developmental genetics vs. bioengineering) and must be weighed for their relative significance in achieving an adequate explanatory framework at the desired level of abstraction. More specific questions involve clade-level differences, which require the study of diverse taxa and the assiduous comparison of results.

The focus of one discipline or a specified configuration of several fields of study on some questions rather than others creates a fruitful division of labor and organizes different lines of investigation in terms of the kinds of questions they concentrate on. For example, because a morphological structure develops based on prior changes in lower-level traits (e.g., gene transcription and cell migration), an account explaining the generation of this structure must account for these mechanistic interactions. And, because the precise phylogenetic pattern preceding a novelty (character transformations at particular junctures) has to be settled prior to assessing which developmental mechanisms contributed to the evolutionary transition, the architecture of the problem agenda not only requires different approaches (paleontology, phylogeny, and developmental biology) but also points to how those contributions from different approaches are to be integrated, such as through answering particular kinds of questions. Thus, both heterogeneity and hierarchical structure in a problem agenda provide a scaffold upon which to insert the relevant disciplinary contributions.

Beyond the necessity of coordinating diverse epistemic components to address multiple related questions remains the evaluation or standards for assessing the contributions. These can be understood in terms of the agenda's associated criteria of explanatory adequacy, which also help to organize research because ongoing inquiry is directed at fulfilling these criteria. First, any adequate explanatory framework for the origin of new characters must be sufficiently abstract and general. The demand of abstraction requires that the necessary disciplinary contributions have been made, such as the developmental generation of variation being investigated using methods from quantitative genetics, developmental genetics, epigenetics, and phenotypic plasticity. A key part of the needed integration is an explicit articulation of the relations among the levels of organization and temporal scales these methods focus on; an understanding of complex evolutionary transitions requires integration among the genetic, developmental, and morphological levels in both developmental and evolutionary time. The demand of generality requires that diverse characters in different clades (i.e., taxonomic scope) are investigated using many methods and that appropriate proxies for extinct taxa are utilized in experimental research with full knowledge of their epistemic limitations. Successful explanatory proposals for particular novelties (e.g., tetrapod limbs) need to be evaluated with respect to their applicability to other characters (e.g., the vertebrate jaw).

A second criterion of adequacy is that any adequate explanatory framework for the origin of new characters must exhibit both intricacy and balance. Although this might seem counterintuitive based on the assumption that theoretical explanations should be governed by a principle of parsimony, the intricacy is about matching the heterogeneous questions in the problem agenda with corresponding answers. It goes hand in hand with the balance of an explanatory framework, which should handle empirical and theoretical questions, not neglect pattern questions for process questions, deal with lower levels of organization as well as higher levels, and address later moments in ontogeny in addition to earlier ones. The work of interdisciplinarity involves the continual evaluation of whether and how different contributions are suited to the multiple, diverse demands of the problem agenda. Convincing explanations require strategic combinations of histology, morphology, physiology, molecular genetics, population genetics, quantitative genetics, and phylogeny.

Importantly, what counts as sufficiently abstract and general or intricate and balanced for an explanatory account of the origin of evolutionary novelties is subject to scientific change through history (Love 2015). Dimensions of problem agendas, such as heterogeneity and hierarchy, take on different shapes and contours as new research uncovers novel empirical results or theoretical perspectives. Although there is clear continuity, such that researchers can recognize how past investigation is linked to the concerns of current inquiry, these changes can be substantial and include whether particular problem agendas are foregrounded over others. Sometimes changes are related to developments within disciplinary approaches themselves. The availability of new methods that emerged independently of specific concerns about research questions in evo-devo were crucial to the modern configurations we observe working collaboratively on its problem agendas. For example, molecular genetic tools that were forged in the context of model organisms to better

understand development (such as *in situ* hybridization to detect spatially localized gene expression), often for biomedical reasons, became central to interrogating the nature of homology. Theoretical advances in phylogenetic reconstruction occurred within systematics and facilitated the use of newly collected molecular data, which made it possible to more rigorously establish character polarity and thereby test hypotheses about the evolution of developmental features (see chapter ▶ "Evo-Devo and Phylogenetics"). Additionally, changes within one discipline can have an impact on others. For example, advances in the application of an engineering perspective to embryos grew out of adopting standards from molecular developmental genetics for establishing causes in developmental processes through the manipulation of experimental variables. This alignment of standards across disciplinary approaches then meant that explanations of the origin of novelties must integrate both genetic and physical causes in order to be adequate (Love et al. 2017), thereby transforming the shape of the interdisciplinary problem agenda.

Thus far we have concentrated on how complex natural phenomena prompt us to tailor our epistemology of scientific investigation to that reality. However, it is crucial to acknowledge, even if only in passing, that this directionality can be reversed. That is, particular features of scientific epistemology, such as methodological commitments or preferred explanatory approaches, guide how investigators in evo-devo study the evolution of development and the developmental basis of evolution. Most obviously, the criteria of explanatory adequacy reflect epistemic values of biological researchers, such as generality, which holds independently of the complexity of evolutionary developmental phenomena. Molecular genetic methods used in developmental biology to identify gene expression relevant to GRN dynamics fostered a particular conception of developmental phenomena and their evolutionary significance that tended to ignore the potential contribution of protein-protein interactions (Lynch and Wagner 2008). Similarly, innovations in the quantification of morphology have made it possible to document and comprehend shape change in developmental sequences and through evolutionary time (see chapter ▶ "Morphometrics in Evolutionary Developmental Biology"). These epistemological and methodological aspects change researchers' focus on features of developmental and evolutionary phenomena. More sociologically, different disciplinary approaches can have incentives for the kind of research that is encouraged, which can nurture some types of interdisciplinary collaboration and discourage others. In the 1980s, an intellectual environment interested in epigenetic dynamics fostered collaborations that included physical science considerations (e.g., Oster et al. 1988). In the decades after, the centrality of GRNs downgraded attention to epigenetic dynamics: "Developmental complexity is the direct output of the spatially specific expression of particular gene sets and it is at this level that we can address causality in development" (Davidson and Peter 2015, 2). Combined attention to genetics and physics has begun to resurface only recently (Love et al. 2017). The complex phenomena have always been there, but the epistemological inclination to explore them has waxed and waned over time.

The task laid out by the different problem agendas found within the evolution of development and the developmental basis of evolution is daunting, and rightly

so. However, several procedural lessons can be drawn about the needed explanatory integration across disciplines. The relevant types of disciplinary contribution can occur in a piecemeal fashion, directed at particular research questions (as opportunity permits), rather than being cast at a global level (the developmental basis of evolution). The concreteness and specificity of these "local" research endeavors facilitate a more transparent picture of what actual intellectual contributions are needed for an adequate explanation; different novelties at different levels of organization may require different explanatory ingredients in different combinations, and thus the intricacy and balance criterion can be secured through a patchwork synthesis of local explanatory integrations of distinct disciplinary approaches. Successful interdisciplinarity coordination involves different integrative relations across fields and for delimited tasks or times (Brigandt 2010). Progress in evo-devo need not be measured by a consensus set of theoretical relations across all relevant fields of study in evolutionary and developmental biology. A good example of this piecemeal progress is found in a classic evo-devo question: the fin-limb transition.

Interdisciplinarity and the Fin-Limb Transition

The transition from fins to limbs in the history of life is a long-standing evolutionary puzzle associated with the origin of tetrapods and the vertebrate invasion of land (Hall 2007; see chapter ▶ "Evo-Devo of the Fin-to-Limb Transition"). Attacking the thorny empirical and conceptual questions that compose this problem requires multiple disciplinary approaches, each with specialized concepts and methods: "the challenge is to continually synthesize knowledge gained from multiple perspectives into an ever more refined understanding" (Hall 2007, 151). The origin of the autopodium (hand/foot) – fins minus fin rays plus digits equal limbs – is informed by new fossil findings (matched with detailed morphological analysis) that shed light on the ancestral character state of the fin and facilitate more refined phylogenetic reconstruction (Cloutier et al. 2020), identification of shared genetic enhancers involved in the development of fin rays in fish and tetrapod digits (Gehrke et al. 2015; Nakamura et al. 2016), and functional morphological analyses of fish locomotion (Kawano and Blob 2013). Studies of the developmental variation and generation of digits are not confined to molecular genetics but include approaches that specifically address physical dynamics (Onimaru et al. 2016; Stewart et al. 2017). Different processes that involve mechanisms across levels of organization include chondrogenesis, osteogenesis, apoptosis, joint formation, postnatal growth, and regeneration.

There is compositional complexity in the form of fins and limbs, such as endochondral versus dermal bone (Nakamura et al. 2016), as well as organizational complexity in how this morphology is generated (endochronal bone arises from aggregations of mesenchymal cartilage cells later replaced by mineral bone; dermal bones mineralize directly from mesenchyme without a cartilaginous intermediary step). The skeletal arrangements in fins and limbs have distinctive patterns of size

and shape that are observable in different fossil and extant taxa. The compositional complexity extends to other tissues (e.g., blood vessels or connective tissues) and both cellular and molecular constituents (e.g., collagen or actinodin). On a developmental time scale, this complex composition arises from common sources (e.g., lateral plate mesoderm) but distinct developmental mechanisms (e.g., differential spatial and temporal patterns of Hox gene expression or alterations to epithelial morphogenesis) – another type of organizational complexity. On an evolutionary time scale, two distinct developmental changes occurred in the origin of the tetrapod limb: a loss of fin rays and the expansion of the endochondral region of bone (tetrapod limbs contain no dermal elements). Additionally, there are interactions between compositional and organizational complexity, such as the evolution of developmental mechanisms relevant to fins and limbs.

The fin-limb transition is situated within the problem agenda of the origin of novelties (Hall 2007, Chap. 4; see chapter ▶ "Developmental Innovation and Phenotypic Novelty"). We see this in more concrete illustrations of examples described in the previous section, such as the heterogeneity aspect of problem agenda structure found in different types of questions, whether empirical ("What changes in gene regulation are responsible for differential spatial and temporal patterns of Hox gene expression?") or theoretical ("How is the functional demand of terrestrial weight-bearing managed in limbs with different skeletal arrangements?"). Questions about the cellular level of organization differ from questions about the anatomical level of organization (see chapter ▶ "Proximate Versus Ultimate Causation and Evo-Devo"); questions about the source of cell populations earlier in ontogeny differ from questions about the morphogenetic trajectories of epithelial sheets later in ontogeny. The hierarchy aspect of problem agendas that refers to relations among component research questions can be detected in abstract questions ("How important are Turing-type reaction–diffusion models compared with GRN models for understanding digit origins?"), as well as more specific component parts ("How does the GRN of Bmp-Sox9-Wnt operate in fins and limbs?"). Questions that are more general ("How does endochondral bone expand in tetrapod limb evolution?") have counterparts that are less general ("how do mesopodial wrist elements emerge in the limbs of stem tetrapods?).

Although we could tease out these correspondences further, it should be clear in this case study how problem agenda structure helps to coordinate and integrate disciplinary contributions and their evaluation. A variety of investigative approaches from different fields of study are required and need integration within the structure of the problem agenda. The relative importance of differential gene expression and biomechanics needs to be synthesized, as do morphological, paleontological, and systematic analyses. Progress in answering the question derives both from new empirical contributions (e.g., new fossils) and an enhanced integration of contributions, such as an increased interweaving of developmental genetics and physical dynamics for digits (Stewart et al. 2017) or the different trajectories of common developmental precursors in fins and limbs (see chapter ▶ "Proximate Versus Ultimate Causation and Evo-Devo"). Together, these contributions and their integration

are on track to yield a sufficiently abstract and general explanatory account that is reciprocally informative for other evolutionary novelties (e.g., the origin of vertebrate jaws), while maintaining an appropriate intricacy and balance that address heterogeneous questions with adequate empirical detail (Love et al. 2017).

Conclusion

Despite different perspectives on what evo-devo is and different formulations of the meaning of interdisciplinarity, there is no question that interdisciplinarity is central to its identity and practices. The primary rationale for why evo-devo investigations exhibit such a diverse mixture of disciplinary contributors is the complexity of the evolutionary and developmental phenomena under scrutiny, which had been somewhat neglected in standard evolutionary biology. This holds for both the diverse spatial levels of organization in focus (e.g., molecular, cell, tissue, organ, anatomical trait) – compositional complexity – and the temporal scales on which causal interactions within and across these levels occur – organizational complexity (see chapter ▶ "Mechanisms in Evo-Devo"). Biologists represent this multifaceted complexity in nature as structured problem agendas that organize diverse research questions and help to coordinate disciplinary approaches (where a contribution is made) and their evaluation (the standards for assessing the contributions). At least two broad features provide the relevant structure for problem agendas: epistemic heterogeneity and nested hierarchies. Both the complexity of the evolutionary developmental phenomena and this representational architecture can be observed in reciprocal interactions through history in the case study of the fin-limb transition.

Although this chapter has concentrated on interdisciplinarity in evo-devo, with special attention to how its research questions are coordinated by problem agendas (e.g., the nature of evolvability), similar considerations could be applied to capture ways in which the interdisciplinarity of evo-devo engages with disciplinary approaches that are in some sense "external." Examples of these types of collaborative interactions can be seen with respect to the developmental basis of evolution where both population and quantitative genetic models in evolutionary theory are brought into contact with how developmental processes affect evolution (see chapters ▶ "Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics" and ▶ "Micro-Evo-Devo"). However, much of this work remains to be done and constitutes ongoing research for those working on evo-devo problem agendas and their connections to problem agendas predominant elsewhere in evolutionary biology.

The above analysis has several epistemological and sociological implications. Epistemologically, both the complexity of the phenomena and the structure of the problem agendas suggest that the enterprise of evo-devo is likely to yield fragmentary patchworks of explanatory frameworks in response to different broad domains of problems, such as the origin of novelties. This implication is reinforced by the subtle feedback effects between problem agendas, preferred methodologies, and social structure of biology. It means there should be caution in demanding a fully

integrated and unified theoretical framework in evolutionary biology that incorporates both the evolution of development and the developmental basis of evolution. Although these patchworks will display different degrees of integration as answers to various questions within a problem agenda are identified and interconnected with one another, it is unclear whether a single or small set of principles will be adequate to account for all of the relevant phenomena. This does not mean that parallels across spatial levels or temporal scales should not be sought; instead, it means expectations should be tempered for the resulting knowledge that derives from interdisciplinary inquiry in evo-devo. Rather than a single, unified theoretical perspective, multiple models will typically persist as a core element of the successful explanations offered by researchers (Love et al. 2017).

These epistemological implications also bear on the sociological structure we should expect in evo-devo. The coordination of disciplinary approaches and their evaluation is a difficult task, and one that is not conducive to the regular operation of scientific disciplines. Disciplinary structure in contemporary science serves a number of purposes that are reflected in professional societies, research journals, dedicated funding, textbooks, and specified job openings. These purposes require a narrowing of methods and approaches in order to sufficiently constrain or "discipline" these units of scientific organization. Standards of evaluation in morphometrics (see chapter ▶ "Morphometrics in Evolutionary Developmental Biology") differ from those in comparative developmental genetics. Maintaining loci where the full range of interdisciplinarity is on display is quite difficult, whether in professional meetings or peer-reviewed journals. Wherever more overt sociological disciplinary structures have emerged within evo-devo (e.g., the *Pan-American Society for Evolutionary Developmental Biology*, or specific journals such as *Evolution & Development*), there has been a constriction (intentional or not) of the kinds of research appearing in these outlets. Work of the kind needed to adequately answer the research questions of problem agendas related to the evolution of development and developmental basis of evolution occurs in other societal contexts and variegated publishing outlets. The same holds for funding different disciplinary facets relevant to evo-devo's problem agendas, which comes from sources other than "The Evolution of Developmental Mechanisms Program" at the National Science Foundation (for example).

These epistemological and sociological implications of the interdisciplinarity of evo-devo serve as reminders that the necessary integration among answers to research questions within problem agendas will not necessarily translate into unified theoretical frameworks or synthesized disciplinary architectures. There is no expected trajectory where every facet of scientific reasoning in evo-devo dovetails in the long run, especially because we can be confident that the problem agendas will alter and shift in their criteria of adequacy, take on new contours in their dimensions, and reconfigure in a variety of ways as disciplinary approaches grow, develop, fragment, and synthesize, sometimes in response to institutional pressures orthogonal to the research questions of evo-devo. However, this is not a reason for pessimism or despair. In fact, it can be taken as the opposite. The last several decades demonstrate that the interdisciplinarity of evo-devo has yielded increasingly integrated explanatory frameworks for complex phenomena represented by structured problem agendas despite the existence of centrifugal forces on the organization of its disciplinary contributors and continued fragmentation of its

epistemological outputs. And it is progress in our understanding of both the evolution of development and the developmental basis of evolution that is the ultimate goal, regardless of how our knowledge is structured or the relevant social manifestations of the science are organized.

Cross-References

- ▶ Complexity in Evo-Devo
- ▶ Computational Modeling at the Cell and Tissue Level in Evo-Devo
- ▶ Developmental Evolutionary Biology (Devo-Evo)
- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Epistasis
- ▶ Evo-Devo and Phylogenetics
- ▶ Evo-Devo of the Fin-to-Limb Transition
- ▶ Evolution of Complexity
- ▶ Explanation in Evo-Devo
- ▶ Form and Function in Evo-Devo
- ▶ Generalization in Evo-Devo
- ▶ Levels of Organization in Evo-Devo
- ▶ Mechanisms in Evo-Devo
- ▶ Mechanisms of Pattern Formation, Morphogenesis, and Evolution
- ▶ Methods and Practices in Paleo-Evo-Devo
- ▶ Micro-Evo-Devo
- ▶ Modeling and Simulation in Evo-Devo
- ▶ Modeling Evolution of Developmental Gene Regulatory Networks
- ▶ Morphometrics in Evolutionary Developmental Biology
- ▶ Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics
- ▶ Proximate Versus Ultimate Causation and Evo-Devo
- ▶ Variational Approaches to Evolvability: Short- and Long-Term Perspectives

Acknowledgments I am grateful to the Centre for Advanced Study at the Norwegian Academy of Science and Letters for an excellent environment to think and write during the 2019–2020 project "Evolvability: A New and Unifying Concept in Evolutionary Biology?" led by Thomas Hansen and Christophe Pelabon. Ingo Brigandt and Laura Nuño de la Rosa provided helpful comments and criticisms on an earlier draft of this chapter.

References

Amundson R (2005) The changing role of the embryo in evolutionary thought: roots of evo-devo. Cambridge University Press, New York

Brigandt I (2010) Beyond reduction and pluralism: toward an epistemology of explanatory integration in biology. Erkenntnis 73:295–311

Brigandt I, Love AC (2012) Conceptualizing evolutionary novelty: moving beyond definitional debates. J Exp Zool (Mol Dev Evol) 318B:417–427

Calcott B (2009) Lineage explanations: explaining how biological mechanisms change. Br J Philos Sci 60:51–78

Carroll SB (2008) Evo-devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. Cell 134:25–36
Cloutier R, Clement AM, Lee MSY, Noël R, Béchard I, Roy V, Long JA (2020) *Elpistostege* and the origin of the vertebrate hand. Nature 579:549–554
Collins JP (2002) May you live in interesting times: using multidisciplinary and interdisciplinary programs to cope with change in the life sciences. Bioscience 52:75–83
Davidson EH, Erwin DH (2006) Gene regulatory networks and the evolution of animal body plans. Science 311:796–800
Davidson EH, Peter IS (2015) Genomic control process: development and evolution. Academic Press, San Diego
De Robertis EM (2008) Evo-devo: variations on ancestral themes. Cell 132:185–195
Feder ME, Mitchell-Olds T (2003) Evolutionary and ecological functional genomics. Nat Rev Genet 4:649–655
Gehrke AR, Schneider I, de la Calle-Mustienes E, Tena JJ, Gomez-Marin C, Chandran M, Nakamura T, Braasch I, Postlethwait JH, Gómez-Skarmeta JL, Shubin NH (2015) Deep conservation of wrist and digit enhancers in fish. Proc Natl Acad Sci 112:803–808
Hall BK (1999) Evolutionary developmental biology. Kluwer Academic Publishers, Dordrecht
Hall BK (ed) (2007) Fins into limbs: evolution, development, and transformation. University of Chicago Press, Chicago/London
Hansson B (1999) Interdisciplinarity: for what purpose? Policy Sci 32:339–343
Kawano SM, Blob RW (2013) Propulsive forces of mudskipper fins and salamander limbs during terrestrial locomotion: implications for the invasion of land. Integr Comp Biol 53:283–294
Kirschner MW, Gerhart JC (2005) The plausibility of life: resolving Darwin's dilemma. Yale University Press, New Haven/London
Kopp A (2011) *Drosophila* sex combs as a model of evolutionary innovations. Evol Dev 13:504–522
Love AC (2008) Explaining evolutionary innovation and novelty: criteria of explanatory adequacy and epistemological prerequisites. Philos Sci 75:874–886
Love AC (ed) (2015) Conceptual change in biology: scientific and philosophical perspectives on evolution and development. Springer, Berlin
Love AC (2017) Evo-devo and the structure(s) of evolutionary theory: a different kind of challenge. In: Huneman P, Walsh D (eds) Challenging the modern synthesis. Oxford University Press, New York, pp 159–187
Love AC, Stewart TA, Wagner GP, Newman SA (2017) Perspectives on integrating genetic and physical explanations of evolution and development. Integr Comp Biol 57:1258–1268
Lynch VJ, Wagner GP (2008) Resurrecting the role of transcription factor change in developmental evolution. Evolution 62:2131–2154
Moczek AP, Sears KE, Stollewerk A, Wittkopp PJ, Diggle P, Dworkin I, Ledon-Rettig C, Mattus DQ, Roth S, Abouheif E, Brown FD, Chiu C-H, Cohen CS, De Tomaso AW, Gilbert SF, Hall BK, Love AC, Lyons DC, Sanger T, Smith J, Specht C, Vallejo-Marin M, Extavour CG (2015) The significance and scope of evolutionary developmental biology: a vision for the 21st century. Evol Dev 17:198–219
Müller GB (2007) Evo-devo: extending the evolutionary synthesis. Nat Rev Genet 8:943–949
Nakamura T, Gehrke AR, Lemberg J, Szymaszek J, Shubin NH (2016) Digits and fin rays share common developmental histories. Nature 537:225–228
National Academy of Sciences, National Academy of Engineering, Institute of Medicine (2005) Facilitating interdisciplinary research. National Academies Press, Washington, DC
Newman SA, Forgacs G, Müller GB (2006) Before programs: the physical origination of multicellular forms. Int J Dev Biol 50:289–299
Onimaru K, Marcon L, Musy M, Tanaka M, Sharpe J (2016) The fin-to-limb transition as the re-organization of a Turing pattern. Nat Commun 7(1):11582
Oster GF, Shubin N, Murray JD, Alberch P (1988) Evolution and morphogenetic rules: the shape of the vertebrate limb in ontogeny and phylogeny. Evolution 42:862–884

Panganiban G, Irvine SM, Lowe CJ, Roehl H, Corley LS, Sherbon B, Grenier JK, Fallon JF, Kimble J, Walker M, Wray GA, Swalla BJ, Martindale MQ, Carroll SB (1997) The origin and evolution of animal appendages. Proc Natl Acad Sci 94:5162–5166
Raff RA (2000) Evo-devo: the evolution of a new discipline. Nat Rev Genet 1:74–79
Raff RA (2007) Written in stone: fossils, genes, and evo-devo. Nat Rev Genet 8:911–920
Repko AF (2008) Interdisciplinary research: process and theory. Sage, Thousand Oaks
Shubin NH, Tabin C, Carroll SB (2009) Deep homology and the origins of evolutionary novelty. Nature 457:818–823
Stewart TA, Bhat R, Newman SA (2017) The evolutionary origin of digit patterning. EvoDevo 8(1):21
Wagner GP, Chiu C-H, Laubichler M (2000) Developmental evolution as a mechanistic science: the inference from developmental mechanisms to evolutionary processes. Am Zool 40:819–831
Wimsatt WC (1997) Aggregativity: reductive heuristics for finding emergence. Philos Sci 64: S372–S384

Proximate Versus Ultimate Causation and Evo-Devo

Rachael L. Brown

Contents

Abstract

Made famous by Ernst Mayr (1961), the distinction between proximate and ultimate causation in biological explanation is widely seen as a key tenet of evolutionary theory and a central organizing principle for evolutionary research. The study of immediate, individual-level mechanistic causes of development or physiology ("proximate causation") is distinguished from the study of historical, population-level statistical causes in evolutionary biology ("ultimate causation"). Since evolutionary developmental biology (evo-devo) is a field that explicitly uses so-called "proximate" sciences such as developmental biology, morphology, and embryology in the study of evolution, it challenges the standard construal of the proximate-ultimate distinction and its associated account of causation. The exact nature of the challenge and its ramifications for the viability of the distinction more broadly are contested, but these conceptual questions are central to the status and significance of evo-devo in contemporary evolutionary biology.

Keywords

Proximate causation · Ultimate causation · Mayr

R. L. Brown (✉)
School of Philosophy, Research School of Social Sciences, The Australian National University, Canberra, ACT, Australia
e-mail: rachael.brown@anu.edu.au

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_97

Introduction

The distinction between "proximate" causes and "ultimate" causes and corresponding differences in biological explanation are commonly invoked when delineating different domains of inquiry within the life sciences. According to this prevalent approach, made famous by Ernst Mayr (1961), explaining any particular trait or its features within an organism involves invoking one of two types of cause – proximate or ultimate. Roughly, proximate causes are individual-level, mechanistic phenomena, while ultimate causes are population-level, evolutionary phenomena. Mayr uses the annual southward migration of warblers to illustrate his point. One possible explanation for the migration of warblers from North America south for the winter references its "proximate" causes. These include, for example, the interaction between the physiology of particular warblers and changes in the environment in the northern hemisphere during autumn that trigger their migration behavior, such as reducing temperature and light levels. Alternatively, the migration could be accounted for by reference to its "ultimate" causes. These include the historical lack of food in the northern hemisphere and its selective impact upon the genetic makeup of ancestral warbler populations over time. These two types of causes, in turn, are said to delineate independent research domains within biology – proximate causation is the focus of fields such as developmental biology, physiology, and anatomy, whereas ultimate causation is the focus of evolutionary biology, especially areas such as population genetics and behavioral ecology.

The proximate-ultimate dichotomy has played a key role in shaping the landscape of biology and evolutionary biology in particular, for the past half century, which is observable in university infrastructure where ecology and evolutionary biology are often separate administrative units from genetics, cell biology, and development.

Evo-devo is a research field that takes seriously the potential for developmental processes within individuals (apparent proximate causes) to play an important role in evolution at the population level (the domain of ultimate causes). This challenges the premise that proximate and ultimate causes are distinct and that inquiry into them is independent from each other. Therefore, understanding the motivation behind evo-devo as a field involves understanding why one might step outside the research framework encapsulated by this distinction. This chapter considers the challenge that evo-devo presents to this traditional conception of causation and its associated division of cognitive labor within biology. Alternative conceptions of causation and explanation found in evo-devo research help to situate its agenda within evolutionary biology, clarify its motivations, and account for the organization of inquiry in this field. To appreciate the specific differences of these alternatives, a more detailed overview of Mayr's distinction, its origins, and roles in contemporary evolutionary biology is needed.

The Dichotomy: Proximate and Ultimate Causation Explained

The historical context in which the idea that there are two, complementary, ways of accounting for the features of organisms – proximate causes and ultimate causes – came to prominence is perhaps as important as the details of the distinction itself for

understanding its relationship to evo-devo. The idea is most commonly associated with Mayr's 1961 *Science* article "Cause and Effect in Biology" although his thinking on the matter goes right back to his PhD thesis and other work on bird migration in the late 1920s (Beatty 1994). The 1961 article came at a time when evolutionary biology was under threat; in the late 1950s and 1960s, following Watson and Crick's discovery of DNA, the central place of evolutionary biology was under threat from the boom in molecular biology and genetics. It is only through this historical lens that Mayr's intent in the 1961 article is really clear; it is an attempt to stamp out a clear domain for evolutionary biology (i.e., the study of ultimate causes).

According to Mayr (1961), *proximate causes* "govern the responses of an individual (and his organs) to immediate factors of the environment" (p. 1503). These causes are mechanistic in nature and account for *how* the individual organisms in question produce the trait of interest or manifest a particular feature. For Mayr, proximate causes offer answers to "how" questions, rather than "why" questions. For example, biologists interested in the single-toed hooves seen in zebras, horses, and other equines might ask questions such as: "How do the limbs and hooves of equines develop?" "How do the hooves work in locomotion?" "Why are the hooves single-toed?" Within the traditional framework of the proximate-ultimate distinction, the first two of these questions require hypotheses that invoke proximate causes, such as the biomechanics of how equine limbs and hooves work or the processes by which adult limbs and hooves arise during maturation. In the framework of the proximate-ultimate distinction, causes of this type (and the questions they are invoked to answer) are understood to be the proper focus of research in fields such as developmental biology, physiology, and anatomy.

In contrast, a concern with the latter question ("why are equine hooves single-toed?") would be a different endeavor – the study of *ultimate causes*. Ultimate causes "are responsible for the evolution of a particular DNA code of information with which every individual of every species is endowed" (Mayr 1961, p. 1503). They are population-level phenomena pertaining to evolution and history and account for *why* the species in question has evolved the trait of interest or exhibits a particular feature. Natural selection is the predominant ultimate cause, but there are other relevant candidates (e.g., drift or migration). For the equine single-toed hoof, an ultimate explanation might appeal to how ancestors of modern-day equines who had smaller second and fourth toes survived and reproduced at greater rates than those that did not (the last common ancestor of all equids lived approximately 4–4.5mya (Orlando et al. 2013)). This fitness difference resulted in diminishment and eventually a loss of function in these digits over evolutionary time.

It naturally follows from the proximate-ultimate distinction that the evolutionary sciences aim to discover and formulate explanations for phenomena in terms of ultimate causes. Although Mayr makes clear that reference to both types of causes is required for a full explanation of any given trait (i.e., the two causal domains are complementary), they are considered to be largely causally autonomous. Although knowledge of one causal domain can inform the other, the causal processes involved in each are distinct and require separate domains of inquiry; functional biologists (e.g., developmental biology or physiology) study proximate causes, and

evolutionary biologists (e.g., population geneticists and behavioral ecologists) study ultimate causes.

More than 50 years after its initial articulation, Mayr's distinction has become prevalent within biology and evolutionary biology especially. This makes it difficult to discern that, rather than reflecting a deep causal chasm between the research domains of evolutionary biology and "proximate" sciences, the distinction reflects an underlying empirical and epistemic claim regarding the explanatory value of different kinds of explanations. Despite the appearance of some minimal causal relevance, it is largely assumed by many biologists that the mechanisms of trait development and physiology play no interesting *explanatory* role in the study of evolution and that the population-level dynamics of selection and drift play no *explanatory* role in the study of development and physiology. One can offer adequate answers to "how" questions without referring to ultimate causes and adequate answers to "why" questions without referring to proximate causes. However implausible this might seem, it is not difficult to see that the distinction can serve as a reasonable and useful idealization, such as to ignore or "black box" developmental mechanisms while undertaking evolutionary biological investigation. The assumption regarding the causal autonomy of proximate and ultimate domains is commonly invoked in evolutionary sciences but is perhaps best reflected within population genetics where little to no attention is paid to the specific mechanisms of variation and heredity within species. Within many genetic and molecular models of evolution, development is idealized away entirely, and the genotype-phenotype relationship is characterized purely mathematically with no reference to mechanism (Laubichler 2010).

Motivating the Black-Boxing of Development

Mayr's dichotomy reflects a mid-twentieth-century consensus within evolutionary biology, sometimes referred to as the Modern Synthesis, regarding the fundamental mechanics of evolution and the explanatory adequacy of population genetics. These ideas are still influential within evolutionary biology today and go a long way to explaining the continued appeal of the proximate-ultimate distinction. However, the exact nature of these empirical and theoretical commitments is complex, and there is not a single perspective that unites all evolutionary biologists apart from a basic commitment to the reality of evolution by natural selection as a phenomenon. Yet there are some key points upon which evolutionary biologists *generally* do agree and help illuminate how evo-devo and standard evolutionary theory converge and diverge.

First, both the Modern Synthesis and standard evolutionary theory are typically adaptationist in nature, although the contours of that commitment have changed over time (see chapter ▶ "Form and Function in Evo-Devo"). Historically, the adaptationism of evolutionary biology could be best summarized as the view that "natural selection is the predominant force in evolution." Sustained discussion in the wake of Gould and Lewontin's famous *Spandrels* critique (1979), coupled with theoretical and empirical advances, such as neutral theory (Kimura 1983), has led to

a far more modest "explanatory" adaptationism in current evolutionary biology. Much contemporary evolutionary research is best characterized as motivated by the view that adaptation is the central evolutionary question and that evolution by natural selection is the primary, though not the only, explanation (Godfrey-Smith 2001; Orzack and Sober 1994).

Second, standard evolutionary theory primarily presents a gene-centric picture of evolution. It assumes that the biological structures that enable natural selection to operate are predominantly genetic and that mapping gene frequencies in populations over time is sufficient to capture the evolutionary process (Dobzhansky 1957, 1971; Mayr 1954; Mayr and Provine 1981). Although advances such as neutral theory have undermined the extent to which gene frequency change over time reflects selection alone, agreement remains among many evolutionary biologists that the evolutionary "action" occurs at a genetic level.

The appeal of this picture of natural selection and the explanatory adequacy of population genetics to many evolutionary biologists derive from two empirically motivated idealizations or simplifying assumptions about the relationship between genotypes and phenotypes. First, many evolutionary models assume that there is a smooth mapping from genotype to phenotype (i.e., small genetic changes map onto small phenotypic changes). Without such a strong correspondence, changes in phenotypes over time would not be accounted for solely by changes in genotypes, and therefore the explanatory adequacy of the gene-centric picture would be limited. Although modern evolutionary biologists are aware that the mapping is not always simple in nature due to factors such as neutrality, pleiotropy, and linkage effects (see chapter ▶ "Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics"), most maintain that macroevolution is merely the summation of microevolution over time. Mutation, selection, migration, and drift are sufficient to explain why populations exhibit specific traits with particular features across the tree of life.

A second common simplifying assumption is that the supply of phenotypic variation available to selection is isotropic over evolutionary timescales (i.e., the products of the developmental processes generating variation are roughly equiprobable in their occurrence). On this view, the mechanisms that produce phenotypic variation are not constrained or sensitive to the adaptive value of the variants that they generate. Again, although there is widespread acceptance that some phenotypic variants are more or less likely in the short term due to linkage effects and other molecular phenomena, the consensus is that these shorter-term effects will be washed out over evolutionary time. The isotropism assumption entails that the final outcomes of selective processes in populations are relatively causally autonomous from the processes responsible for phenotypic variation and development in organisms. The effectiveness of natural selection is dependent upon the presence of phenotypic variation within populations, but if that variation is assumed to be blind with respect to adaptation and more broadly unconstrained, then population-level processes account for the outcomes of the evolutionary process. This warrants black-boxing individual-level processes, such as developmental plasticity, which are believed to be of little to no explanatory importance. Despite the challenges this assumption faces due to recent empirical findings in

the study of phenotypic plasticity and developmental constraint (West-Eberhard 2003), those working within the framework of standard evolutionary theory assume that these processes only play an important explanatory role in a small number of cases. Even when they are identified as playing a role, it is common to argue that their contribution nonetheless results from changes in gene frequency change over time (Wray et al. 2014; Welch 2017).

Both of these simplifying idealizations, and the associated broader assumptions about the process of evolution, are best understood as principled empirical "bets." No evolutionary biologist believes them to be exceptionless generalizations – it is implausible that there are law-like generalizations in biology (Beatty 1995). Rather, they are claims about what is taken to hold in the majority of circumstances. Given this, it is unsurprising that the proximate-ultimate distinction and the entailed understanding of the causal autonomy of evolution from development are generally accepted among biologists. Importantly, the rejection of these principled empirical "bets" motivates evo-devo and therefore involves a direct challenge of the dichotomy between proximate and ultimate causation (Laland et al. 2011).

Evo-Devo: A Different Perspective

Evo-devo is the current iteration of a much older historical movement that challenged the Modern Synthesis with its emphasis on the autonomy of the mechanisms of inheritance and variation from evolution (Laubichler 2010; Laubichler and Maienschein 2003; Love 2015a, b). This movement is motivated by a variety of empirical phenomena including phenotypic novelty, the persistence of homology, and genetic assimilation (see chapters ▶ "Developmental Homology," ▶ "Developmental Innovation and Phenotypic Novelty," and ▶ "Developmental Plasticity and Evolution"). Those within evo-devo argue that these phenomena fail to fit within the picture of evolution presented by the Modern Synthesis, and more specifically, they contradict the empirical simplifying assumptions regarding the genotype-phenotype map and the nature of the supply of phenotypic variation to selection.

Consider homologies – traits that are shared between species in virtue of sharing a common ancestor. According to standard evolutionary theory, these are simply "ancestral characters that happened by circumstance to survive" (Williams 1992, p. 99). They are the reflection of a shared genetic inheritance through common ancestry and eventually will be "washed out" of the genotype through the steady appearance of random mutations and selection (Amundson 2005). This view is, however, contradicted by evidence that many homologies are the product of more than just residual genetic inheritance. There are conserved, shared developmental mechanisms that are both causally responsible for homologies and appear to facilitate their persistence over evolutionary time by protecting the phenotype from environmental and genetic perturbation (Wagner 2014; see chapters ▶ "Developmental Homology" and ▶ "Typology and Natural Kinds in Evo-Devo"). This understanding of the mechanisms of homology challenges the notion that homologous traits would be "washed out" by mutation and selection; it also undermines the

isotropism assumptions about variation in standard evolutionary theory. Although the supply of mutations within populations may be isotropic, the supply of phenotypic variation to selection is not. The developmental mechanisms underwriting many homologues serve to make particular phenotypic possibilities more likely than others (see chapter ▶ "Dispositional Properties in Evo-Devo"). Moreover, homologies are ubiquitous across the tree of life and not exceptional cases. Evolutionary developmental biologists take this type of evidence to undermine the justification for a principled empirical bet in favor of an isotropic supply of phenotypic variation to selection and motivate the study of the relationship between the developmental mechanisms underwriting homologies and evolution more generally.

Phenomena like this have led evo-devo biologists to emphasize, rather than downplay, the complexity of the relationship between genes and phenotypes and reject the proximate-ultimate distinction. Rather than adopting the empirical bets of standard evolutionary theory, evo-devo works from a different perspective, one that focuses on the construction of the phenotype and the ways in which the developmental processes responsible for the phenotype can influence evolution.

Given this alternative perspective, it is unsurprising that evo-devo proponents often directly challenge the proximate-ultimate distinction. Although there is some controversy regarding the exact nature of the challenge (Minelli 2010; Laubichler 2010; Laland et al. 2011, 2014; Wray et al. 2014; Welch 2017), there are two important commitments in evo-devo as a research program that contradicts Mayr's account of biological causation.

The most obvious and salient of these commitments is the important role that evo-devo biologists see development playing in evolution (Laland et al. 2011, 2014). According to the traditional proximate-ultimate distinction, the mechanistic, individual-level causal processes of development are assumed to be relatively independent of the population-level processes of evolution by natural selection. Evo-devo biologists clearly reject this assumption. Developmental processes are central to understanding a number of important evolutionary "why" questions, such as the origin of phenotypic novelties (innovation), trait identity in the tree of life (homology), and the distribution of phenotypes across that tree (disparity and evolvability). Returning to the single-toed equine hoof, an adequate explanation of why horses have the hooves they do – from an evo-devo perspective – involves reference not just to selection but also to the developmental mechanisms that made the diminishment of the second and fourth digits possible (Alberch and Gale 1985). It requires understanding how the supply of phenotypic variation required for the evolution of the single-toed hoof arose. Appealing to the simple accumulation of mutation is inadequate. In this sense, evo-devo biologists deny the strong causal autonomy entailed by the proximate-ultimate distinction: answering evolutionary questions requires reference to development.

The second evo-devo commitment challenges the proximate-ultimate distinction more directly. Evo-devo biologists engage in a particular type of explanation that sits entirely outside of the dichotomous picture Mayr's distinction presents (Calcott 2013; Laubichler 2010). Evo-devo researchers emphasize the complexity that the construction of the organism presents to evolutionary processes. The question of how one evolves a

limb or another bodily trait cannot be answered by simple reference to mutation and selection (Kirschner and Gerhart 2005). Unsurprisingly, explanations in evo-devo often consist in offering a step-by-step account of how a trait evolved via small changes to the mechanisms underwriting it. In the case of the equine single-toed hoof, the stepwise progression of cumulative developmental changes required to get from a three-toed to single-toed hoof would comprise such an explanation. These so-called lineage explanations (Calcott 2009) fall outside of the "how" and "why" dichotomy of the proximate-ultimate distinction. They are neither mechanistic answers to "how" questions or historic population-level answers to "why" questions. They rather explain the evolution of a trait by reference to changes in the mechanisms that are responsible for it without reference to selection or populations.

The picture of causation and explanation that this leaves us with differs dramatically from that originally presented by Mayr. Mayr emphasized a dichotomy between individual-level causes, for which we give mechanistic explanations, and population-level causes, for which we give historical explanations. In contrast, evo-devo biologists demonstrate the relevance of individual-level causes and mechanistic explanations to evolutionary questions. Historical explanations, contra Mayr, can be both mechanistic and individual level in nature. Although further empirical work is required to cash out the empirical bets of evo-devo, new techniques and advances within developmental genetics and embryology now make it possible to more rigorously test this picture of evolution and thereby its associated account of evolutionary inquiry.

Cross-References

- ▶ Canalization: A Central but Controversial Concept in Evo-Devo
- ▶ Developmental Homology
- ▶ Developmental Plasticity and Evolution
- ▶ Dispositional Properties in Evo-Devo
- ▶ Evo-Devo's Contributions to the Extended Evolutionary Synthesis
- ▶ Explanation in Evo-Devo
- ▶ Form and Function in Evo-Devo
- ▶ Mechanisms in Evo-Devo
- ▶ Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics
- ▶ Typology and Natural Kinds in Evo-Devo

References

Alberch P, Gale EA (1985) A developmental analysis of an evolutionary trend: digital reduction in amphibians. Evolution 39:8–23

Amundson R (2005) The changing role of the embryo in evolutionary thought: the roots of evo-devo. Cambridge University Press, Cambridge

Beatty J (1994) The proximate/ultimate distinction in the multiple careers of Ernst Mayr. Biol Philos 9(3):333–356

Beatty J (1995) The evolutionary contingency thesis. In: Wolters G, Lennox JG (eds) Concepts theories, and rationality in the biological sciences. University of Pittsburgh Press, Pittsburgh
Calcott B (2009) Lineage explanations: explaining how biological mechanisms change. Br J Philos Sci 60(1):51–78
Calcott B (2013) Why how and why aren't enough: more problems with Mayr's proximate-ultimate distinction. Biol Philos 28(5):767–780
Dobzhansky TG (1957) On methods of evolutionary biology and anthropology. Am Sci 445:381–392
Dobzhansky TG (1971) Genetics of the evolutionary process. Columbia University Press, New York
Godfrey-Smith P (2001) Three kinds of adaptationism. In: Orzack SH, Sober E (eds) Adaptationism and optimality. Cambridge University Press, Cambridge, pp 335–357
Gould SJ, Lewontin RC (1979) The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. Proc R Soc Lond B 205:581–598
Kimura M (1983) The neutral theory of molecular evolution. Cambridge University Press, Cambridge
Kirschner MW, Gerhart JC (2005) The plausibility of life: resolving Darwin's dilemma. Yale University Press, New Haven/London
Laland KN, Sterelny K, Odling-Smee J, Hoppitt W, Uller T (2011) Cause and effect in biology revisited: is Mayr's proximate-ultimate dichotomy still useful? Science 334:1512–1516
Laland KN, Uller T, Feldman M, Sterelny K, Müller GB, Moczek A, Jablonka E, Odling-Smee J (2014) Does evolutionary biology need a rethink? Point: yes, urgently. Nature 514:161–164
Laubichler MD (2010) Evolutionary developmental biology offers a significant challenge to the neo-Darwinian paradigm. In: Ayala FJ, Arp R (eds) Contemporary debates in philosophy of biology. Wiley-Blackwell, Malden, pp 199–212
Laubichler M, Maienschein J (eds) (2003) From embryology to Evo-Devo: a history of developmental evolution. MIT Press, Cambridge, MA
Love AC (2015a) Conceptual change in evolutionary developmental biology. In: Love AC (ed) Conceptual change in biology: scientific and philosophical perspectives on evolution and development. Boston studies in philosophy of science. Springer, Berlin, pp 1–54
Love AC (2015b) Evolutionary developmental biology: philosophical issues. In: Heams T, Huneman P, Lecointre G, Silberstein M (eds) Handbook of evolutionary thinking in the sciences. Springer, Berlin, pp 265–283
Mayr E (1954) Change of genetic environment and evolution. Evol Process 157:180
Mayr E (1961) Cause and effect in biology. Science 134:1501–1506
Mayr E, Provine WB (1981) The evolutionary synthesis. Bull Ame Acad Arts Sci 17–32
Minelli A (2010) Evolutionary developmental biology does not offer a significant challenge to the neo-Darwinian paradigm. In: Ayala FJ, Arp R (eds) Contemporary debates in philosophy of biology. Wiley-Blackwell, Malden, pp 213–226
Orlando L et al (2013) Recalibrating *Equus* evolution using the genome sequence of an early middle Pleistocene horse. Nature 499:74–78
Orzack SH, Sober E (1994) Optimality models and the test of adaptationism. Am Nat 143(3):361–380
Wagner G (2014) Homology, genes, and evolutionary innovation. Princeton University Press, Princeton
Welch JJ (2017) What's wrong with evolutionary biology? Biol Philos 32:273–279
West-Eberhard MJ (2003) Developmental plasticity and evolution. Oxford University Press, New York
Williams GC (1992) Natural selection: domains, levels and challenges. Oxford University Press, Oxford
Wray GA, Futuyma DA, Lenski RE, MacKay TFC, Schluter D, Strassman JE, Hoekstra HE (2014) Does evolutionary biology need a rethink? Counterpoint: no, all is well. Nature 514:161–164

Complexity in Evo-Devo

Robert C. Richardson

Contents

Abstract

Complexity comes in many forms. All involve some kind of hierarchical organization. We can distinguish two distinct dimensions to what is called "complexity": one concerns complexity in composition, while the other emphasizes complexity in causal organization. The latter, in turn, has at least three aspects, which allows us to understand the variety among complex systems. These dimensions – and especially the aspects reflecting causal organization – inform how we understand the developmental evolution of complexity.

Keywords

Evolution of complexity · Hierarchical organization · Modularity

R. C. Richardson (✉)
Department of Philosophy, University of Cincinnati, Cincinnati, OH, USA
e-mail: robert.richardson@uc.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_170

Introduction

Many different things are described as complex. Galaxies are complex. Stars are complex. Economies are complex. People are complex. Minds are complex. Ecologies are complex. Ant societies are complex. Organisms are complex. Cells are complex. Sometimes processes are described as complex. Speciation is complex. Combustion is complex. Gene expression is complex. Development, of course, is complex. It is not clear that complexity means the same thing in all these cases. Complexity is complex. It takes many forms.

I will largely focus here on the many forms of complexity as they are manifested in the life sciences – including biology generally, but more specifically genetics, development, evolution, ecology, and related fields such as evo-devo. I will for the most part set aside complexity as it is encountered in physics or chemistry, or even economics, without adjudicating whether complexity as it is manifested in the life sciences is similar or different to complexity as it is encountered in other areas of science.

Hierarchical Organization and Complexity

Complexity in the most interesting cases is hierarchical. Perhaps all complex systems are hierarchical in the sense that they are composed of simpler entities, which should be thought of as composing them. Macroscopic and microscopic systems are composites, consisting of interacting constituents. Galaxies are complex systems, consisting of stars, stellar matter, interstellar dust and dark matter, bound together by gravitational forces. Economies are complex systems, consisting of consumers, producers, institutions, and bound together by trade and commerce. Cells are complex systems, composed of genes, proteins, nucleic acids, mitochondria, and more, all enclosed in a semi-permeable membrane. These things all are "many body" systems, consisting of large numbers of constituents and coupled together by a few kinds of relations.

These systems are organized hierarchically according to some ranking or principle, such as composition. As Herbert Simon explains, a hierarchical system is "a system that is composed of interrelated subsystems, each of the latter being, in turn, hierarchic in structure until we reach some lowest level of elementary subsystem" (Simon 1962, 196). The elementary, fundamental entities can often be ignored because it is the *organization* of these elements at different levels or scales that does the work. The hierarchy of organization is exhibited by these different levels or scales (see chapter ▶ "Levels of Organization in Evo-Devo").

Hierarchical systems, which are complex systems, come in a variety of forms. On the one hand, there is complexity we encounter when we take seemingly simple systems and examine their parts or compositional organization. An enzyme is a simple entity that consists of an array of amino acids arrayed in specific order and manifesting a specific three-dimensional structure. Think of this as vertical

complexity. On the other hand, there is the complexity we encounter as we examine the causal organization of these systems and the way they are embedded in and interact causally with other entities. A given enzyme within a cell interacts with an array of other enzymes and substrates forming a causal network. Think of this as the horizontal dimension to complexity.

I will focus on dynamic, evolving systems in which there are variable constraints on constituents of the system. These are hierarchical systems in which there is a significant change in the overall state of the system, and this change in state tends to promote some degree of "quasi-independence" for the system as a whole (Simon 1981, 12). A system consisting of relatively simple components with just a few possible states and simple relations can have a literally astronomical number of possible states. So, with just ten constituents, each of which can take just one of two states, there will be 1024 possible configurations and with 20 there will be over a billion. The number of independent variables describing the system can be used to describe a phase space in which the system evolves. The dynamics may be quite simple. Under conditions approaching equilibrium, a state may be attained or maintained independently of environmental variation; out of equilibrium, the state of the system changes in regular ways. Variation in overall state is a consequence of variable constraints on constituent subsystems; in this way, the variability is essential to understanding system functioning.

The basic contrast for dynamic hierarchies is *structural* hierarchies, in which change of state is incidental to systemic integrity, or inimical to it, and in which the constraints on constituent behavior are marginally variable. For example, a crystalline structure is hierarchical but not dynamic. By way of contrast, development is both complex and dynamic. During embryogenesis, a single pluripotent cell replicates and is specialized into the array of adult cell types. This can be envisioned as a complex tree, which reflects cell fates. In the zebrafish, partial trees have been developed using selected genes; more recently, RNA sequencing has allowed for the construction of more comprehensive trees describing cell fate (Sagar and Grün 2020). This involves thousands of cell lineages, differentiating over time.

Dimensions of Complexity

As noted, complexity can take a number of forms in hierarchically organized and dynamic systems. The first is vertical or compositional complexity, and the second is horizontal or causal organization complexity (see chapter ► "Mechanisms in Evo-Devo").

Compositional Complexity

Compositional or vertical complexity is the simplest to recognize. The systems we encounter have parts and, as the number of those parts increases in a system, we

naturally think of it as more complex. A hierarchical system has multiple subsystems, so one obvious difference is in the sheer number of constituent subsystems. More complex systems are ones that have more parts. *C. elegans* has 302 neurons. A fruit fly has roughly 250,000. Chimpanzees or baboons have around 10^{10}. There is something on the order of 100 billion neurons in a human brain. As the sheer numbers go up, we recognize greater complexity, at least within a common category. However, these relationships are not always easy to interpret. *C. elegans* has roughly 20,400 protein-coding genes and the fruit fly has around 15,500; humans have roughly 20,000–25,000. Thus, the number of parts in a category is a defeasible indicator of compositional complexity for a system.

Another way of thinking of complexity emphasizes not the number of parts, but diversity in the ways something may be constructed. Sometimes we are interested not in an exact description of a system but in the classes of systems that fit some general description. This is still vertical complexity, because it emphasizes composition, though it emphasizes not just the number of elements but the number of ways a system may be constituted. Cancer is a complex disease. Oncologists once hoped there would be a kind of magic bullet to cure cancer. If there is a single cause, it was thought, then there should be a single treatment. Chronic myeloid leukemia is associated with a unique chromosomal abnormality. That encouraged the search for a single treatment that could inhibit the spread of the cancer in all patients, with partial success. More common forms of cancer, however, come in a variety of forms and are enormously heterogeneous. Treatment, accordingly, is not simple. Some breast cancers respond to hormone treatments, while others do not. We now know there is a bewildering variety of genetic mutations associated with breast cancers. Comparisons of individuals yield very different sets of mutations even within a single type of tumor. And even in a single case, there are typically many genes involved. Likewise, there are pathways that characterize particular forms of cancer, but these too are heterogeneous. This kind of complexity is common, rooted in the variety of underlying causes.

Similar features of compositional complexity are easily recognizable in the context of the problem of homology in evo-devo (see chapter ▶ "Developmental Homology"). For example, among insects there is a similarity of body type that might be taken to suggest that the continuity is due to some common underlying cause. So understood, homologies would be reflected in similarity of underlying mechanisms. Multiple examples show this turns out not to be the case. In salamanders, if the lens is removed from the eye, then the lens can regenerate. In these cases, the lens is derived from cells in the iris, whereas in embryonic development lens is derived from cells in the epidermis. Likewise, in insects the characteristic body plan sometimes develops directly (as in grasshoppers) and sometimes indirectly (as in flies). The mechanisms of development are variable, even though the homology of the characters is not controversial. "We can safely assume that some level of variation in the developmental mechanisms of homologous characters is the rule rather than the exception" (Wagner 2014, 90).

Organizational Complexity

The number of parts and the number of kinds of parts both affect our judgments of the complexity of a system. However, complexity is also affected by the kind of *organization* exhibited by a system. This is challenging because there does not seem to be a single metric which would order things in terms of the degree of complexity. Instead, there appear to be a least three, independent aspects of organizational complexity. This arises from differences in the composition and organization of subsystems, which are themselves heterogeneous composites. Moreover, each of these aspects seems to be more or less independent of one another. The three aspects are: degree of intersubstitutability, degree of aggregativity, and degree of decomposability. Complexity can be thought of as increasing along any of these three dimensions; they jointly define a three-dimensional space with the most complex systems maximizing all three dimensions (Fig. 1).

Within dynamic systems, then, in addition to the number of constituents that compose a system, there can be variation in the number of *kinds* of components involved. This would mean that different components behave differently within the system and have different functional profiles. Some dramatic effects can be seen even with only a few different kinds of constituents, or even in the absence of much variability in the kinds of components. The spectacular "self-organizing" displays of schooling fish or flocks of birds in unconstrained spaces do not depend on variability among individuals. The organization is a consequence of local interactions of many individuals. This occurs in pattern formation during ontogeny, such as in lateral inhibition to generate evenly spaced tracts of feathers (Bailleul et al. 2019). With variability in the (kinds of) individuals, there is even greater complexity. Assuming that we hold the total number of constituents constant, increasing the number of

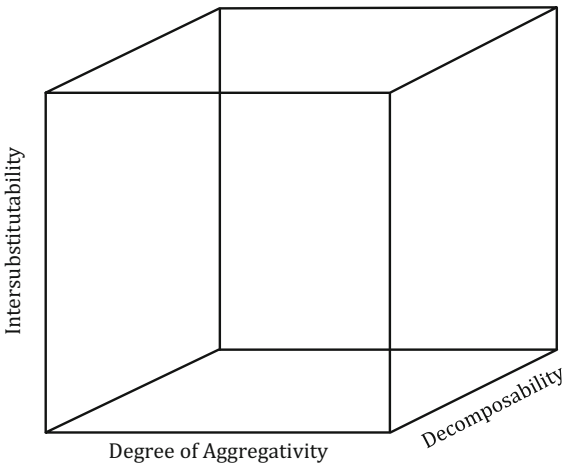


Fig. 1 A three-dimensional space representing systems differing with respect to their organizational complexity. The most tightly integrated systems minimize all three aspects, while purely aggregative systems maximize all three aspects

kinds of constituents also increases system complexity and affects its characteristic behaviors. Ant colonies, for example, are complex in part because there is variation among the various "castes" that define the colonies and the various contributions they make to the colony. Ecological systems likewise are complex assemblies of species occupying a discrete space. In ontogeny, as cells become more specialized, acquiring distinctive functions and morphologies, the resulting complexity provides serious challenges for constructing and understanding cell fates.

The significance of variation in kinds of constituents can be illustrated using Stuart Kauffman's elegant work on genetic regulatory networks (Kauffman 1993; see chapter ▶ "Modeling and Simulation in Evo-Devo"). These are networks with large numbers of "nodes," each of which is identified in terms of a Boolean function: a node will be active or inactive depending on both its inputs and intrinsically determined function. Kauffman examines networks with relatively large numbers of nodes ($10{,}000<N<250{,}000$) and also varies the number of connections (K) among nodes. Some of Kauffman's most interesting results emerge when K is relatively low (and so the number of Boolean functions is relatively few). This is the realm of what he describes as self-organization. Since with low K values there will be relatively few alternative functions attributable to the nodes, this means that the number of kinds of constituents will also be low even when the number of constituents is relatively high. The organization that results is, though, striking. Kauffman thinks of it as "order for free." The order he describes is a relative stability in patterns of activation, and it is "free" because it occurs without any master control.

More generally, a genetic regulatory network typically will consist of a large number of distinctive genes, embedded within a broader regulatory network – including RNA, mRNA, ribosomes, proteins, and more – that act to govern gene expression (both stimulation and inhibition). Complexity in these situations is not just a consequence of the large numbers of genes, but of the array of different specialized contributions these various factors offer (e.g., transcription factor binding versus protein-protein interactions). As the number of kinds of elements increases, the complexity of the system increases even if the total number of constituents is unchanged.

Intersubstitutability

Conversely, as the number of kinds of elements decreases, the parts will become increasingly intersubstitutable. The simplest dynamical systems are those in which the constituents of the system are all the same in kind. Given a decomposition of a system into constituent parts, the behavior of the system would be unaffected by substitutions of constituents provided the overall organization of the system is otherwise unaffected. These systems would exhibit a degree of equipotentiality to the degree that the substitution of parts leaves the systemic behavior relatively unaffected. In these circumstances, this will mean that there are relatively few kinds of constituents with little variation in their individual behaviors. (With n constituents and m kinds, the ratio of m to n may approximate the degree of intersubstitutability, though that will also depend on how disparate or qualitatively distinct the kinds are.)

As the number of constituents increases, or as the number of kinds of constituents increases, our ability to understand, model, or predict systemic function may be affected. Thus, degree of intersubstitutability is one aspect of organizational complexity. If the constituents are relatively simple in their behavior (think of perfectly elastic collisions of point masses in an ideal gas), systemic behavior may be statistically predictable even though it would be impossible to predict or describe the behavior of individual constituents, either individually or collectively. As the constituents become more complex or variable in their behavior, the behavior of the system as a whole may become more unpredictable. With a large number of constituents that interact frequently but differ in their intrinsic properties, systemic behavior may still be statistically predictable.

Aggregativity

There also may be variability in the degree of aggregativity within the system, or the amount of interaction among the constituents in determining system behavior. In a limiting case, we have strictly aggregative systems where systemic properties are statistical functions of constituent properties or in which the specific organization of the system is not a primary determinant of the relevant systemic properties. These are aggregative systems in a natural sense and include common bulk materials (e.g., granite) where changes in the mass of a particular instance do not alter systemic properties (e.g., hardness) or, if so, alter them linearly (e.g., weight). William Wimsatt (2007) captures this idea with four conditions, including invariance of system function under intersubstitutability of parts, size scaling, reaggregation of parts, and linearity (see also Levins 1970).

There may be relatively few cases in which systemic behavior is simply an aggregative effect of the constituent parts and their properties, and though simple growth might be nearly aggregative, developmental change certainly is not. An aggregative property would be one in which the organization of the constituents is not a primary determinant of the relevant systemic properties. Accordingly, systemic properties would generally change only quantitatively with changes in the numbers of parts. The flow of semi-viscous fluids across a surface or the movement of herds across a landscape might appear to be such cases. At the other extreme, there are systems in which systemic behavior is determined, in large part, by constituent organization. Systemic behavior then is organizationally dependent or, alternatively, the organization is part of the boundary conditions for constituent behavior. This is a second aspect of complexity. Insofar as systemic behavior depends on the specific organization of constituents, a system will be more complex.

Decomposability

Finally, systems may be more or less decomposable. If a system consists of a number of distinct parts with distinctive functions, then the system will be decomposable to the extent that the functioning of one or more parts is independent of the functioning of other parts. Sometimes this is thought of as analogous to impenetrability; the issue is then whether the actual behavior of one part is influenced by the behavior of other parts. Simon (1962, 210) provides two conditions for *near decomposability:*

1. The short-run behavior of each of the component subsystems is approximately independent of the short-run behavior of the other components.
2. In the long run, the behavior of any one of the components depends in only an aggregate way on the behavior of the other components.

Wimsatt treats this as a parameter that describes the relative strength of intra- and inter-systemic interactions. Equivalently, it may be an estimate of the likely error of predictions based on models that assume interactions among constituents are of minimal importance to systemic function. There are two different ways to understand these conditions. The first is essentially Simon's: given an analysis of a system into parts or subsystems, the system will be decomposable provided that the actual behavior of the subsystems does not depend on the behavior of other subsystems. Even a sequential organization would be likely to fail such a condition, since the inputs to subsystems are a function of other subsystems.

An alternative (somewhat less elegant) understanding of this condition would be: given an analysis of a system into parts or subsystems, a system is decomposable if the capacities of components do not depend on the capacities of other components. As an analogy, we can think of the system capacities described in a functional profile, such as by a machine table in a Turing machine. The actual output of the system will depend critically on the input, but the functional profile will not. Organizational properties do affect systemic behavior. If constituent behavior is relatively independent of the organizational properties, and therefore independent of the behavior of other constituents, then we can think of constituent behavior as intrinsically determined. Likewise, if constituent capacities are relatively independent of the organizational properties of the system, and therefore independent of the behavior of other constituents, we can think of constituent capacities as intrinsically determined. Decomposability, in either of these forms, is a matter of degree, so it would be better to say that insofar as constituent capacities or dispositions are independent of other constituents, then those are intrinsically determined. The internal state and the component behavior may depend on inputs from elsewhere in the system, but the capacities do not. More generally, given an analysis of a system into parts or subsystems, the system will be decomposable to the extent that component capacities are relatively independent of the capacities (and behavior) of other components in the system.

Within developmental biology and evo-devo, decomposability is often raised in terms of *modularity*. Raff (1996) relied on the case of the tetrapod limb to illustrate that developmental modules should be both largely under internal genetic control and dynamically independent of other entities with which it interacts. Modularity has been the subject of a good deal of discussion within evo-devo since (see Bolker 2000; Schlosser and Wagner 2004). It can be directly related to Simon's conditions for near decomposability because developmental modules, such as the tetrapod limb, will exhibit short-run behavior during ontogeny that is approximately independent of the short-run behavior of other component subsystems and depend primarily in an aggregate way on the behavior of the other components in the long run (i.e., subsequent to embryogenesis).

Complex Systems

If we assume these three aspects of organizational complexity (intersubstitutability, aggregativity, and decomposability) are independent of one another, then we can use them to construct a three-dimensional space for complex systems, locating complex systems in that space depending upon the relevant values for the aspects that describe the system (see Fig. 1). At one extreme, there may be complex systems that minimize all three aspects. These are highly interactive systems that may be treated efficiently as single units.

> This is a system in which the component subsystems have evolved together, and are not even obviously separable; in which it might be possible to decide which are the really relevant component subsystems. Thus, for example, we might consider that a simpler multicellular organism is composed of cells and yet the cells may be regarded, under other circumstances, as simply spatial subdivisions, partly isolated, of an organism (Levins 1970, 77).

These are fully integrated systems in which "component" functions are sufficiently interdependent such that the "components" are not independent of one another. Systemic behavior is dependent upon the network of constituents, but the behavior of the constituents is not insulated from that of other constituents in the system.

At the other extreme would be systems that maximize all three aspects. The component parts are independent of one another, not differentiated with respect to their functions, and determine systemic properties *en mass* (e.g., bulk materials, such as granite). These are purely aggregative systems because they are organizationally simple.

Clearly, there is room for systems that are intermediate between these extremes. An example may be illustrative. Some ant species "farm" aphids, which excrete a substance ("honeydew") that is rich in sugars and which the ants harvest. The aphids are taken in by ants and gain virtual immunity from predation and parasites. This relationship benefits both the ants and the aphids. The colony contains both ants and aphids, and each has their own contributions to the colony. Decomposability is relatively high, even though aphids depend on the ants for access to their food sources, and both intersubstitutability and aggregativity are relatively low.

The Evolution of Complexity

Although the evolution of complex systems is discussed more fully elsewhere in this volume (see chapter ▶ "Evolution of Complexity"), it may nonetheless be useful to see, at least in outline, how complexity considerations might be applied to some well-known cases. Organisms are integrated systems, but it is generally accepted that some exhibit a degree of modularity or near decomposability. As Wagner et al. (2007) explain, modularity is an "abstract" concept that captures the degree of functional interaction for an entity. For example, if the effects of mutation are

relatively circumscribed to some entities rather than others, that indicates a modular organization, whereas pleiotropic effects indicate more systemic integration (i.e., more functional interaction across parts). A modular organization would mean that developmental constraints would be reduced and, as a result, independent adaptive responses among modules would be more likely. That is, modularity would enhance "evolvability" (see chapter ▶ "Evolvability"). For example, beak size and shape are important for Darwin's finches. If beak length and depth can change independently, that would increase evolvability; if, on the other hand, depth and length are correlated (i.e., somehow interacting functionally), then that could reflect developmental constraints (Wagner et al. 2007, 923).

Conclusion

The variation in kinds of complex systems is substantial, whether the focus is on the number of constituents in a hierarchical system or the various organizational differences encountered. It is useful to recognize the variety because they are likely to yield to different kinds of scientific investigation. For the simplest organization, a complex system might be explored fully by overstimulating or deleting parts (cf. Bechtel and Richardson 1993). This is unlikely to be as successful with more tightly integrated systems. The fact that developing organisms exhibit a combination of different degrees of both compositional and organizational complexity means they will require different kinds of methods or disciplinary approaches to formulate an adequate explanatory account. Given that the evolution of developing organisms affects these degrees of complexity implies that interdisciplinarity will be essential to comprehending the complex phenomena under scrutiny in evo-devo (see chapter ▶ "Interdisciplinarity in Evo-Devo").

Cross-References

- ▶ Developmental Homology
- ▶ Evolution of Complexity
- ▶ Evolvability
- ▶ Interdisciplinarity in Evo-Devo
- ▶ Levels of Organization in Evo-Devo
- ▶ Mechanisms in Evo-Devo
- ▶ Modeling and Simulation in Evo-Devo

References

Bailleul R, Curantz C, Desmarquet-Trin Dinh C, Hidalgo M, Touboul J, Manceau M (2019) Symmetry breaking in the embryonic skin triggers directional and sequential plumage patterning. PLoS Biol 17(10):e3000448

Bechtel W, Richardson RC (1993) Discovering complexity. Princeton University Press, Princeton. (reprinted by MIT Press, 2010).
Bolker JA (2000) Modularity in development and why it matters to evo-devo. Am Zool 40:770–776
Kauffman S (1993) The origins of order: self-organization and selection in evolution. Oxford University Press, London
Levins R (1970) Complex systems. In: Waddington CH (ed) Towards a theoretical biology. Edinburgh University Press, Edinburgh, pp 73–88
Raff RA (1996) The shape of life. University of Chicago Press, Chicago
Sagar S, Grün D (2020) Deciphering cell fate decision by integrated single-cell sequencing analysis. Annu Rev Biomed Data Sci 3(1):1–22
Schlosser G, Wagner GP (eds) (2004) Modularity in development and evolution. University of Chicago Press, Chicago/London
Simon H (1962) The architecture of complexity. Proc Am Philos Soc 106:467–482
Simon H (1981) The sciences of the artificial, 2nd edn. MIT Press, Cambridge
Wagner GP (2014) Homology, genes and evolutionary innovation. Princeton University Press, Princeton
Wagner GP, Pavlicev M, Cheverud JM (2007) The road to modularity. Nat Rev Genet 8(12):921–931
Wimsatt WC (2007) Re-engineering philosophy for limited beings: piecewise approximations to reality. Harvard University Press, Cambridge

Levels of Organization in Evo-Devo

Markus I. Eronen

Contents

Abstract

Levels of organization is an ambiguous concept but typically refers to entities at a higher level being composed of entities at a lower level. Understood in this way, levels of organization have played an important role in various branches of biology, including evo-devo. However, sustained attempts at defining or characterizing levels precisely have been rare. The most significant approaches are Wimsatt's theory of levels of organization, Craver's account of levels of mechanisms, and recent skeptical or deflationary perspectives. Although many definitions of levels of organization face various difficulties, their flexibility and intuitive appeal suggest that the concept is likely to remain active in biological research, including in evo-devo.

Keywords

Levels · Levels of organization · Composition · Scale · Evo-devo

M. I. Eronen (✉)
Faculty of Philosophy, University of Groningen, Groningen, The Netherlands
e-mail: m.i.eronen@rug.nl

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_93

Introduction

The concept of levels of organization is deeply rooted in contemporary biology. It typically refers to entities at a higher "level" being composed of entities at a lower level. Standard levels of organization include the molecular level, cells, organisms, and groups. Levels of organization are usually taken to form nested, structural hierarchies: groups are composed of individual organisms, those individual organisms are in turn composed of cells, and cells are composed of molecules.

The idea that nature, or some part of it, is structured into levels of organization has played an important (albeit often implicit) role in biology at least since the early twentieth century. It continues to figure prominently in many areas of evolutionary biology, such as debates on levels of selection, evolutionary transitions, and homologies. However, in spite of the ubiquity and the intuitive appeal of this picture, attempts at precisely defining levels of organization have been few and far between. In recent years, this has started to change. On the one hand, new arguments against the very idea of levels of organization have been advanced (Potochnik and McGill 2012; Eronen 2013, 2015). On the other hand, constructive attempts have been made at defining levels in a local and more scientifically plausible way (Bechtel 2008; Craver 2007, 2015; Love 2012). The study of levels of organization is currently experiencing a revival both in biology and philosophy.

It should be noted that the terms "levels" and "hierarchy" have a wide range of different uses and meanings in biology. Even the more specific term "levels of organization" is ambiguous. For example, when things at higher levels (or wholes) impose control or constraints on things at lower levels (or parts), authors sometimes refer to these as "levels of organization." Different conceptions of levels come with different sets of issues and problems. Here, the focus will be on levels of organization characterized by compositional (part-whole) relationships, which also play a central role in evo-devo and its philosophy (Love 2006; Winther 2006).

The structure of this chapter is as follows. I begin with some examples of how levels of organization figure in biology generally and evo-devo specifically. Next, I review the most significant attempts at defining levels of organization: Wimsatt's comprehensive account of levels of organization, Craver's levels of mechanisms, and skeptical approaches to levels. I conclude with some general remarks on the role and significance of levels in biology and evo-devo.

Levels of Organization in Biological Science

Perhaps the most salient context where levels of organization regularly appear in biology is textbooks and other introductory texts. There it is common to depict nature or a part of it as forming several hierarchical levels of organization. For example, in the first chapter of a standard ecology textbook, one finds: "The living world can be viewed as a biological hierarchy that starts with subcellular particles, and continues up through cells, tissues and organs. Ecology deals with the next three levels: the individual *organism*, the *population* . . . and the *community*" (Begon et al.

2006, p. xi). When levels of organization are presented in this way, their main function seems to be twofold (Eronen and Brooks 2018). First, they illustrate the hierarchical structure of biological systems or nature as a whole, and second, they help in delineating the scope and aims of the discipline by pointing out its focal level(s). In these contexts, the idea of levels of organization is also used to express explanatory and methodological pluralism: to fully understand nature or some part of it, we need to investigate it at multiple levels of organization. In this vein, Hall (1999, p. xv) presents evo-devo as a hierarchical investigative approach that concerns multiple levels, ranging from the molecular to the population level.

Besides textbooks, levels of organization also play a role in biological theorizing. A prominent example is the multilevel selection debate, where many researchers aim to identify at which levels of the biological hierarchy natural selection is operating (Lloyd 2017). In Darwin's original theory, selection primarily occurred at the level of organism. However, as Lewontin (1970) noted, the principle of natural selection can be formulated in an entirely abstract way that allows for selection to take place at multiple levels. In the following decades, a heated debate ensued between authors defending a gene-centered view of evolution (e.g., Dawkins 1976), where natural selection is taken to operate (almost) exclusively at the level of genes, and those defending the view that selection also operates at higher levels.

Group selection is probably the most extensively debated candidate for higher-level selection. Some phenomena, most prominently altruistic behavior, are puzzling from the point of view of natural selection at the level of the organism but arguably make perfect sense when the level of the group is taken into account (Sober and Wilson 1998). For example, vervet monkeys make alarm calls that significantly reduce the chances of survival for the individual making the call, but these are beneficial for the group as a whole. On this basis, it seems plausible that groups with more altruists can be more likely to survive than groups with fewer or no altruists. Thus, natural selection would favor the more altruistic groups, and altruistic behavior would be maintained through evolution.

The idea of higher-level selection is often embedded into a broader framework of multilevel evolutionary theory. Since the 1980s, Niles Eldredge and colleagues have been developing a "hierarchy theory of evolution" that aims to explain the organization of nature and evolutionary processes in terms of levels and hierarchies (Eldredge et al. 2016; Vrba and Eldredge 1984). One of their core hypotheses is that a crucial explanatory factor for evolution is the interplay of two biological hierarchies (Vrba and Eldredge 1984): The *ecological hierarchy*, which is a hierarchy of compositional levels of organization roughly in the traditional sense, and the *genealogical hierarchy*, which is based on reproductive units related as parts to wholes (e.g., cells, organisms, demes, species). Interactions between entities at a level in the ecological hierarchy (e.g., the level of organisms) can result in corresponding changes in the genealogical hierarchy (e.g., differential replication of organisms); phenomena such as higher-level selection can be embedded into this framework.

Another closely related area of biology where levels of organization play a key role is the question of *evolutionary transitions*. In the context of the levels of selection debate, the organization of nature into levels is typically taken as given.

However, the emergence of this organization is something that evolutionary biology also needs to explain. How did (units at) higher levels of organization emerge from (units at) lower levels of organization (Okasha 2006; Maynard Smith and Szathmáry 1995)? For example, single-celled organisms evolved into multicellular organisms, and individual animals evolved into colonies, resulting in the complex hierarchical organization we witness today (see chapter ▶ "Evolution of Complexity"). In their highly influential book, Maynard Smith and Szathmáry (1995) proposed that the characteristic feature of major evolutionary transitions is that entities capable of replicating independently before a transition are only capable of replicating as parts of higher-level wholes after the transition. Thus, the levels of selection and evolutionary transitions are tightly intertwined: selection at higher levels seems to be an important factor in the evolution of higher-level units and thus in the emergence of new levels of organization (Maynard Smith and Szathmáry 1995; Okasha 2006). These debates have also close connections to evo-devo. For example, it has been argued that the emergence of new levels of organization crucially depends on the evolution of developmental mechanisms (Buss 1987; Hall 1999).

Levels of organization also play an important role in many other contexts in evo-devo (Love 2006). As an example, consider the debate on homology, which is a central concept in evolutionary biology generally and evo-devo specifically (Hall 2003; Wagner 2014; see chapter ▶ "Developmental Homology"). Roughly speaking, homology refers to similarity (or sameness) of characteristics due to shared evolutionary ancestry, one standard example being the forelimbs of humans and bats. The precise definition of homology has turned out to be elusive and a subject of much debate, but it is widely agreed that homologies can be identified at multiple levels, such as genes, developmental processes, or morphological features (Hall 2003). Importantly, characteristics can also be homologous at one level but not at others. For example, salamander digits and frog digits are arguably homologous at the morphological level, but not at the developmental level (Hall 2003). It is also important to note that the "developmental level" can in fact consist of multiple levels, as developmental processes can themselves take place at several different levels of organization (Love 2006). As in the other contexts discussed above, also in evo-devo the precise nature of levels has so far received little explicit attention.

Defining Levels of Organization

As the above examples illustrate, the idea of compositional hierarchies that form levels of organization is an ubiquitous background assumption in biology and also plays a direct role in biological theorizing. However, while this idea is intuitively appealing, it needs to be spelled out in more detail. Things can be decomposed into parts in many different ways, so what exactly is meant by "composition"? How do compositional hierarchies fit together with the idea of horizontal levels in nature? What are the properties and defining features of levels of organization? Surprisingly, attempts at precisely spelling out how levels of organization should be understood are few and far between. The most significant ones are Oppenheim and Putnam's

(1958) classic but outdated account of levels in the context of reduction and unity of science, Wimsatt's (1994/2007) theory of levels of organization, and Craver's (2007) account of levels of mechanisms. I will focus here on the latter two before turning to recent skeptical approaches to levels.

Wimsatt on Levels of Organization

More than 25 years ago, William Wimsatt (1994/2007) presented an analysis of levels of organization that is still unparalleled in its scope and audacity. Instead of giving a straightforward definition of levels, Wimsatt characterized the key features that levels of organization typically (but not necessarily) exhibit. First, levels are compositional and form nested hierarchical structures so that wholes at lower levels function as parts at higher levels. Second, levels of organization are a "deep, non-arbitrary, and extremely important feature of the ontological architecture of our natural world" (Wimsatt 1994/2007, p. 203). Third, they are "constituted by families of entities usually of comparable size and dynamical properties, which characteristically interact primarily with one another" (ibid., p. 204). Fourth, "[l]evels of organization can be thought of as local maxima of regularity and predictability in the phase space of alternative modes of organization of matter" (ibid., p. 209).

This last point requires some explanation. The idea is roughly that patterns and regularities, which can be used as a basis for prediction and explanation, are found clustered around certain spatial or temporal scales and that such clusters indicate levels of organization. As an example, with regard to size scale, levels of organization should appear as peaks or local maxima of regularity and predictability on that scale. For instance, if the "level" of cells is a level of organization as suggested by Wimsatt, then around the size scale of cells we should find more regularity and predictability than at scales somewhat smaller or larger.

Wimsatt goes on to discuss (inter alia) how processes at higher levels tend to happen at slower rates than processes at lower levels, that higher level properties can typically be realized by several different lower level properties (e.g., hardness can be due to bone or enamel), and higher-level causal relationships tend to be autonomous. By including so many possible characteristics of levels, Wimsatt's account is extremely versatile and wide in scope. However, at the same time, it is difficult to apply in practice. It contains a multitude of common but not necessary criteria that levels satisfy, which implies that almost any set of entities that are in some respect similar could be said to form a level. Moreover, the individual criteria raise many questions (Eronen and Brooks 2018). Most importantly, the core idea of "local maxima of regularity and predictability" remains vague. It is not clear how the amount of regularity or predictability could be measured or estimated, or even how these terms should be precisely understood (Craver 2007). It is also difficult to combine this idea of peaks or "local maxima" with Wimsatt's other criteria, such as the part-whole organization of levels (Eronen and Brooks 2018). In order to render the account useful for biological research, and in particular for understanding the kinds of evo-devo levels described in the previous section, it would need to be worked out in more detail.

Levels of Mechanisms

Carl Craver (2007) has proposed a different approach to levels that is far more local and minimalistic (see also Bechtel 2008 for a similar account). He argues that many of the central features associated with levels of organization in the life sciences can be captured with the notion of "levels of mechanisms" (see chapter ▶ "Mechanisms in Evo-Devo"). These are specific kinds of compositional levels: lower level *components* are organized together to form higher level *mechanisms*. In other words, there are mechanisms at higher levels and their components at lower levels. As the components can also be mechanisms themselves, levels of mechanisms form nested compositional hierarchies that are characteristic of levels of organization. For example, molecules and ions are components of receptor mechanisms, receptor mechanisms are components of neurons, and neurons are components of the hippocampal spatial map mechanism (Craver 2007).

As Craver (2007) highlights, levels of mechanisms seem to have many of the features that have been attributed to levels of organization. They are compositional by definition; entities at higher levels are typically larger than entities at lower levels; and, levels of mechanism can correspond to local peaks of regularity and predictability. However, these similarities are overshadowed by differences. Most importantly, levels of mechanisms are decidedly and extremely local (Craver 2007; Bechtel 2008). Levels can only be decided on in a case-by-case basis, and different mechanisms can have entirely different sets of levels. For example, levels in the mechanism of protein folding are very different from levels in the spatial memory mechanism in the human brain. Even within one mechanism, there is often no clear answer to whether things are at the same or different levels (Craver 2007; Eronen 2013).

It is beyond doubt that levels of mechanisms are important in explaining complex biological systems. However, they are quite different from the levels of organization that are involved in the biological debates described above. For example, levels in the context of evolutionary transitions seem to be very broad in scope, and not just restricted to one system or mechanism. Similarly, in the debate on homology in evo-devo, levels in different species are compared (e.g., the level of genes in the salamander and the level of genes in the frog), which is not possible with levels of mechanisms due to their local nature. In other words, levels of mechanisms do not seem to provide a sufficiently general framework for these debates.

Skeptical Approaches to Levels

The actual compositional organization in nature seems to be more complex than is assumed in the traditional picture of levels of organization found in textbook presentations. Consider a paradigmatic level of organization – organisms – that turns out to be quite heterogeneous and messy. For example, blue whales and bacteria should both be located at the level of organisms but are very different kinds of

entities with radically different properties (Eronen and Brooks 2018; Potochnik and McGill 2012). The complexity increases as we move to the next lower level. According to the traditional picture, the next level should be made up of the components of organisms (Potochnik and McGill 2012), but for blue whales this includes organs, tissues, and cells, whereas for bacteria it includes none of these. Instead, we find a cell membrane, nucleus, and microfilaments. There is no scientifically relevant sense in which these two sets of components would be "at the same level."

Moreover, even within one organism or mechanism, compositional organization does not result in neatly defined horizontal layers or slices through systems or organisms. Instead we find complex downward-branching hierarchies (Craver 2007; Love 2012). This becomes clear when we consider the components of unicellular organisms. The cell membrane is composed of lipid molecules and proteins, whereas the nucleus is composed of structures such as the nuclear membrane and nuclear matrix. Again, there is no clear sense in which all these things would be "at the same level." Indeed, according to Craver (2015), the idea of being "at the same level" is unimportant or even meaningless in the context of levels of mechanisms.

On the basis of these kinds of observations, it has been argued that we should not characterize the complexity and organization of nature in terms of levels, but rather use more well-defined and coherent concepts (Eronen 2013, 2015; Potochnik and McGill 2012; Thalos 2013). For example, Potochnik and McGill (2012) and Eronen (2013, 2015) have argued that *scale* functions better as an organizing concept for biological sciences (see also Green and Batterman 2017). There are many scales that are relevant for biology, perhaps the most important being size scale (i.e., how big things are: proteins are at the scale of nanometers; insects at the scale of millimeters; human organs at the scale of centimeters, and so on) and the temporal scale (i.e., the rate at which processes occur or interact: ontogenetic processes take place at a much faster rate than phylogenetic processes; see chapter ▶ "Heterochrony" on the importance of temporal scales for evo-devo).

Scales have the advantage of being entirely continuous and therefore, unlike levels, they do not require arranging things into discrete strata (Potochnik and McGill 2012; Green and Batterman 2017). The traditional idea of levels of organization combines vertical criteria (composition) with horizontal criteria (similarity in scale), implicitly assuming that they go together, but we have seen that this leads to problems when defining levels. If we consider scale and composition as distinct notions for analyzing biological organization that should be applied separately, these problems can be avoided (Eronen 2015). For example, in evo-devo, scientists study processes at different temporal and size scales and study the part-whole structure of organisms and developmental mechanism (Winther 2006), but perhaps there is no need for a further idea of "levels" over and above these notions. However, whether this "deflationary" approach is satisfactory or sufficient for understanding the levels in biological debates discussed above remains to be seen (see also Eronen and Brooks 2018).

Concluding Remarks

Above, levels of organization have been understood primarily as objective features of nature. However, it is also plausible that the concept "levels of organization" has many heuristic uses in science that do not require committing to ontological levels (Brooks and Eronen 2018). For example, immensely complex systems or problems can perhaps be made more tractable by initially thinking of them in terms of levels, without assuming that these correspond to actual and fully stratified organization in nature (Brooks 2019). "Levels of organization" also can be seen as a concept that is typically vague but can be given a precise and consistent meaning in a definite context. For example, in the debate on homologies, researchers refer to levels such as the gene level, the developmental level, and the morphological level (Hall 2003). As such, these levels are likely to be vague and mainly heuristic, but perhaps in each specific case (e.g., the digits of salamanders and frogs), they could be defined more precisely.

In conclusion, the concept of levels of organization figures prominently in various areas of biology, including evo-devo, and seems to be an intuitive and flexible tool for understanding the complexity of nature. However, there is no consensus on how we should understand or define levels of organization, and much further research is needed to clarify their meaning and significance. If the idea of levels of organization is taken with a grain of salt and a dose of healthy skepticism, it can play an increasingly fruitful role in biology and evo-devo in particular.

Cross-References

- ▶ Developmental Homology
- ▶ Evolution of Complexity
- ▶ Explanation in Evo-Devo
- ▶ Heterochrony
- ▶ Interdisciplinarity in Evo-Devo
- ▶ Mechanisms in Evo-Devo

Acknowledgments I would like to thank Laura Bringmann, Dan Brooks and the editors (Laura Nuño de la Rosa and Alan Love) for their very helpful comments on earlier versions of this chapter.

References

Bechtel W (2008) Mental mechanisms. Philosophical perspectives on cognitive neuroscience. Routledge, London

Begon M, Townsend CR, Harper JL (2006) Ecology. From individuals to ecosystems, 4th edn. Blackwell, Malden

Brooks DS (2019) A new look at 'levels of organization' in biology. Erkenntnis (online first) 1–26

Brooks DS, Eronen MI (2018) The significance of levels of organization for scientific research: a heuristic approach. Stud Hist Phil Biol Biomed Sci 68:34–41

Buss L (1987) The evolution of individuality. Princeton University Press, Princeton
Craver CF (2007) Explaining the brain: mechanisms and the mosaic unity of neuroscience. Oxford University Press, Oxford
Craver CF (2015) Levels. In: Metzinger T, Windt JM (ed) Open MIND. MIND Group. http://open-mind.net/papers/levels
Dawkins R (1976) The selfish gene. Oxford University Press, Oxford
Eldredge N, Pievani T, Serrelli E, Temkin I (eds) (2016) Evolutionary theory. A hierarchical perspective. University of Chicago Press, Chicago
Eronen MI (2013) No levels, no problems: downward causation in neuroscience. Philos Sci 80:1042–1052
Eronen MI (2015) Levels of organization: a deflationary account. Biol Philos 30:39–58
Eronen MI, Brooks DS (2018) Levels of organization in biology. In: Zalta EN (ed) The Stanford encyclopedia of philosophy (Spring 2018 Edition). https://plato.stanford.edu/archives/spr2018/entries/levels-org-biology/
Green S, Batterman R (2017) Biology meets physics: reductionism and multi-scale modeling of morphogenesis. Stud Hist Phil Biol Biomed Sci 61:20–34
Hall BK (1999) Evolutionary developmental biology, 2nd edn. Kluwer, Dordrecht
Hall BK (2003) Descent with modification: the unity underlying homology and homoplasy as seen through an analysis of development and evolution. Biol Rev 78:409–433
Lewontin RC (1970) The units of selection. Annu Rev Ecol Syst 1:1–18
Lloyd E (2017) Units and levels of selection. In: Zalta EN (ed) The Stanford encyclopedia of philosophy (Summer 2017 Edition). https://plato.stanford.edu/archives/sum2017/entries/selection-units/
Love AC (2006) Evolutionary morphology and evo-devo: hierarchy and novelty. Theory Biosci 124:317–333
Love AC (2012) Hierarchy, causation and explanation: ubiquity, locality and pluralism. Interf Focus 2:115–125
Maynard Smith J, Szathmáry E (1995) The major transitions in evolution. Oxford University Press, Oxford
Okasha S (2006) Evolution and the levels of selection. Oxford University Press, Oxford
Oppenheim P, Putnam H (1958) The unity of science as a working hypothesis. In: Feigl H, Scriven M, Maxwell G (eds) Concepts, theories, and the mind-body problem. University of Minnesota Press, Minneapolis, pp 3–36
Potochnik A, McGill B (2012) The limitations of hierarchical organization. Philos Sci 79:120–140
Sober E, Wilson DS (1998) Unto others: the evolution and psychology of unselfish behavior. Harvard University Press, Cambridge, MA
Thalos M (2013) Without hierarchy: the scale freedom of the universe. Oxford University Press, Oxford
Vrba ES, Eldredge N (1984) Individuals, hierarchies, and processes: towards a more complete evolutionary theory. Evolution 50:967–976
Wagner GP (2014) Homology, genes, and evolutionary innovation. Princeton University Press, Princeton
Wimsatt WC (1994) The ontology of complex systems: levels of organization, perspectives, and causal thickets. Can J Philos S20:207–274. Reprinted in: Wimsatt WC (2007) Re-engineering philosophy for limited beings. Piecewise approximations to reality. Harvard University Press, Cambridge, MA, pp 193–240
Winther RG (2006) Parts and theories in compositional biology. Biol Philos 21:471–499

Form and Function in Evo-Devo

Ron Amundson

Contents

Abstract

The distinction between form and function has a long and complex history among biologists and philosophers. During some historical periods, the concepts are typically taken to name two distinct but consistent aspects of design. Let us term these compatibilist periods. During other periods it is argued that one member of the pair is more basic, central, or meaningful than the other. Term these adversarial periods. This chapter will begin by discussing adversarial periods and how the form-function dichotomy then plays out against other important biological concepts. The most recent adversarial episode was approximately the final quarter of the twentieth century, when "adaptationist" neo-Darwinists favored function and advocates of what became evo-devo argued for the centrality of form. The conflict was often labeled "adaptation versus developmental constraint." By the turn of the century, advances in molecular developmental genetics and paleontology led to advances in evo-devo (though not necessarily to a reduction in the status of adaptationist studies). The chapter will conclude with a report on the kind of compatibilism that has resulted. Classical texts that had

R. Amundson (✉)
Philosophy Department, University of Hawaii at Hilo, Hilo, HI, USA
e-mail: ronald@hawaii.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_91

supported earlier adversarial views have received criticism, and functional analyses have come to be applied to the mechanisms of genetics. The strict separation of form and function seems to be dissolving. New philosophical work on the form-function relation is called for.

Keywords

Adaptationism · Developmental genetics · Form · Function · Form/function dichotomy · Homology · Structuralism

Introduction

The distinction between form and function has a long history in biological science. In some contexts, the relation is uncontroversial; form and function merely name two aspects of organisms. In other contexts, however, theoretical and ideological differences have given rise to debates in which form and function are partisan labels, with adversaries typically favoring one or the other. This chapter begins with historical sketches of some adversarial exemplars before turning to late twentieth century debates between advocates of mainstream adaptationism and early advocates of evo-devo. It concludes with recent arguments about how our understanding of the form-function dichotomy should be changed as a result advances in biological understanding.

Form and Function in History

An early version of the form-function distinction can be seen in Aristotle who claimed that knowledge about things in the world required knowledge of four distinct causes: *efficient*, *formal*, *material*, and *final*. These constitute different kinds of answers to "why" questions. Efficient causes approximate our modern concept of cause – a force propels something to happen. Formal causes designate a pattern or design to which an object might conform, and material causes explain a property of an object by reference to what the object is made of. Final causes are akin to purposes or functions; they designate what a thing is *for*. Controlled explosions inside an automobile's internal combustion engine cause the pistons to move (efficient); the rigidity of the crankshaft is because it is made out of steel (material); the shape of various parts arise from a design pattern (formal); and the automobile moves forward for the purpose of transportation (final).

Causes ordinarily come before their effects, yet final causes do not. The automobile cannot fulfill its purpose until after it is constructed. So how can transportation be a cause of the automobile? For artifacts like automobiles, we can say (loosely) that the purpose (transportation) *existed in the minds* of the automobile's designers before the design was carried out. So far, so good, at least for items designed by human beings. But how can such a formulation apply to natural occurring objects,

such as teeth? Arrangements of teeth serve a purpose. Human teeth have sharp incisors in the front of the mouth and molars in the back. We bite off pieces of food with incisors and then grind them with molars. Different species of animals have different arrangements of teeth, each of which is suited to the food eaten by that species. Do arrangements of teeth in animals have a final cause or purpose in the various modes of eating of various animals? Aristotle responded affirmatively, saying that purposes were imminent in nature. Some affirmed the existence of natural purposes and believed that this proved the existence of a divine mind that had planned those purposes. Others were skeptical and held that animals could use whatever teeth they found in their mouths but denied that suitable teeth were *for the purpose* of providing them with food. The basic dichotomy between functionalists, who believed in natural purposes, and structuralists, who favored non-finalist explanations persisted, with different sides being favored in different historical eras.

Nineteenth-Century Transformations

One historically significant event – the Cuvier-Geoffroy debate – displays the strong influence of the form-function contrast (Appel 1987). George Cuvier claimed that the diversity of animal form was due to the different ways that animals had found to make their livings in the world. Differences among animals were essentially different manners of adaptation to the environment, and similarities in body parts of species were due solely to similarities in the ways the various species were adapted. Cuvier was thus strongly functionalist in his understanding of biology, emphasizing final causes – the purposes that each body part served. This contrasted sharply with the views of Etienne Geoffroy St. Hilaire, who claimed that animals came in types independent of adaptation. Animals put their body parts to use, but the similarities of body parts of different species existed due to unknown typological laws of structure. The functionalist Cuvier claimed that the deepest truths about organisms were seen in their conditions of existence, especially the tightly integrated organization of anatomical parts; for the structuralist Geoffroy, the unity of type described the deepest correspondences among organisms. In 1830, a debate took place between the two. In contradiction to Cuvier's claims, Geoffroy proposed homologies (structural correspondences) between body parts that couldn't possibly serve the same function. For example, Cuvier had identified the *furcula* bone as serving an important function in bird flight, but Geoffroy claimed to have discovered furcula bones in fish. If Geoffroy was correct, then animals could have corresponding body parts even when these were functionally distinct. Geoffroy favored form over function, while Cuvier accepted correspondences only between body parts that served identical functions.

By the end of the decade, Cuvier was generally considered to have won the debate, in part because of the popularity of a broader functionalism in the intellectual culture of the time. The functionalist bias of British natural theology was a leading influence, which unlike Cuvier primarily emphasized the close adaptive fit between organism and environment. William Paley's "Argument from Design" was originally

published in his 1802 *Natural Theology*. An influential updating of Paley's argument was titled the *Bridgewater Treatises*, a series of volumes that began being published 1829 to update Paley, whose scientific examples were becoming dated. However, the science of the 1830s began to shift the preference for function over form. In embryology, Karl Ernst von Baer showed that all vertebrate embryos began with similar forms and that the forms gradually diverged during embryonic development in a pattern that corresponded with their taxonomic diversity. For example, embryonic fish diverge from other embryonic tetrapods while still very early embryos. Later in development reptiles diverged from fish, and even later mammals diverged from reptiles. These structural or typological patterns in embryology fit neatly with the patterns discovered from comparative morphology. Structuralism in morphology waxed and functionalism waned in subsequent decades. Additionally, geological discoveries of an ancient earth filled with fossils expanded the structuralist interpretation of contemporary species.

The morphologist Richard Owen published a formalization of the concept of *homology* that captured Geoffroy's claim that the correspondence of body parts in different species is independent of function. Homologs were "the same traits in different organisms under every variety of form and function" (Owen 1843). This definition violates Cuvier's rule that corresponding organs must serve the same functions. It assumes that "the same trait" has a clear meaning and that the sameness of homologs persisted while their forms (shapes) and uses varied. Although Owen and other structuralists showed some leanings towards evolutionary thinking, religious conservatives discouraged this style of thought. Not surprisingly, Darwin put Owen's many examples of homology to very good use.

One might expect that Darwin's *Origin* would have served as a watershed to the form-function dichotomy, possibly even a resolution. Ironically, it did not. Most educated people were convinced of descent with modification, which offered an evolutionary perspective on the typology expressed in morphology. Homological "sameness" was due to traits having originated from "same" structures in ancestral species. However, Darwin was a functionalist and immensely impressed with adaptation. His most unique contribution – natural selection – is primarily an explanation of adaptation. But most of his early followers were practitioners of morphology and strong structuralists. They had little interest in the study of adaptation and little use for natural selection. The dichotomous interpretation of form and function continued into the twentieth century.

The First Century of Darwinian Evolution

At the 50th anniversary of the publication of his *Origin of Species* in 1909, Darwin was esteemed by most naturalists and nearly all considered themselves to be Darwinians (Richmond 2006). Still, natural selection remained controversial, perhaps even marginal, as an evolutionary mechanism. During this period, Edward Stuart Russell (1887–1954) published his remarkable book *Form and Function* (Russell 1916), insightfully characterizing methodological conflicts that would be

neglected during much of the following century. The problem for natural selection was heredity; no one yet knew what kind of heredity theory *could* make natural selection work as Darwin needed it to work. Nineteenth century thinkers (including Darwin) conceived of heredity as integrated with embryological development. To explain heredity, one must also explain *how inherited characters and traits arose* in the offspring. Thomas Hunt Morgan (1866–1945) and his associates introduced a key maneuver that altered the structure of the problem. They redefined heredity in such a way that it *was independent from* embryological development. Genes were defined not in terms of their embryological activity, but as patterns of phenotypic correlation between ancestral and descendent generations. This facilitated integration with both the recently rediscovered laws of Mendel and with cytological discoveries about chromosomes and their activity during meiosis. Genes were defined phenotypically not developmentally. Morgan admitted that he could not explain the role of genes in development (what he now called "developmental genetics"). But, he said, if one was concerned with *heredity* (now called "transmission genetics"), then embryology was irrelevant.

With the rise of mathematical population genetics, the new Morgan-Mendelian-chromosomal approach to genetics was shown to fit nicely with natural selection. Finally, a heredity theory fit well with natural selection. This innovation eventually overcame the antagonism among schools of evolutionists. It was gradually coordinated with disciplines like field biology (natural history) and paleontology. Julian Huxley dubbed this new evolutionary theory the "Modern Synthesis" of evolution and insisted on its status as "Darwinian" (Huxley 1942). Huxley's "Darwinian" label was controversial, because many of his colleagues were sensitive to the fact that Darwin had affirmed both use inheritance (usually termed "Lamarckian") and blending inheritance – both of which were firmly rejected by the new outlook. Although the new view was dissonant with Darwin's beliefs in blending heredity and use heredity, it was consonant with Darwin's functionalism and emphasis on natural selection. Huxley's new version of Darwinism gradually won the day, as biologists began to forget that the beloved Darwin had been committed to views that had lost their luster.

By the 100th anniversary of Darwin's *Origin* in 1959, Darwinians were Modern Synthesis evolutionists. Not all was well, however. Two trends from about this period illustrated a continuing conflict. One came from Ernst Mayr, a Synthesis "architect," who argued that "population thinking" was the proper style of evolutionary reasoning and was conceptually opposed to "typological thinking" exhibited by structuralist morphologists and others. The other trend was the description coined by paleontologist Stephen Jay Gould (1941–2002) as the "hardening of the Synthesis" – a firmed up commitment to adaptation by means of natural selection. Mayr criticized any structuralist challenges to adaptation by natural selection, while Gould regretted the growing strength of adaptationism. To be sure, some aspects of midcentury biology remained neutral with respect to the form-function dichotomy (e.g., random genetic drift). And it must be recognized that the Darwinian functionalism of the 1970s was far different from the theologically based version of the 1830s. Still, it shared the view that organic structure was explained by adaptation to

environment rather than by any structural principles that existed independently of adaptation. Twentieth century adaptationism was a stable tradition that included not only a wide range of scientific fields but also historians and philosophers who worked in coordination with biologists. This produced a tradition of "Synthesis Historiography" that interpreted the history of evolution theory in a way that tended to justify the correctness of the Modern Synthesis (Amundson 2005). This became more evident from the perspective of a minority but growing scientific field that challenged central features of the Modern Synthesis: evolutionary developmental biology (evo-devo). Interpretations of earlier biological thought from Synthesis Historiography were biased against structuralist forms of thinking that led to a different picture of the evolutionary process. An example is the mistaken view that species fixism was an ancient belief endorsed by nearly all thinkers prior to Darwin. This was recognized by E.S. Russell but by no one else for almost a century. Recognizing species fixism as an innovation of the eighteenth century was an important step in reinterpreting Darwin's achievement from an evo-devo, rather than a Modern Synthesis, perspective.

Evo-Devo and the Re-emergence of Form

Although functionalism was the majority view among evolutionary biologists through the twentieth century, a minority showed structuralist tendencies. Conrad Hal Waddington (1905–1975) and Ivan I. Schmalhausen (1884–1963) argued for the relevance of development to evolution but had little influence on the field. Around 1980 the disagreement came into public view and resurrected many contours of the Cuvier-Geoffroy debate. Stephen Jay Gould and Richard Lewontin claimed that adaptationism had become a biased doctrine that forbade scientific study of developmental constraints (Gould and Lewontin 1979). Viktor Hamburger (1900–2001) claimed that embryology had become a black box, the contents of which were invisible to Synthesis evolutionists, and urged that the box should be reopened. Many iconoclastic theorists criticized mainstream adaptationism for its failure to account for structural facts observable in developmental biology and comparative morphology.

Mainstream adaptationists began to argue that the use of developmental data within evolutionary theorizing was methodologically flawed, exemplifying essentialism, typology, and other faulty ways of thinking. John Maynard Smith dismissed outright the significance of development: "One consequence of Weismann's concept of the separation of the germline and soma was to make it possible to understand genetics, and hence evolution, without understanding development" (Maynard Smith 1982, p.6). Similar refutations of the relevance of development used the genotype-phenotype distinction and another dichotomy from Mayr – proximate versus ultimate causation (see chapter ▶ "Proximate Versus Ultimate Causation and Evo-Devo") – in addition to that of typological versus population thinking.

However, by the turn of the century, molecular genetics began to show the power and relevance of development to evolutionary thought. Shocking numbers of

molecular homologies were found in early development, corresponding to von Baer's embryological principles, and illustrated the value of the perspective of evolutionary morphology. The new field of evolutionary developmental biology (evo-devo) grew substantially. Its methods, as compared with those of mainstream adaptationism, showed an impressive similarity to those of Geoffroy as compared to Cuvier. The metaphysics underlying each approach had changed. The metaphysics of adaptation had changed from natural theology to natural selection. Similarly, the metaphysics of structuralism had changed from abstract typology to molecular genetics. And, although the form-versus-function pattern persists, today's conceptual divide is not as dichotomous as in earlier times. Most evo-devo practitioners accept the legitimacy of natural selection and population genetics. And virtually none of the adaptationists of the 2020s are willing to argue that embryological development is irrelevant to the understanding of evolution. An understanding of *ontogeny as form* is necessary to understand evolution.

Evo-Devo and New Questions of Form Versus Function

To this point we have examined the form-function dichotomy historically. We will now consider the present context of evo-devo and consider some possible revisions. The first is an innovative defense of the concept of *functional homology* (Love 2007). The expression "functional homology" occurs in recent biology and psychology literature, but it appears to be a contradiction in terms. Owen defined homological traits as the same in different organisms *under every variety of form and function*. Homology itself was a kind of *structural* sameness. When an evolutionary biologist wanted to talk about function, she would refer to analogy rather than homology. Analogous structures are similar because of their separate shaping (towards a similar function) by natural selection. In the absence of structural evidence for homological "sameness," what could justify a claim of sameness (not just analogous similarity) for function?

Love proposes *organization* as one possible conceptual basis for a broader conception of homology and analyzes it in terms of molecular genetic organization with its "hierarchically interconnected interdependencies (similar to relative position and connection for skeletal elements in structural homology)" (Love 2007, p. 691). The parallels in organization between the two domains, skeletal organization in anatomy and genetic network organization in ontogeny, suggest a justification for functional homology. On this account, functional homologs have the kind of individuation and causal persistence shown by structural items like tetrapod forelimbs (of which bat wings and human arms are instances). This transferal of homology to a functional category can be observed in evo-devo discussions of particular molecular discoveries:

> When studying the molecular evolution of regulatory genes, their biochemical and developmental function must be considered separately. The biochemical function of *PAX–6* and *eyeless* are as general transcription factors (which bind and activate downstream genes), but

> their developmental function is their specific involvement in eye morphogenesis. (Abouheif 1997, p. 407)

The molecular identity of the genes *PAX-6* and *eyeless* was among the early shocking discoveries of deep genetic homologies. The same "gene" (transcription factor) stimulated the development of eyes in fruit flies and mice, but this stimulation was indirect. Abouheif distinguished between the biochemical causal action of regulating the expression of other genes and the eventual developmental product of eyes in both fruit flies and mice. Both the biochemical and developmental roles were functions but functions of different kinds. Love called the first *activity functions* and the second *use functions* (following Wouters 2003). The activity function is the DNA binding and subsequent "switching on" of genes in certain contexts. The use function is the developmental goal of the process, the eventual development of eyes.

This modified form-function semantics is possible only because of our increased understanding of the biochemical context of DNA. It does not act alone but participates in embryological development and interacts with other molecules. The notion of a transcription factor requires a conceptualization of genes very different than merely being *for* phenotypic traits or trait differences, as genes were seen during most of the twentieth century. Transcription factors are involved in determining where (in the embryo) and when (during embryogenesis) genes are expressed. Ironically, the first genes identified as shared between remotely related species turned out not to be *for* any particular phenotype at all. Instead they controlled the expression of other genes in a hierarchical sequence of activation and repression. The growing understanding of molecular genetics revealed an analogy between the biochemical activity of a transcription factor (e.g., *PAX-6*) and the structure of a morphological character (e.g., the vertebrate forelimb); each item maintains a persistent identity that exhibits correspondence across species despite the existence of variation in form and in (use) function.

Overall, the identification of molecular developmental activity functions for certain genes permits us to see a nested pair of form/function relations. A particular DNA sequence (e.g., *PAX-6*) has a molecular genetic function (transcription factor) that it performs in several different contexts of a developing organism. One of these is the initiation of eye morphogenesis; others occur in the development of vertebral columns and the pancreas. One implication of Love's account of functional homology is the novelty of a hierarchy of function categories. Transcription factors serve as *functions* with respect to their DNA sequences (forms) and as *forms* with respect to their biological roles. This nesting of form and function is a new implication that arises from evo-devo.

Evo-devo will surely offer many other changes in our understanding of biological form, function, and the relation between the two. A quick example is the recent discovery of homologies between genes found in highly derived organisms (e.g., mammals and birds) and genes in more basal groups, which (it is argued) existed in the groups that were ancestral to today's derived species. These homologies are especially surprising because the ancestral forms of the genes are found in organisms

that are so phenotypically simple that they lack the body parts (i.e., they lack the phenotypes) that their homologs are responsible for producing in derived species. An example is choanoflagellates, the single-celled organisms that many believe to be the closest living relatives of metazoans. Choanoflagellates possess genes that are homologous to the genes that are responsible for cell adhesion molecules in metazoans (King et al. 2008). However, single-celled organisms seemingly have no need for cell adhesion molecules at all, at least not to stick their cells together! A similar discovery was made concerning jellyfish and placozoa, which have no nervous systems. Yet they share genes that are used by metazoans to build nervous systems (Arendt 2008). Hemichordates such as acorn worms have no brain but share the genetic signaling centers used by vertebrates to grow their brains (Pani et al. 2012). What need do these simple animals have for genes that are central to the construction of body parts in much more complex organisms? We hope eventually to understand what functions are served by those genes in those organisms, but clearly their (use) functions in complex organisms differ substantially from those of their ancestral homologs in simple organisms.

In some ways, this may not be surprising. It was clear to Owen that homologous features served distinct functions ("every variety of form and function") and to Darwin that ancestral homologs changed their functions during evolution. But for much of the twentieth century, prominent adaptationists believed that evolutionary novelties would require genetic novelties – new phenotypes required new genes (see chapter ► "Developmental Innovation and Phenotypic Novelty"). They did not expect that old genes could somehow be repurposed to serve new functions. These discoveries highlight changes of function within ontogeny itself. The form-function relation is constantly shifting.

The second set of discoveries that indicates an increasing complexity of the form-function relationship begins with a well-known puzzle about homology (see chapter ► "Developmental Homology"). Many early embryologists expected that homologs would share their embryological origins, such as growing out of the same germ layers. But it was soon discovered that some obviously homologous structures, such as the alimentary canals of various groups of vertebrates, grew out of distinct germ layers in different taxa. Gavin de Beer (1899–1972) observed that the canals developed out of different germ layers in sharks, lampreys, fish, and birds. Apparently, the function of supplying cellular material for the growth of the canal had meandered during evolutionary time. This phenomenon has recently been documented at the genetic level, even in closely related species. Several homologous traits in related species of nematodes, such as sex determination, early embryonic patterning, and excretory physiology, exhibit substantial variation in their developmental genetic underpinnings. The phenomenon has been termed developmental system drift: "[I]t appears that gene regulatory networks are constantly being reconfigured even when phenotypes are not" (Haag 2014; see chapter ► "Developmental System Drift"). Recent empirical and theoretical work has explored whether there is some network of genes that is retained and confers identity on homologs despite the pervasive nature of developmental system drift (e.g., Wagner 2014).

Evo-devo has shown us that evolutionary history is densely packed with complex, changing relationships between form and function. These patterns of change include the redeployment of functional homologs in ontogeny, the retention of homologous genes that have radically shifted in their developmental roles, and the decoupling of traits from specific materials and construction rules seen in developmental systems drift. Not only do old forms come to have new functions, but also phenotypes (the "functions" of existing forms) have evolved such that different "forms" (developmental causes) produce them. The traditional distinction of form and function would seem to be insufficient to characterize the relations among sets of homologous genes and the very many different phenotypes to which members of these sets are attributed.

The relations between form and function were at the center of several debates up until evo-devo achieved its autonomy as a field of evolutionary study at about the beginning of the twenty-first century. Evo-devo itself has inspired innovative interpretations of the form-function dichotomy. In addition, it has offered innovative empirical discoveries that challenge the traditional interpretations of, e.g., adaptationism. Exactly how to philosophically parse these complications is a task for the future.

Cross-References

- ▶ Conrad Hal Waddington (1905–1975)
- ▶ Developmental Homology
- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Developmental System Drift
- ▶ Gavin de Beer (1899–1972)
- ▶ Ivan I. Schmalhausen (1884–1963)
- ▶ Proximate Versus Ultimate Causation and Evo-Devo
- ▶ Stephen Jay Gould (1941–2002)

References

Abouheif E (1997) Developmental genetics and homology: a hierarchical approach. Trends Ecol Evol 12:405–408

Amundson R (ed) (2005) The changing role of the embryo in evolutionary thought: roots of evo-devo. Cambridge University Press, New York

Appel TA (ed) (1987) The Cuvier-Geoffroy debate: French biology in the decades before Darwin. Oxford University Press, New York

Arendt D (2008) The evolution of cell types in animals: emerging principles from molecular studies. Nat Rev Genet 9:868–882

Gould SJ, Lewontin RC (1979) The spandrels of san Marco and the Panglossian paradigm: a critique of the adaptationist programme. Proc R Soc Lond Biol Sci 205(1161):581–598

Haag ES (2014) The same but different: worms reveal the pervasiveness of developmental system drift. PLoS Genet 10(2):e1004150

Huxley J (ed) (1942) Evolution: the modern synthesis. Harper and Brother Publishers, New York

King N, Westbrook MJ, Young SL, Kuo A, Abedin M, Chapman J et al (2008) The genome of the choanoflagellate *Monosiga brevicollis* and the origin of metazoans. Nature 451(7180):783–788
Love AC (2007) Functional homology and homology of function: biological concepts and philosophical consequences. Biology & Philosophy 22(5):691–708
Maynard Smith J (ed) (1982) Evolution and the theory of games. Cambridge University Press, Cambridge
Owen R (ed) (1843) *Lectures on the Comparative Anatomy and Physiology of the Invertebrate Animals*. Longman Brown Green and Longmans, London
Pani AM, Mullarkey EE, Aronowicz J, Assimacopoulos S, Grove EA, Lowe CJ (2012) Ancient deuterostome origins of vertebrate brain signalling centres. Nature 483(7389):289–294
Richmond M (2006) The 1909 Darwin celebration in Cambridge: reexamining evolution in the light of Mendel, mutation, and meiosis. Isis 97:447–484
Russell ES (1916) Form and function. John Murray, London
Wagner GP (2014) Homology, genes, and evolutionary innovation. Princeton University Press, Princeton
Wouters A (2003) Four notions of biological function. Stud Hist Philo Biol Biomed Sci 34:633–668

Dispositional Properties in Evo-Devo

Christopher J. Austin and Laura Nuño de la Rosa

Contents

Abstract

In identifying intrinsic molecular chance and extrinsic adaptive pressures as the only causally relevant factors in the process of evolution, the theoretical perspective of the Modern Synthesis had a major impact on the perceived tenability of an ontology of dispositional properties. However, since the late 1970s, an increasing number of evolutionary biologists have challenged the descriptive and explanatory adequacy of this *chance alone, extrinsic only* understanding of evolutionary change. Because

C. J. Austin
Faculty of Philosophy, Radcliffe Humanities, University of Oxford, Oxford, UK
e-mail: Christopherja@gmail.com

L. Nuño de la Rosa (✉)
Department of Logic and Theoretical Philosophy, Complutense University of Madrid, Madrid, Spain
e-mail: lauranun@ucm.es

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_64

morphological studies of homology, convergence, and teratology have revealed a space of possible forms and phylogenetic trajectories that is considerably more restricted than expected, evo-devo has focused on the causal contribution of intrinsic developmental processes to the course of evolution. Evo-devo's investigation into the developmental structure of the modality of morphology – including both the possibility and impossibility of organismal form – has led to the utilization of a number of dispositional concepts that emphasize the tendency of the evolutionary process to change along certain routes. In this sense, and in contrast to the perspective of the Modern Synthesis, evo-devo can be described as a *science of dispositions*. This chapter discusses the recent philosophical literature on dispositional properties in evo-devo, exploring debates about both the metaphysical and epistemological aspects of the central dispositional concepts utilized in contemporary evo-devo (e.g., variability, modularity, robustness, plasticity, and evolvability) and addressing the epistemological question of how dispositional properties challenge existing explanatory models in evolutionary biology.

Keywords

Developmental constraints · Dispositions · Evo-devo · Evolvability · Modularity · Robustness · Teleology · Variational structuralism

Introduction: Evo-Devo as a Science of Dispositions

One dominant tradition in the history of metaphysics views the denizens of our universe as fundamentally passive; like so many billiard balls, the activity of each ultimately amounts to the degree to which they are *pushed and pulled* by some other. This is in some ways enshrined in contemporary scientific paradigms: what an entity can or must do is conceived as functionally derivative, due either to the contingencies of its extrinsic causal context or as a necessary consequence of the *laws of nature* which govern it. Another tradition views these modalities as intrinsically grounded in those entities. According to this perspective, the entities which populate our world play an active role in erecting its causal structure. This activity shapes the course of events in virtue of those entities possessing properties that specify their potential for (or dispose them to) change.

The former tradition is manifested today by those who defend the idea that the only genuine properties are *categorical properties*. Categorical properties place no causal or modal constraints on the world. If the world is merely a collection of categorical properties, those constraints are contingent and extrinsic, imbued upon it from a set of higher-order natural laws (which may have been different), or reducible to abstracted reflections of brute facts about its regularities (which may not have occurred). A world constructed from dispositional properties is radically different. Dispositional properties are intrinsically dynamic, essentially defined or individuated by what they do. Their dynamic nature consists in being causally responsible for reliably and repeatedly bringing about a particular type of state of affairs (a *manifestation*) upon the obtaining of a particular set of conditions (a *stimulus*).

Because they are individuated by their functional role in bringing about specific end-states, one and the same dispositional property can be possessed by any number of compositionally or structurally distinct systems (i.e., they are *multiply realizable*). Furthermore, as the causal roles intrinsic to these properties consist in the potential for the specified production of particular states, dispositions are often understood as teleological, causally directed toward or for those end-states. The intrinsically directed natures of these properties deliver and delimit the space of possibilities for the entities that possess them and thereby underwrite patterns of causal regularities we observe.

Although contemporary metaphysics is currently experiencing a neo-Aristotelian revival of sorts, especially within the philosophy of science, dispositional ontologies have historically been viewed with suspicion in theoretical biology. From the perspective of the long-standing paradigm of the Modern Synthesis, there are several reasons why this might be the case. The most obvious stems from a general distrust of teleological analyses of natural phenomena: it considered the commitment to a thoroughly mechanistic, nonpurposive view of nature, as ushered in by the Scientific Revolution, as incompatible with assigning any explanatory role to the goal-directedness of organisms (Grene and Depew 2004). This demanded a rejection of teleological analyses such as the theory of orthogenesis – the view that the evolutionary history of organisms was guided by an intrinsic impetus oriented toward some predefined goal (Mayr 1992). It is often claimed that Darwin's nonteleological explanation of adaptation effectively dissolved this problematic perspective. However, according to Mayr, the viability of a teleological characterization of life only vanished with the advent of the Modern Synthesis in which biological disciplines collectively rejected the existence of all (phenomenal or causal) finalism. With palaeontology proving the absence of privileged evolutionary trends and molecular biology demonstrating the intrinsic randomness of the process of genetic change, the study of evolution by natural selection became reduced to the statistical analysis of changes in population gene frequencies (Walsh 2015).

In identifying intrinsic molecular chance and extrinsic adaptive pressures as the only causally relevant factors in the process of evolution, the Modern Synthesis perspective strongly suggested that an ontology of dispositional properties in biology was untenable. On one hand, its dependence upon the absolute randomness of variation effectively disavowed the study of *the possible* as a legitimate epistemological goal of evolutionary theory. Because every and any form can theoretically arise from random mutations, the proper study of evolution via population genetics had to be focused solely on actual variations. On the other hand, its insistence that the evolutionary trajectories of populations were explicable only as the result of the process of natural selection effectively relegated the explanatory power of intrinsic factors to a position of theoretical irrelevancy. From the outlook of the Modern Synthesis, if the central *explanandum* was adaptive change, then functionally designated developmental processes were passive, being *pushed and pulled* by the selective forces of their environments. As a result, evolutionary biology was framed as a science of the external.

However, since the late 1970s, an increasing number of evolutionary biologists have challenged the descriptive and explanatory adequacy of this *chance alone,*

extrinsic only understanding of the process of evolution. Recent morphological studies of homology, convergence, and teratology have revealed that the space of possible forms and phylogenetic trajectories is considerably more restricted than expected. Evo-devo, in contrast to the approaches of evolutionary biology founded in the Modern Synthesis, focuses on the causal contribution of intrinsic developmental processes in shaping that space, and with it the course of evolution. Evo-devo's investigation into the developmental structure of the modality of morphology – the possibility and impossibility of organismal form, as well as the directionality and speed of morphological changes – has led to the utilization of dispositional concepts that emphasize the *inherency* of the evolutionary process, its proclivity to privilege particular pathways (see chapter ► "Inherency"). In this sense, and in contrast to the perspective of the Modern Synthesis, evo-devo can be described as a *science of dispositions* (Austin 2017).

This chapter discusses recent philosophical literature on dispositional properties in evo-devo. The first section explores debates about the metaphysical and epistemological aspects of the central dispositional concepts utilized in evo-devo: developmental constraints and variability, modularity, robustness, plasticity, and evolvability. The second section addresses the epistemological question of how dispositional properties challenge existing explanatory models in evolutionary biology.

Dispositional Concepts in Evo-Devo

From Constraints to Variational Properties

The differences between the explanatory agendas of standard evolutionary biology and evo-devo might be seen as a translation of the relationship between actual and possible. In the 1980s, with the rise of molecular biology and the explanatory project of adaptationism, the concept of *developmental constraints* was understood *negatively* as the observable limits of molecular variation and selective optimization within populations (Brigandt 2015b). In contrast to the insistence on the ability of natural selection to explain the shape of any morphological form as one causally carved from extrinsic, adaptive forces, morphologists and developmental biologists became increasingly interested in the apparent intrinsic *resistance to change* that certain morphologies, such as homologues and body plans, seemed to exhibit over evolutionary timescales, and the corresponding *forbidden* areas of morphospace that remained unoccupied in existing phylogenies.

In recent years however, evo-devo biologists have highlighted the generative power of constraints. On this understanding, the causal architectures of developmental systems not only constrain the set of possible forms but also actively provide new opportunities for evolutionary change (Brigandt 2015b). The role of development in the process of evolution is not simply to constrain but to determine what is morphologically possible, and among these possibilities, what is more likely. On this *positive* conception of constraints, developmental systems facilitate morphological change and thus are capable of playing a central role in the explanation of

macroevolutionary transformation and evolutionary novelty. In contemporary evo-devo, concepts like *variability* (as opposed to *variation*) capture this positive role of constraints as generative capacities; while variations are *the actually realised differences between individuals, variability is a term that describes the potential or the propensity to vary* (Wagner and Altenberg 1996). Indeed, some authors have advocated abandoning the notion of developmental constraints altogether in favor of conceptualizing developmental systems as possessing *variational properties*: diverse generative capacities causally responsible for the production of a wide range of phenotypes (see chapter ▶ "Mechanisms of Pattern Formation, Morphogenesis, and Evolution").

While the concept of developmental constraint situates the conflict between populational and developmental approaches to evolutionary change in the context of the dichotomy between externalism and internalism, utilizing the concept of variability allows one to emphasize the differences between the epistemological aims of population genetics and evo-devo, thereby making clear their distinct theoretical commitments – the former to the *actual*, the latter to the *possible* (Eble 2003). Evo-devo biologists explicitly recognize the tension between these two perspectives, noting that while variation *can be directly observed as a property of a collection of items*, variability *belongs to the group of "dispositional" concepts*, as the *variability of a phenotypic trait describes the way it changes in response to environmental and genetic influences* (Wagner and Altenberg 1996, p. 969). For this reason, Gunter Wagner coined the term *variational structuralism* to denote the ontological commitments of evo-devo in contrast to those of the Modern Synthesis according to which *all that is real are the realised differences among organisms, and not their underlying variational tendencies* (Wagner 2014, p. 19; see chapter ▶ "Typology and Natural Kinds in Evo-Devo").

Modularity

The development of an organism's morphological features does not happen all at once, or even all together; it is a process of compartmentalized collaboration. Each *developmental module* that participates in that process is characterized by a high degree of internal causal connectivity among its constituents, a prominent form of which consists in the tightly knit regulatory domains of a genetic regulatory network's transcription factors, cis-regulatory sites, and signaling cascades. Because these semiautonomous organismal subsystems govern the generative specificities of morphological development and have their own traceable intra- and interspecies phylogenetic histories, they are of prime importance in evo-devo research.

From a developmental perspective, these subsystems appear to operate dispositionally, as centers of specified morphological potentiality. They causally mediate the influence of chemical stimuli – from cellular signaling to downstream genetic expression pattern – to reliably and regularly initiate the production of particular morphological features. These modules exhibit a high degree of robustness such that mutational variations among their component elements and epigenetic

variations in their regulatory structure generally have little to no effect on their generative competence to produce morphological structure. Over time then, and in successive generations, the generative role played by a particular complex of genetic elements in a specific regulatory configuration can become *autonomized*, gaining a kind of independence from its original underlying genetic architecture. This autonomy is manifested via multiple realization by a number of distinct generative structures over developmental and eventually evolutionary time-scales (see chapters ▶ "Developmental Homology" and ▶ "Developmental System Drift").

Because of this autonomy, the storied pasts of these modules can only be deciphered with some difficulty, and it is conceptually advantageous to individuate them functionally, according to the specific causal role they perform in the production of organismal morphology. Given the molecular heterogeneity of a single module's multiple genetic underpinnings over time, that generative role is quite complex; it encompasses not only the production of one or a few morphological configurations but an entire *morphospace* of quantitative and qualitative variations of them (see chapter ▶ "Morphological Disparity"). Importantly, however, the generative potential which is mapped-out in these morphospaces are reflections of the intrinsic capacities of these modules, capacities which channel the exploration of these spaces over evolutionary time (see chapter ▶ "Inherency"). As multiply realized, intrinsic centers of specified potentiality that both conform and constrain the character of morphological regularities in the phylogenetic record, developmental modules are best conceptualized dispositionally (Austin 2017).

One of the major goals of evo-devo is unraveling the developmental basis of variational modularity or the variational independence among characters. In this context, modularity is understood as a property of the whole genotype-phenotype map which determines how variation is structured. Therefore, variational modularity is not a disposition to generate particular morphological features but a disposition to generate variation in a particular way. The modularity of the genotype-phenotype map seems crucial for the ability of developmental systems to evolve, insofar as the organization of development into semiautonomous processes leads to the independent variation of characters which can function as *building blocks* of phenotypic adaptation (see the section on "Evolvability").

Furthermore, although this developmental and evolutionary autonomy of modules is secured by the high degree of causal integration among their constituents, they are at the same time typified by their tolerance to alterations in this dynamic architecture. Modular systems thus possess the capacity to preserve their generative competency throughout modifications to their causal-temporal structure, an ability secured by a number of complex network features (see the section on "Robustness"). This dispositional aspect of modular systems is of major evolutionary significance as it is correlated with the phenomenon of developmental plasticity: the ability of a single module to produce an array of differing phenotypes crucially depends upon this capacity for compositional flexibility (Walsh 2015; see also the section on "Plasticity"). Moreover, this capacity is itself subject to evolutionary modification, as illustrated in recent studies on the developmental basis for variational independence among particular traits in particular species (see references in Wagner et al.

2007). For instance, forelimbs and hindlimbs appear to be ancestrally correlated, but in bats, this correlation is broken – a separation which subsequently facilitated the specialization of the forelimb for flying. Thus, understanding how interconnections within and among modules originate, break down, and change throughout evolutionary time is a major research agenda for evo-devo (Bolker 2000).

Robustness

In developmental biology, robustness (also referred to as canalization or developmental stability) refers to the ability of developmental trajectories to buffer against environmental or genetic perturbations that would otherwise affect a phenotypic outcome (see chapter ▶ "Canalization: A Central but Controversial Concept in Evo-Devo"). Robust systems are both persistent – able to maintain the causal production of an end-state by means of compensatory changes within the system – and pleonastic – able to bring about that end-state via a number of alternative pathways. Because these homeostatic phenomena represent a system's causal bias toward some particular state, they are standard marks for those systems being *goal-directed* (Walsh 2015; see chapter ▶ "Teleology in Evo-Devo").

The pleonastic robustness of biological systems is exhibited in their privileging of a limited set of developmental trajectories. As illustrated in Waddington's epigenetic landscapes, and more recently in biological models developed from dynamic systems theory, these systems promote morphological invariance because their constitutive causal architecture is dynamically oriented toward the production of a limited set of developmental fates from a wide variety of initial (and intermediate) states (Brigandt 2015a). In grounding the intrinsic developmental stability of a system by dynamically privileging the production of a particular morphology, and thus the regularity of the system's operation in pursuit of that end within a wide range of environmental and developmental contexts, pleonastic robustness is a system-level exhibition of dispositionality.

The homeostatic behavior exhibited in persistence-based robustness is typically secured in one of two ways. First, biological systems can be constituted by a regulatory network that possesses a number of *redundant* elements, which can take-up the causal slack of missing, mutated, or disabled elements. Second, these systems exhibit a *degenerative* capacity to *rewire* their regulatory architecture, producing new causal connections in novel configurations to maintain their function under significant perturbation. Importantly, in these more extreme exhibitions of homeostatic phenomena, the system maintains a generative proclivity toward a specific morphological end-state that persists throughout the alteration of its compositional elements and their causal architecture. This multiply realized, goal-directed capacity to produce and dynamically preserve the production of an end-state occupies a principal position in the explanatory framework of evo-devo, providing an important link between ontogeny and phylogeny.

The project of analyzing and understanding developmental systems' propensity for invariance is central to evo-devo: not only does this feature of systems illustrate one of its central tenets – that the modality of organismal morphology has an

intrinsic, developmental basis – but it also serves as an important theoretical tool with which it conceptualizes the stability (and thus, selectability) of phenotypic traits over evolutionary time-scales. On one hand, these systems' generative stability throughout a variety of structural perturbations undergirds the ability of organisms to maintain their morphological integrity within various environmental conditions – a capacity which is positively correlated with their potential for selective success. On the other hand, the generative stability of these systems throughout mutational variations at the same time allows for their accumulation of developmental resources which, while not immediately divertive of their generative function, may eventually contribute to the production of morphological novelties when the system undergoes further genetic or environmental alterations – this amassing of *cryptic variation* thus enhances the evolvability of these systems (see chapters ▶ "Canalization: A Central but Controversial Concept in Evo-Devo," ▶ "Developmental System Drift" and ▶ "Developmental Innovation and Phenotypic Novelty").

Plasticity

The ability of organisms to produce distinct phenotypes in distinct environments is an extremely pervasive phenomenon, one evidenced in everything from simple seasonal-based changes in coloration to the complex predator-based alterations of defensive morphologies. In developmental biology, phenotypic plasticity is invariably described in dispositional terms as the ability of an organism to alter its phenotype in response to changes in its environment or as the potential of a genotype to produce a number of alternative phenotypes in the context of distinct environmental conditions (see chapter ▶ "Developmental Plasticity and Evolution"). While in a certain sense the plasticity of developmental systems can be understood as an inverse measure of their generative robustness, the two phenomena are in another sense both exemplifications of those systems' ability to develop a functional phenotype (Brigandt 2015a). In this latter sense, both are exhibitions of persistent and pleonastic processes and together serve as *the very paradigm of goal-directed activity* (Walsh 2015).

The phenomenon of phenotypic plasticity demonstrates that the goal-directedness or causal privileging of developmental systems toward the production of a particular phenotype is not a unidimensional affair; alterations in the environmental conditions and thus heterochronical and heterotopical alterations in the upstream signaling of these systems (that is, changes in their *stimulus* conditions) can result in downstream qualitative alterations – in shape, size, pigmentation, etc. – of its phenotypic product (that is, its *manifestation*). The goal-directed capacities of developmental systems are thus *multi-track* dispositions able to produce a range of quantitatively and qualitatively distinct manifestations in response to a variety of distinct stimulus conditions (Vetter 2013). The set of potential manifestations of a developmental system's multi-track disposition may form a continuous, gradient-like stratification of phenotypic variation – as exemplified in the *reaction norms* of experimental genetics – or consist of a

collection of discrete phenotypic forms (so-called *polyphenisms*). Thus, the developmental dispositions of these systems cannot be individuated according to their generative competency with respect to the production of a single, particularized phenotypic end-state but rather to their potential to produce an entire *phenotypic repertoire* (Austin 2017).

Developmental plasticity has been shown to have a major role in adaptive evolution and has even led to the foundation of a new discipline, ecological developmental biology (or eco-evo-devo) that incorporates the central role of the environment in our understanding of developmental evolution (see chapter ▶ "Eco-Evo-Devo"). While evolution is typically regarded as a process through which organisms passively generate random variation and become better adapted to the external demands of their environment, developmental plasticity integrates the constitutive role of the environment in the very generation of variation. Like robustness, plasticity is an adaptive response to external conditions. However, plastic responses to environmental changes can also lead selection, preceding and enabling adaptive change. Thus, in *plasticity-first* models of evolution, novel phenotypes are conceptualized as originating by means of environmental changes that only subsequently are genetically stabilized. In this way, the intrinsic capacities of organisms for specified phenotypic variation can be seen as a significant factor in determining the directionality of the process of evolution.

Evolvability

Evolvability, or the capacity of biological systems to evolve, is widely understood as a cornerstone of evo-devo (Hendrikse et al. 2007; see chapters ▶ "Evolvability" and ▶ "Variational Approaches to Evolvability: Short- and Long-Term Perspectives"). Efforts have been made to precisely define evolvability and philosophers have shown an interest in conceptualizing it dispositionally. While some have argued for a unified concept (Sterenly 2007; Brown 2014), biologists by and large understand evolvability as having a multiplicity of referents (Pigliucci 2008; Nuño de la Rosa 2017). Evo-devo research on evolvability concentrates on how the properties of developmental systems, such as modularity, plasticity, or robustness, affect the generation of heritable phenotypic variation on which natural selection can act (Wagner and Altenberg 1996; Kirschner and Gerhart 1998). In this sense, the concept of evolvability refers not only to the generation of possible forms but also to possible adaptations. Thus, whereas developmental constraint and variability are primarily morphological concepts that articulate the causal relationship between genetic and phenotypic variation, the concept of evolvability incorporates a functional dimension to the explanatory framework of developmental evolution, operating as a conceptual tool to understand the relation between variability and adaptation.

Philosophers of biology typically construe evolvability as a probabilistic dispositional property. In contrast to ordinary, deterministic dispositions, which manifest in the presence of appropriate stimulus conditions, a lineage may fail to evolve even

when it has the capacity to do so. If we understand evolvability as a population-level property, its probabilistic character is evident: the manifestation of evolvability is a function of various stochastic processes within populations, and therefore, natural selection may or may not select the most evolvable (from a developmental perspective) populations (Love 2003; Sterenly 2007; Brown 2014). However, in evo-devo research, the probabilistic character of evolvability can also be understood as an intrinsic consequence of organism-level, genotype-phenotype mappings that determine whether *the probability that random mutation will improve the phenotype has increased during evolution* (Pavlicev and Wagner 2012). The capacity to evolve is generally understood as an intrinsic property of biological systems in evo-devo which is dependent on their genetic composition and associated developmental architecture. However, philosophers of biology have challenged this assumption arguing that, given their causal influence on mutation rates, environmental (extrinsic) factors, such as geographic range or temperature, must be included in the *causal base* of evolvability, a requirement which plausibly calls into question the intrinsicality of the capacity (Brown 2014; Love 2003).

While the fact that phenotypes vary in their propensities to respond to natural selection is widely accepted, the idea that evolvability itself is the subject of selection and can therefore evolve remains highly contested. The mainstream position is that the evolution of evolvability would entail an untenable teleological approach to evolution according to which natural selection produced adaptations for future, unknown environments (Sniegowski and Murphy 2006). However, this is not necessarily the case: evolvability may evolve simply as a by-product. For instance, the fitness advantages conferred by developmental modularity may have led to its selection, which would in turn have had long-term consequences for adaptive evolution. In these sorts of cases, natural selection would act on the effects associated with the variational capacities of developmental systems, thus indirectly enhancing evolvability.

Dispositional Properties and Explanatory Models in Evolutionary Biology

Evo-devo is defined not only by its adoption of a novel set of conceptual tools but also by its utilization of them in constructing explanatory frameworks to understand both development and evolution). Because its conceptual toolbox is thoroughly dispositional, the explanatory models of evo-devo stand in stark contrast to those of the Modern Synthesis: they appeal to the efficacy of the intrinsic, dynamic capacities of developmental systems to shape the course of evolution. In doing so, these models follow in the spirit of the proposal to re-conceptualize *fitness* as a propensity, or disposition to survive and reproduce, rather than as a measure of the actual number of offspring of an organism (Mills and Beatty 1979).

One of the most central schemas employed to conceptualize the process of evolution that arose from the ontological commitments of the Modern Synthesis was the distinction between proximate and ultimate causation (see chapter

► "Proximate Versus Ultimate Causation and Evo-Devo"). According to Mayr, proximate causes (e.g., developmental and physiological factors) and ultimate causes (e.g., natural selection, selective drift) necessarily address distinct *how* and *why* questions. These distinct question types constitute the explanatory agendas of independent biological disciplines: functional biology (how) and evolutionary biology (why). Although Mayr acknowledged that developmental processes have a unique causal role to play in biological explanations (even one involving the goal-directedness of their operation), he nevertheless considered them irrelevant to the explananda of evolutionary biology; answers to the *how* questions of development are unable in principle to feature in the answers to the *why* questions of evolution (Mayr 1992). According to this dichotomy, embedded in the theoretical framework of the Modern Synthesis, developmental systems are intrinsically inert with respect to the process of evolution. They are *pushed and pulled* by the extrinsic forces of selection and hence incapable of playing an important explanatory role with respect to that process.

Philosophers of biology have recognized that Mayr's proximate-ultimate dichotomy is a framework into which the explanatory agenda of evo-devo cannot be situated comfortably. The aim of evo-devo is to integrate the causal influences intrinsic to the process of development into our understanding of evolution. This is a goal that can only be accomplished by adopting a more *reciprocal* model of biological causation wherein the proximate factors operative within those processes, together with natural selection, are understood as proper, active causes of evolutionary change. For example, in offering *lineage explanations* which provide detailed information about the actual sequential changes in developmental mechanisms which have causally undergirded phenotypic changes over time, the explanatory framework of evo-devo necessarily includes aspects of both proximate and ultimate explanations (Calcott 2009; see chapters ► "Explanation in Evo-Devo," ► "Evo-Devo and Phylogenetics," and ► "Proximate Versus Ultimate Causation and Evo-Devo"). Furthermore, *ultimate*, lineage-based explanations that appeal to actual evolutionary trajectories do not exhaust the kind of evolutionary explanations (indeed, the most characteristic kinds of explanations) employed in evo-devo, precisely because the latter depend upon the dispositional properties of biological systems. Explanations involving *evolvability* appeal to the intrinsic disposition of a population to evolve, to *differences in the internal (rather than external) features of populations that increase the probability of a particular evolutionary outcome in the future (for example, adaptedness, diversity)* (Brown 2014, p. 560). These types of explanations, characteristic of and crucial to evo-devo, are ones in which the intrinsic potentialities of developmental systems shape the contours of an adaptive landscape and possess the relevant explanatory power with respect to the actual characteristics of a particular lineage.

From a developmental perspective, one of the most prominent and successful explanatory programs employed in evo-devo research consists in the utilization of mechanistic models. Mechanistic explanations offer predictive utility about some target phenomenon in virtue of conceptualizing it as the causal product of a stepwise, temporally successive series of state-changes in a structurally organized set of

discrete elements. While an explanatory schema that appeals to the actual organization and regular operation of mechanistic components is powerfully predictive in many molecular contexts, *in evo-devo . . . there are important scientific questions that are not just about the actual behaviour of a mechanism, but also its dispositions* (Brigandt 2015a, p. 162). This is because explaining robustness, plasticity, and modularity (inter alia) requires an appeal to the capacities of developmental systems to either react to perturbations or permit modifications. Genuinely explanatory models of these phenomena must not only represent their mechanistic structure but also their dynamic potentiality for the alteration of that structure. Properly capturing the dynamics of developmental systems, and thus adequately measuring their role in shaping the modal structure of the evolution of morphology, may even require the utilization of nonmechanistic models like those of *dynamical systems theory*, which are increasingly prevalent in evo-devo analyses of everything from robustness to evolutionary novelty (see chapters ▶ "Modeling and Simulation in Evo-Devo" and ▶ "Modeling Evolution of Developmental Gene Regulatory Networks").

Cross-References

▶ A Macroevolutionary Perspective on Developmental Constraints in Animals
▶ Developmental Homology
▶ Developmental Innovation and Phenotypic Novelty
▶ Developmental Plasticity and Evolution
▶ Evo-Devo and Phylogenetics
▶ Evo-Devo's Contributions to the Extended Evolutionary Synthesis
▶ Evolvability
▶ Explanation in Evo-Devo
▶ Inherency
▶ Interdisciplinarity in Evo-Devo
▶ Mechanisms in Evo-Devo
▶ Modeling and Simulation in Evo-Devo
▶ Modeling Evolution of Developmental Gene Regulatory Networks
▶ Proximate Versus Ultimate Causation and Evo-Devo
▶ Teleology in Evo-Devo
▶ Typology and Natural Kinds in Evo-Devo
▶ Variational Approaches to Evolvability: Short- and Long-Term Perspectives

References

Austin CJ (2017) Evo-devo: a science of dispositions. Eur J Philos Sci 7:373–389

Bolker JA (2000) Modularity in development and why it matters to evo-devo. Am Zool 40:770–776

Brigandt I (2015a) Evolutionary developmental biology and the limits of philosophical accounts of mechanistic explanation. In: Braillard P-A, Malaterre C (eds) Explanation in biology: an enquiry into the diversity of explanatory patterns in the life sciences. Springer, Dordrecht, pp 135–173

Brigandt I (2015b) From developmental constraint to evolvability: how concepts figure in explanation and disciplinary identity. In: Love A (ed) Conceptual change in biology. Boston studies in the philosophy and history of science, vol 307. Springer, Dordrecht

Brown RL (2014) What evolvability really is. Br J Philos Sci 65:549–572
Calcott B (2009) Lineage explanations: explaining how biological mechanisms change. Br J Philos Sci 60:51–78
Eble GJ (2003) Developmental and non-developmental morphospaces in evolutionary biology. In: Crutchfield JP, Schuster P (eds) Evolutionary dynamics: exploring the interplay of selection, accident, neutrality, and function. Oxford University Press, Oxford
Grene MG, Depew DJ (2004) The philosophy of biology: an episodic history. Cambridge University Press, New York
Hendrikse JL, Parsons TW, Hallgrímmson B (2007) Evolvability as the proper focus of evolutionary developmental biology. Evol Dev 9(4):393–401
Kirschner M, Gerhart J (1998) Evolvability. Proc Natl Acad Sci 95:8420–8427
Love A (2003) Evolvability, dispositions, and intrinsicality. Philos Sci 70:1015–1027
Mayr E (1992) The idea of teleology. J Hist Ideas 53:117–135
Mills SK, Beatty JH (1979) The propensity interpretation of fitness. Philos Sci 46:263–286
Nuño de la Rosa L (2017) Computing the extended synthesis: mapping the dynamics and conceptual structure of the evolvability research front. J Exp Zool Part B Mol Dev Evol 328:395–411
Pavlicev M, Wagner GP (2012) Coming to grips with evolvability. Evol Educ Outreach 5(2):231–244
Pigliucci M (2008) Is evolvability evolvable? Nat Rev Genet 9:75–82
Sniegowski PD, Murphy H a (2006) Evolvability. Curr Biol 16:R831–R834
Sterenly K (2007) What is evolvability? In: Philosophy of Biology. Elsevier, Amsterdam, pp 163–173
Vetter B (2013) Multi-track dispositions. Philos Q 63(251):330–352
Wagner GP (2014) Homology, genes, and evolutionary innovation. Princeton University Press, Princeton
Wagner GP, Altenberg L (1996) Perspective: complex adaptations and the evolution of Evolvability. Evolution 50:967–976
Wagner GP, Pavlicev M, Cheverud JM (2007) The road to modularity. Nat Rev Genet 8:921–931
Walsh DM (2015) Organisms, agency, and evolution. Cambridge University Press, Cambridge

Typology and Natural Kinds in Evo-Devo

Ingo Brigandt

Contents

Abstract

The traditional practice of establishing morphological types and investigating morphological organization has found new support from evolutionary developmental biology (evo-devo), especially with respect to the notion of body plans. Despite recurring claims that typology is at odds with evolutionary thinking, evo-devo offers mechanistic explanations of the evolutionary origin, transformation, and evolvability of morphological organization. In parallel, philosophers have developed nonessentialist conceptions of natural kinds that permit kinds to exhibit variation and undergo change. This not only facilitates a construal of species and higher taxa as natural kinds, but also broadens our perspective on the diversity of kinds found in biology. There are many different natural kinds relevant to the investigative and explanatory aims of evo-devo, including homologues and developmental modules.

Keywords

Essentialism · Evolvability · Natural kinds · Mechanistic explanation · Morphological organization · Variational structuralism

I. Brigandt (✉)
Department of Philosophy, University of Alberta, Edmonton, AB, Canada
e-mail: brigandt@ualberta.ca

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_100

Introduction

Although typology and natural kinds are conceptually distinct issues, they have converged prominently in discussions of the nature of species and higher taxa. This is because natural kinds are traditionally deemed to be defined by essences, and critics of typology have opposed essentialism about species, arguing that species and higher taxa are not natural kinds but individuals. However, more recently, skepticism about typology and natural kinds in evolutionary biology has subsided. Part of the reason derives from scientific developments. The use of such notions as body plan in evolutionary developmental biology (evo-devo) has made the idea of morphological type reputable again (or at least more reputable) by embedding it within the agenda of mechanistically accounting for the origin and subsequent evolvability of morphological organization. In a parallel trend, philosophers have advanced nonessentialist conceptions of natural kinds that can capture variation and evolutionary change. While these conceptions permit biologists to theorize species and higher taxa as natural kinds, the more general lesson emerging from these parallel developments is that there are a diversity of biological kinds beyond taxa relevant to the investigative and explanatory aims of evo-devo.

Typology and Body Plans

The establishment and investigation of types has been a central practice in comparative morphology, especially in the tradition of structuralism, which emphasizes form and structural commonalities across taxa independent of functional considerations (see chapter ▶ "Form and Function in Evo-Devo"). Although typology has a long and venerable history, it has been controversial ever since Ernst Mayr (1959) contrasted "*typological thinking*" with "population thinking." Mayr objected to characterizing a species by means of a set of typical traits, for example, using particular plumage features and vocal traits to characterize a bird species such as *Elaenia obscura* as a whole. Instead, he argued that species should be conceived in terms of variation across individuals, which is subject to the operation of natural selection. He credited Darwin with having introduced population thinking, according to which the (statistically characterized) traits of entire species are derived exclusively from the traits of individuals. As a consequence, species traits are subject to constant change. Mayr acknowledged that some morphologists who invoked types did accept evolution, but he objected that only saltational evolution is possible from the perspective of typological thinking, whereas gradual evolution was ruled out by definition. Based on a similar criticism of traditional taxonomic methodology made by David Hull (1965), who dubbed the flawed approach "essentialism," typological thinking and essentialism often came to be viewed as synonymous labels for a misconceived (if not erroneous) way of reasoning about species,

which fails to comport with Darwinian evolutionary theory (Amundson 2005; Winsor 2006).

Essentialism links directly to the topic of *natural kinds*. Traditionally, any species taxon was deemed to be a class, defined by some set of shared properties, or a natural kind, which philosophers often viewed as having an essence. It is certainly appropriate to construe chemical elements as natural kinds, where the specific atomic number can be seen as an essence shared by all elements of the same type (e.g., oxygen atoms). However, Michael Ghiselin (1974) and David Hull (1978) challenged the view that species are classes or kinds and introduced the now dominant species-as-individuals thesis. On this view, a species taxon is an individual, a complex whole that has organisms as its parts, very much like an organism is an individual composed of various cells. Any account of the ontological nature of species has to capture three features: (i) a species is denoted by a proper name, (ii) a species is a concrete thing that occupies a certain region of space and exists during a particular period of time, and (iii) a species exhibits variation at any point in time and can undergo change across time. Ghiselin and Hull argued that abstract classes (or kinds) fail to capture these features. However, the notion of an individual appears to capture all three. Given within-species variation and evolutionary change, neither a phenotypic nor a genotypic trait could be the essence of a species construed as a natural kind. The individuality thesis, in contrast, is not committed to shared traits. In fact, the parts of any complex individual can be quite different, just as different cells that compose an organism can belong to different cell types.

In his detailed discussion of the historical roots of evo-devo, Ron Amundson (2005) addresses how Mayr and other neo-Darwinians promoted the "essentialism story" as both a historical account of previous biological traditions (with a special emphasis on their flaws), and also used it as a tool to criticize contemporary approaches. This account has been shown to be historically inaccurate. For example, although there were species fixists before Darwin, these biologists did not appeal to species possessing essences as the explanation of fixism. More important for the issue of typology, Amundson notes that whereas neo-Darwinian objections to reasoning in terms of types pertain to species, previous comparative morphologists never invoked species types; instead, morphological types were formulated for *higher* taxa. And, just like structuralist morphological investigation in general, the notion of the *unity of type* pertained to relations across various species. This was already present in the period before Darwin, such as within the tradition of transcendental morphology in Germany and Geoffroy St. Hilaire in France, and continued with subsequent traditions, some of which conducted morphological investigation and explanation explicitly within an evolutionary framework (Hall 1999; Russell 1982[1916]). Notably, soon after Darwin proposed his theory, the evolutionary morphology of Gegenbaur and Haeckel used comparative anatomical and embryological studies to establish relationships among taxa in the form of phylogenetic trees. Moreover, some conceptions of morphological types were not simply abstract descriptions of anatomical traits, but also could be invoked to explain why some structural pattern is present within a taxon or why some structural

transitions between taxa are possible (and others impossible). For example, mammals do (and must) have fewer jawbones than birds because they have more ear ossicles, which are homologous to avian jawbones (Amundson 2005). Although the vertebrate archetype advanced by Richard Owen in 1848 has been repeatedly (though inaccurately) portrayed as a Platonic type, Owen was quite open to historical transformations between species, albeit not by means of natural selection but by developmental forces guided within the explanatory framework of the archetype (Rupke 1993).

Earlier, upon its initial introduction in the eighteenth century, the Linnaean taxonomic system tended to be viewed as an arbitrary human convention. Yet the establishment of the idea of the unity of type in the first half of the nineteenth century fostered a different view – that the taxonomic system reflects relations, such as homologies, that really exist between species (Amundson 2005). This basic vision has been reinforced with the advent of modern phylogenetic systematics. Comparative studies in molecular and developmental biology have uncovered the phenomenon of "deep homology": developmental genes and mechanisms are widely conserved across taxa, including animals, plants, and other eukaryotes (see chapter ▶ "Developmental Homology"). Contemporary accounts of *body plans* contribute to the traditional idea of the unity of type by adding developmental processes and features to the structural traits shared by a taxon, e.g., the phylotypic stage of development (Hall 1999; Raff 1996; Slack et al. 1993). This evo-devo vision contrasts with what Amundson (2005) dubs the "residual conception of homology" that is common among neo-Darwinians. A residual conception looks backward phylogenetically at homologies as nothing but traits that have not (yet) been modified by natural selection. From an evo-devo perspective, however, homologous developmental processes and overall body plans are governed by *developmental constraints*, and thus have an important impact and shaping influence on future evolutionary trajectories.

In summary, although typology and the study of types has meant many different things in the complex history of comparative morphology, it is fair to say that current ideas of deep homology, developmental constraints, and body plans demonstrate that theorizing higher taxa in terms of shared developmental and morphological features is a reputable biological practice: "typology naturally emerged from the facts of evolutionary developmental biology and it would be seriously problematic to try to avoid it" (Wagner 2014: 5). Furthermore, neo-Darwinian criticisms of "typological thinking" as leading to erroneous ways of thinking about the variation and evolution of species do not carry over to higher taxa. However, the tenet that natural kinds are not the appropriate ontological category for species because they undergo evolutionary change also has been invoked for higher taxa and homologues. Some argue that an evolutionary perspective mandates that each higher taxon and each homologue be conceptualized as an individual (Ereshefsky 2009; Grant and Kluge 2004; Jenner 2006). Therefore, we need to see more clearly how recent conceptions of natural kinds can capture variation and evolutionary change.

Conceptions of Natural Kinds Capturing Variation and Evolutionary Change

Although some biologists have recently endorsed the notion of natural kinds, it is an idea originally developed by philosophers. A kind is a grouping of several objects (the members of the kind). The key issue is to distinguish between kinds where the grouping conforms to reality – so-called *natural* kinds – and where the grouping merely reflects an arbitrary human convention – sometimes called *nominal* kinds (Khalidi 2013). Thus, a kind is a "natural" kind not because its *members* are natural objects (as opposed to artifacts), but rather because the *grouping* corresponds to a division in nature (i.e., reality). The metaphor that a natural kind carves nature at its joints is often used to express this basic idea. For traditional philosophical accounts, a natural kind is characterized by an *essence* (usually a microstructural property), which has two functions. First, the essence constitutes the kind's identity: an object is a member of this kind if and only if the object possesses the essential property. Second, the essence is causally basic: this property accounts for the presence of other properties characteristic of the kind. In this respect, the essence also fulfills an explanatory function. Something is an oxygen atom if and only if it has eight protons; moreover, the atomic number essence also accounts for or explains the various chemical properties common to oxygen atoms, such as the ability to form particular bonds and participate in certain chemical reactions.

In the last three decades, philosophers of science have developed new conceptions of natural kinds that relax the emphasis on essences and are deliberately meant to capture the kinds found in biology and other special sciences (Khalidi 2013; Magnus 2012; Wilson et al. 2007). These approaches do not assume that kinds have sharp boundaries or that they are governed by exceptionless generalizations (e.g., classical laws of nature), which would prevent members of the kind from varying among each other. The most influential of these newer approaches is Richard Boyd's (1999a) account of natural kinds as *homeostatic property clusters* (HPCs). A property cluster is any set of properties that exhibit correlations with one another. Correlations are usually not perfect, so that kind members need not possess each of these properties from the cluster. This allows for internal diversity and vague boundaries for kind membership, while at the same time corresponding to the fact that a simple correlation is sufficient for the purpose of prediction, generalization, or explanation in many scientific contexts. The property correlations of an HPC must be due to some underlying features of reality in order to qualify as a natural kind, as opposed to a nominal or conventional kind. Boyd calls these underlying features "homeostatic mechanisms," which, in contrast to a classical essence, need not be a single property, but can consist in complex processes or assemblages of properties.

Importantly, the HPC kind account asserts explicitly that the properties defining a kind need not be intrinsic properties (e.g., internal structure or microstructure). Often the relevant properties are *relational* (Griffiths 1999). A good example of this situation is a higher taxon, which is defined in terms of descent from a particular ancestral species. An extant species possesses the property of "being descended from ancestor A" not because of its intrinsic, internal features, but because of how it is

(historically) related to another species in a lineage. Although this defines who is a member of the kind, the relational property of common ancestry permits variation in *other* properties, such as the genetic and phenotypic traits of different species within this higher taxon. Thus, an HPC conception of natural kinds in terms of clustered (yet imperfectly correlated) properties that includes relational properties can capture the variation exhibited by biological kinds synchronically (i.e., at one point in time) and diachronically (i.e., across time), including evolutionary change through phylogenetic history (Brigandt 2009; Wilson et al. 2007).

Using a naturalistic philosophical framework, Boyd (1999b) and many other contemporary philosophers of science base their account on actual scientific kinds and eschew any general theory of natural kinds put forward in an a priori fashion. The same orientation holds for an account of the features of a particular kind (e.g., stem cells); the composition of the homeostatic property cluster characterizing a kind is always an empirical question. Conceptualizing species as HPC kinds is a good illustration of this approach even though species taxa motivated the claim that they are individuals and could not be natural kinds. There are a number of different species concepts that could result in species taxa with different boundaries. However, whatever underlying criteria are used to define a species taxon in a certain context, the HPC approach can rely on them (Brigandt 2009). Interbreeding is one prominent way to define species. The property "being able to interbreed with" is relational; an organism possesses it only in comparison to and interaction with other organisms. The homeostatic mechanisms underlying such an HPC kind therefore include a relation, which is an interaction that permits dynamic change in intrinsic properties (such as an organism's genotype and phenotype). Thus, Boyd's label "homeostatic" should not be taken too literally – interbreeding and gene flow fully permit new variation and evolutionary change. The key point is that due to interbreeding a newly introduced genetic variant will spread within the species, resulting in some degree of species cohesion at any point in history, so as to make the kind natural rather than nominal.

The same situation obtains for other species criteria used by biologists, which can then serve as features characterizing a species taxon as an HPC kind. For instance, the ecological species concept focuses on members of a species occupying the same adaptive zone, which accounts for some degree of cohesion while permitting evolutionary change. Biologists and philosophers who maintain that species are not kinds but individuals have been largely silent on the features and mechanisms that underlie the cohesion and identity of a species. Yet proponents of the species-as-individuals thesis need to account for what makes certain organisms (but not others) count as parts of a species conceived of as an individual. (Ironically, in the history of philosophy, the ontological category of individual often has also been characterized by an essence, where an individual's essence accounts for why the individual's parts are not just a heap of objects but form a unified whole.) Foregoing a discussion of such features and mechanisms is more problematic scientifically than merely shying away from the ontological label "essence," as the next section details.

Kinds Answering to the Aims of Evo-Devo: Beyond Taxa

Following Richard Boyd's seminal account of natural kinds as homeostatic property clusters, which departs from traditional, essentialist accounts, a number of philosophers have adopted and developed the view that species and higher taxa are HPC natural kinds, and a few biologists have followed suit (Assis 2011; Franz 2005; Rieppel 2005b, 2009; Wagner 2014), even though the individuality thesis remains the dominant position. Those taking a natural kind approach typically have assumed that species can be considered as both kinds and individuals (Boyd 1999a; Brigandt 2009; LaPorte 2004; Rieppel 2007b), which brings us to the broader issue of the epistemic role of natural kinds in science.

Regardless of whether several entities are viewed as members of a natural kind or as parts of a whole (for the individuality account), there are *epistemic* benefits to grouping entities together and representing their features (Boyd 1999a; Brigandt 2009). Shared features and property correlations can be used for the purpose of generalization and prediction (see chapters ▶ "Generalization in Evo-Devo"). Some of the features may be causal dispositions or stand in causal-dynamical relations to one other (e.g., the relational properties and homeostatic mechanisms acknowledged by the HPC account). As a consequence, they can be used for the purpose of scientific explanation. A core motivation for the HPC account has been that natural kinds (in contrast to merely nominal or conventional kinds) are central to scientific inference, generalization, and explanation. Whether natural kinds play one or more of these roles depends on the particular kind at hand (e.g., whether it is primarily used for generalization or for explanation) and how concrete explanations are understood (e.g., as the description of a mechanism). In a similar attempt to move beyond the traditional "metaphysics of essentialism," Alan Love (2009) calls for an "epistemology of representation." Scientific representations and typologies (e.g., normal stages of development) may abstract away from some features and variation found in nature, which is licit as long as this is conducive to the epistemic purpose at hand. Other representations or kind concepts are used in different contexts where the features originally abstracted away from are now scientifically relevant and therefore included.

In addition to representations of natural phenomena, such as the knowledge embodied in a kind concept, an important aspect of this epistemic dimension is what particular scientific *aims, needs, and purposes* a kind concept is to serve (Brigandt 2009; Reydon 2016). Natural kinds traditionally have been assumed to cut nature's joints. However, whether something is a natural kind depends not only on metaphysical divisions in nature, but also on whether particular divisions are conducive to our epistemic agendas. As Jessica Bolker (2013: 126) puts it: "the relevance (or naturalness) of a concept depends on the epistemological context. What the term 'natural' in 'natural kinds' ought to describe is the fit between the classification scheme, and the work we want it to do." The diversity of scientific aims – and the various needs of evo-devo in particular – is a motivation to consider kinds other than species and higher taxa.

A good case in point is developmental modules, which several biologists have described as natural kinds (Rieppel 2005a; Wagner 1996). Their significance for evo-devo lies in the phenomenon of modularity, which pertains to an organism's organization both in terms of its component modules and the relations among those modules. While a module exhibits extensive internal developmental-causal connections (i.e., a form of integration), the developmental relations between modules are such that changes in one module need not significantly affect other modules (i.e., modules display relative autonomy), and one module can become developmentally linked to another module it was not connected to before. Therefore, modularity in development enables the evolutionary transformation of organismal organization and the generation of morphological novelty. These questions comprise central explanatory aims of evo-devo's research agenda. Modules (as natural kinds) may be individuated based on developmental criteria, but the choice of suitable criteria in evo-devo should be made with an eye to the evolutionary potential of modules.

Another closely related issue is the treatment of characters and homologues as natural kinds. Evo-devo researchers can view an individual homologue as a unit of evolutionary transformation, where this evolutionary potential is due to the developmental organization and processes of organisms (Brigandt 2007; Rieppel 2007a; Wagner 2001). Although some have argued that a homologue – the overall transformation lineage consisting of a part of an ancestor and the corresponding parts in the descendants – can be construed only as an individual (Ereshefsky 2009; Grant and Kluge 2004), this is another instance where a nonessentialist conception of natural kinds can capture evolutionary change. In this case, developmental processes that account for a homologue being a sufficiently individualized organismal part form the mechanistic basis of an HPC kind that can undergo evolutionary transformation independently of other homologues. Amundson's (2005) notion of "developmental types" (e.g., the vertebrate limb and its development) aligns with a natural kinds perspective on characters (Lewens 2009; Wagner 2014), while fruitfully highlighting that developmental types are hierarchically structured.

Günter Wagner (2014) uses the label "*variational structuralism*" for a modern form of typology that is enriched by evo-devo knowledge. It is explicitly dubbed "variational" because it includes the generation of structural variation across individuals and species that results in morphological evolution. As noted, some traditional approaches that endorsed typology certainly had evolution in view, but variational structuralism adds a mechanistic explanation of how morphological variation and transformation occurs developmentally to engender evolutionary change in morphological types. The research agenda is to understand the causal basis of *morphological organization*, including its hierarchical structure and developmental mechanisms. This then serves the aims of evo-devo biologists in explaining how morphological organization originates in evolution, how individual characters can vary and undergo evolutionary change (while the overall morphological organization of a taxon remains stable), and how morphological organization is transformed, such as by the addition of novel characters (Brigandt 2007; Wagner 2014; Wagner and Stadler 2003). Whether the focus is on an individual homologue (transformational character) or a body plan (organized, variational type), construing

these as natural kinds yields kinds that exhibit *evolvability* (see chapter ▶ "Evolvability"). Accounting for evolvability is a core concern for evo-devo. Evolvability is a dispositional property – it is not about the variation already found within a population (on which neo-Darwinism focuses), but about the potential to generate phenotypic variation and novelty. Given that evo-devo biologists are trying to understand how developmental potential facilitates morphological evolution, dispositional properties are common in evo-devo theorizing (see chapter ▶ "Dispositional Properties in Evo-Devo"). This is not in conflict with a natural kind perspective because many kinds are characterized by causal dispositions (even a traditional essence, e.g., a microstructural feature, can have causal capacities).

In addition to kinds that play roles in the investigation and explanation of evolutionary phenomena, natural kinds defined by purely developmental criteria also are of interest to evo-devo researchers (Nathan and Borghini 2014). Stems cells, even when restricted to those found in a single species, must be construed as a kind that exhibits significant internal variation; a similar situation holds for genes (Reydon 2016; Slater 2013; Wilson et al. 2007). Although normal stages of development obscure evolutionarily significant within-species variation in development (and an evolutionary explanation of development must view stages as subject to transformation), standardized developmental stages provide an important reference point for explanations of a species' ontogeny (Bolker 2013; Love 2009). In general, different biological kinds may be individuated by quite different conditions, including causal features that enable transformation, causal features that maintain stability, and even noncausal criteria. This ontological diversity has led philosophers to argue that not all natural kinds found in biology are HPC kinds (Ereshefsky and Reydon 2015; Khalidi 2013).

Another ontological category of relevance to evo-devo is developmental processes. The general biological significance of processes within organisms (e.g., gene regulatory networks) and between organisms (e.g., ecological interactions involving multiple microbial species) has motivated some to call for a process ontology for biology (Bapteste and Dupré 2013). A process ontology views processes as a core ontological category, often with the claim that processes are more fundamental than structures. Processes are inherently dynamic. Indeed, even though the phenomenon of developmental robustness (which is likewise an investigative and explanatory concern of evo-devo) pertains to maintaining a certain phenotypic trait in ontogeny, this stability is often due to underlying dynamic processes that reconfigure a developmental system in the face of perturbations. Processes are open-ended, without clear-cut boundaries (Rieppel 2009). This makes it difficult to individuate processes. Often epistemic considerations are used to make a contextual individuation choice (Brigandt 2017). Thus, there may be no principled way to count several processes as being of the same type or belonging to the same kind. Instead, only pragmatic considerations may suffice. More work is required to understand the significance of an ontology of processes for illuminating the epistemology of evo-devo and other biological sciences. The complexity of biological phenomena makes it plausible that ontological categories beyond the notion of natural kinds will be necessary.

Cross-References

- ▶ A Macroevolutionary Perspective on Developmental Constraints in Animals
- ▶ Developmental Homology
- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Dispositional Properties in Evo-Devo
- ▶ Evolvability
- ▶ Form and Function in Evo-Devo
- ▶ Generalization in Evo-Devo

Acknowledgments The work on this essay was supported by the Social Sciences and Humanities Research Council of Canada (Insight Grant 435-2016-0500). I thank Alan Love and Laura Nuño de la Rosa for detailed comments on an earlier version of this paper.

References

Amundson R (2005) The changing role of the embryo in evolutionary thought: roots of evo-devo. Cambridge University Press, Cambridge

Assis LCS (2011) Individuals, kinds, phylogeny and taxonomy. Cladistics 27:1–3

Bapteste E, Dupré J (2013) Towards a processual microbial ontology. Biol Philos 28:379–404

Bolker J (2013) The use of natural kinds in evolutionary developmental biology. Biol Theory 7:121–129

Boyd R (1999a) Homeostasis, species, and higher taxa. In: Wilson RA (ed) Species: new interdisciplinary essays. MIT Press, Cambridge, MA, pp 141–185

Boyd R (1999b) Kinds as the "workmanship of men": realism, constructivism, and natural kinds. In: Nida-Rümelin J (ed) Rationality, realism, revision: proceedings of the 3rd international congress of the Society for Analytic Philosophy. de Gruyter, Berlin, pp 52–89

Brigandt I (2007) Typology now: homology and developmental constraints explain evolvability. Biol Philos 22:709–725

Brigandt I (2009) Natural kinds in evolution and systematics: metaphysical and epistemological considerations. Acta Biotheor 57:77–97

Brigandt I (2017) Bodily parts in the structure-function dialectic. In: Lidgard S, Nyhart LK (eds) Biological individuality: integrating scientific, philosophical, and historical perspectives. University of Chicago Press, Chicago, pp 249–274

Ereshefsky M (2009) Homology: integrating phylogeny and development. Biol Theory 4:225–229

Ereshefsky M, Reydon TAC (2015) Scientific kinds. Philos Stud 172:969–986

Franz NM (2005) Outline of an explanatory account of cladistic practice. Biol Philos 20:489–515

Ghiselin MT (1974) A radical solution to the species problem. Syst Zool 23:536–544

Grant T, Kluge AG (2004) Transformation series as an ideographic character concept. Cladistics 20:23–31

Griffiths PE (1999) Squaring the circle: natural kinds with historical essences. In: Wilson RA (ed) Species: new interdisciplinary essays. MIT Press, Cambridge, MA, pp 208–228

Hall BK (1999) Evolutionary developmental biology, 2nd edn. Springer, Dordrecht

Hull DL (1965) The effect of essentialism on taxonomy – two thousand years of stasis. Br J Philos Sci 15:314–326 and 16:1–18

Hull DL (1978) A matter of individuality. Philos Sci 45:335–360

Jenner RA (2006) Unburdening evo-devo: ancestral attractions, model organisms, and basal baloney. Dev Genes Evol 216:385–394

Khalidi MA (2013) Natural categories and human kinds: classification in the natural and social sciences. Cambridge University Press, Cambridge

LaPorte J (2004) Natural kinds and conceptual change. Cambridge University Press, Cambridge
Lewens T (2009) Evo-devo and "typological thinking": an exculpation. J Exp Zool B Mol Dev Evol 312B:789–796
Love AC (2009) Typology reconfigured: from the metaphysics of essentialism to the epistemology of representation. Acta Biotheor 57:51–57
Magnus PD (2012) Scientific enquiry and natural kinds: from planets to mallards. Palgrave Macmillan, New York
Mayr E (1959) Darwin and the evolutionary theory in biology. In: Meggers BJ (ed) Evolution and anthropology: a centennial appraisal. Anthropological Society of Washington, Washington, DC, pp 1–10
Nathan MJ, Borghini A (2014) Development and natural kinds. Synthese 191:539–556
Raff RA (1996) The shape of life: genes, development, and the evolution of animal form. University of Chicago Press, Chicago
Reydon TAC (2016) From a zooming-in model to a co-creation model: towards a more dynamic account of classification and kinds. In: Kendig C (ed) Natural kinds and classification in scientific practice. Routledge, London, pp 59–73
Rieppel O (2005a) Modules, kinds, and homology. J Exp Zool B Mol Dev Evol 304B:18–27
Rieppel O (2005b) Monophyly, paraphyly, and natural kinds. Biol Philos 20:465–487
Rieppel O (2007a) Homology: a philosophical and biological perspective. In: Henke W, Tattersall I (eds) Handbook of paleoanthropology. Springer, Berlin, pp 217–240
Rieppel O (2007b) Species: kinds of individuals or individuals of a kind. Cladistics 23:373–384
Rieppel O (2009) Species as a process. Acta Biotheor 57:33–49
Rupke NA (1993) Richard Owen's vertebrate archetype. Isis 84:231–251
Russell ES (1982[1916]) Form and function: a contribution to the history of animal morphology. With a new introduction by G.V. Lauder. University of Chicago Press, Chicago
Slack JMW, Holland PWH, Graham CF (1993) The zootype and the phylotypic stage. Nature 361:490–492
Slater MH (2013) Cell types as natural kinds. Biol Theory 7:170–179
Wagner GP (1996) Homologues, natural kinds and the evolution of modularity. Am Zool 36:36–43
Wagner GP (2001) Characters, units and natural kinds: an introduction. In: Wagner GP (ed) The character concept in evolutionary biology. Academic, San Diego, pp 1–10
Wagner GP (2014) Homology, genes, and evolutionary innovation. Princeton University Press, Princeton
Wagner GP, Stadler PF (2003) Quasi-independence, homology and the unity of type: a topological theory of characters. J Theor Biol 220:505–527
Wilson RA, Barker MJ, Brigandt I (2007) When traditional essentialism fails: biological natural kinds. Philos Top 35:189–215
Winsor MP (2006) The creation of the essentialism story: an exercise in metahistory. Hist Philos Life Sci 28:149–174

Teleology in Evo-Devo

Denis M. Walsh

Contents

Abstract

Teleological explanations explain by appealing to purposes. Evolutionary developmental biology distinguishes itself from the orthodox Modern Synthesis by its emphasis on the contribution of organismal development to evolution. Organismal development is a manifestation of purposiveness. This suggests that organismal purposiveness has an explanatory role to play in evolutionary theory. There is thus a putative role for teleological explanations. In order to make the case for genuine teleology in biology, we must demonstrate three things: (i) that there are purposes in nature, (ii) purposes make a difference to evolution, and (iii) purposes can figure in genuine scientific explanations.

Keywords

Explanation · Teleology · Invariance · Plasticity · Novelty

D. M. Walsh (✉)
Department of Philosophy, Institute for the History and Philosophy of Science and Technology, Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, Canada
e-mail: denis.walsh@utoronto.ca

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_98

Introduction

Teleology is a mode of explanation in which the nature or presence of an event or feature is explained by appeal to the goal or purpose it subserves. It is prevalent in explanations of agents' actions and in the functional accounts of the features of artifacts. We explain, for example, why an agent performed a particular behavior, say buying a can of paint, by citing her goals (e.g., she wanted to paint a placard), and the way the behavior conduces to the fulfillment of the goal (e.g., the paint was required to make the sign). Alternatively, teleological explanations elucidate the functions of artifacts by adverting to their designers' intentions (e.g., the function of the orange dashboard light is to signify that the petrol level is low). It is widely thought that teleological explanation has no remit in the natural sciences. The nonhuman natural world has neither purposes nor designers, and so no call for explanations that cite them. The world is a world of causes. To explain the occurrence of any non-intentional natural occurrence, it is sufficient to cite its causes.

Things were not always so. Aristotle's biology, for example, and the natural philosophy of the Scholastics were imbued with purposes and goals. The science that emerged in the sixteenth and seventeenth centuries eschewed all references to natural goals and purposes. It advocated studying the natural world as one studies the workings of a machine, by investigating the causal interaction among its parts. While there was a considerable amount of debate concerning the appropriateness of the mechanical model for explaining biological phenomena (Riskin 2016), its advocates strongly argued for the applicability of mechanism to biology, and largely prevailed. Descartes, for example, explicitly invokes the notion of mechanical law, rather than purpose, in explaining the anatomy of animals.

> The number and the orderly arrangement of the nerves, veins, bones and other parts of an animal do not show that nature is insufficient to form them, provided you suppose that in everything nature acts in accordance with the laws of mechanics. (Descartes 1647 [1985] §134)

Much of the evolutionary biology of the twentieth century likewise pursued a comprehensively mechanistic program that ceded no theoretical role to purpose. Allied to the molecular revolution, the emerging Modern Synthesis theory cast evolution as the consequence of the mechanical activities of genes. More recently, however, some biologists have begun to question the adequacy of this gene-centered conception of evolution (Walsh 2015; Nicholson 2013, 2014). The impetus has largely come from an increased understanding of the active role of organisms as participants in evolution, as emphasized by evolutionary developmental biology and related disciplines (Laland et al. 2015). Organisms bias the origin of evolutionary novelties (Uller et al. 2018; Hu et al. 2019), respond adaptively to stresses in their environments (Herman et al. 2016), and pass those responses on to descendants (Lind and Spagopoulou 2018). The active role of organisms as robust, adaptive systems challenges the adequacy of the mechanism-inspired, gene-centered approach to evolution. In doing so, it calls for a reassessment – and perhaps a

rehabilitation – of teleology (Walsh 2015). The reason is that organisms are evidently purposive systems, and as evo-devo appears to demonstrate, their purposiveness makes a difference to evolution.

Rehabilitating teleology in evolutionary biology requires demonstrating three things: (i) that there are purposes in nature, (ii) that purposes make a difference to evolution, and (iii) that purposes can figure in genuine scientific explanations.

Organism and Purpose

Organisms are singular among natural things in being purposive (Walsh 2006). Indeed, the very concept of an organism is closely linked to that of purpose. Ludwig von Bertalanffy makes this point vividly:

> You cannot even think of an organism . . . without taking into account what variously and rather loosely is called adaptiveness, purposiveness, goal seeking and the like. (von Bertalanffy 1969: 45)

The challenge that the purposiveness of organisms raises to the natural sciences has long been acknowledged. Immanuel Kant, for example, argued that the defining features of organisms appear to place them beyond the ambit of scientific explanation. Kant characterized organisms as *self*-building, *self*-nourishing, *self*-reproducing entities. They are "both causes and effects of themselves." By this he meant that the component parts and processes of an organism are not merely appropriate to the production of its characteristic life activities, organisms possess their parts and process precisely *because* of their pursuit of their own purposes. The problem, as Kant sees it, is that explanations in the natural sciences do not appeal to purposes. For Kant, the paradigm of scientific explanation is to be found in Newton's physics, in which the motions of bodies are explained exclusively by appeal to mechanical laws. But, because of their purposiveness, the nature of organisms cannot be fully understood in this way.

> . . . it is quite certain that we can never adequately come to know the organized beings and their internal possibility in accordance with merely mechanical principles of nature, let alone explain them; and indeed this is so certain that we can boldly say that it would be absurd for humans even to make such an attempt or to hope that there may yet arise a Newton who could make comprehensible even the generation of a blade of grass according to the natural laws that no intention has ordered; rather, we must absolutely deny this insight to human beings. (Kant 1793 [2000]: 400)

Other thinkers agree with Kant about the purposive nature of organisms but are decidedly more sanguine about giving it a naturalistic account. The general idea is that purposive is an observable feature of a system's gross behavior. Purposive systems have a distinctive behavioral profile. They are capable of attaining and maintaining their end states robustly across a range of circumstances. They achieve

this by regulating the activities of their parts, enlisting them in the attainment of their ends. Again, von Bertalanffy argues for the naturalness of purpose:

> . . . teleological behaviour directed toward a characteristic final state or goal is not something off limits for a natural science and an anthropomorphic misconception of processes which, in themselves, are undirected and accidental. Rather it is a form of behaviour which can be well defined in scientific terms and for which the necessary conditions and possible mechanisms can be indicated. (von Bertalanffy 1969: 46)

The purposiveness – the goal-directedness – of organisms is an observable phenomenon. It is manifest in the robust, adaptive, responsiveness of organisms manifested in their development, physiology, behavior, metabolism, and immunology.

But accepting that organisms are fundamentally purposive and that their goal-directedness is an unproblematically natural, explainable phenomenon, falls well short of a rehabilitation of teleology. Advocates of natural teleology in evolution must further demonstrate that organismal purposiveness makes a difference to the evolutionary process.

Purpose in Evolution

Twentieth-century evolutionary biology is distinguished by a preoccupation with the dynamics of supra-organismal assemblages (populations) of suborganismal entities (genes). The distinctive properties of organisms, particularly those manifested in their development, have played at best a peripheral role in the explanation of evolutionary dynamics. The Modern Synthesis solution to the problem of the purposiveness of organisms is to pass it off as illusory, or simply to treat it in the manner of any other organismal property. That is to say, the orthodox Modern Synthesis approach seeks to explain the adaptive purposiveness of organisms by appeal to the selection of variant genes.

Perhaps the marginalization of organisms was motivated by Ernst Mayr's famous distinction between proximate and ultimate explanations (see chapter ► "Proximate Versus Ultimate Causation and Evo-Devo"). Explanations of why populations of organisms exhibit the range of character traits they do advert to what Mayr called "ultimate causes" (selection and drift inter alia), the processes that bring about change to populations in evolutionary time. These processes tell us why populations are structured in the way they are. By contrast, the processes by which individual organisms attain their characteristics, in particular development, merely tell us *how* organisms come to be, and play no part in the explanation of "ultimate" processes. This division of explanatory labor between the ultimate, so-called "evolutionary" processes and the proximate merely "biological" ones served to debar development (a "biological" process) from any serious contribution to the study of evolution. The great boon of gene-centered evolutionary biology lay in the fact the study of evolution had no need to consider the complexities of

those processes that occurred within organisms, particularly development. Gene-centered evolutionary biology made it "...possible to understand genetics, and hence evolution, without understanding development" (Maynard Smith 1982: 6). It is hardly surprising the prevailing sentiment at the time was that the "... problems concerned with the orderly development of the individual are unrelated to those of the evolution of organisms through time..." (Wallace 1986: 149).

Evo-devo is set apart from the orthodox Modern Synthesis by its insistence upon the significance of those processes that occur within an individual organism's ontogeny to the process of evolution. In this it sets itself apart from the adaptationist, gene-centrist thinking that dominated much of the later twentieth-century evolutionary biology. Evo-devo has ushered in a marked shift in the perceived significance of the processes that occur within individual organisms to the process of evolution (see chapter ▶ "Evo-Devo's Contributions to the Extended Evolutionary Synthesis").

> The explanation of adaptive change as a population-dynamic event was the central goal of the Modern Synthesis. By contrast, evo–devo seeks to explain phenotypic change through the alterations in developmental mechanisms (the physical interactions among genes, cells and tissues), whether they are adaptive or not. (Müller 2007: 945–946)

Under the auspices of evo-devo, biologists have come to see development as an integral contributor to evolution. This altered perspective requires taking seriously the contribution to evolution of those purposive processes that distinguish individual organisms (Walsh 2010).

Adaptive evolution requires a number of conditions. It needs relative constancy of form across generations (inheritance), a source of evolutionary novelties, the development of organisms, and a process that biases form. One of the principal findings of evo-devo and related disciplines is that the purposiveness of organisms is implicated in each of these conditions (Walsh 2015; see chapter ▶ "Dispositional Properties in Evo-Devo").

A prominent feature of organismal development is its plasticity (see chapter ▶ "Developmental Plasticity and Evolution"). "Phenotypic plasticity is a ubiquitous, and probably primal phenomenon of life" (Wagner 2011: 216). Phenotypic plasticity consists in the capacity of the developing organism to make compensatory adaptive responses to its conditions. Genetic, epigenetic, or environmental perturbations that might otherwise cause the organism to fail are accommodated during development. Plasticity is manifested not just in the ability of an organism to change in response to its conditions, but also to maintain its structure and function across a range of circumstances. This feature of plasticity is usually called "robustness." "Robustness is the capacity of organisms to maintain constant form and function across a range of circumstances" (Kitano 2004). It is integral to the viability of organisms.

> The organism is not robust because it is built in such a manner that it does not buckle under stress. Its robustness stems from a physiology that is adaptive. It stays the same, not because it cannot change but because it compensates for change around it. The secret of the phenotype is dynamic restoration. (Kirschner and Gerhart 2005: 108–109)

The plasticity and robustness of development are facets of an organism's purposiveness.

These purposive capacities are manifest at practically all levels of organization, from gene networks, to genomes, to cells to entire organisms. And their importance for evolution is increasingly becoming recognized.

Novelty

According to Modern Synthesis orthodoxy, evolutionary novelties arise only through the mutation and recombination of genes (see chapter ▶ "Developmental Innovation and Phenotypic Novelty"). These processes, especially mutation, are unbiased with respect to the fitness of the organism in which they occur. Recent developmental biology challenges this genes-first, random approach to the origin of evolutionary novelties. As organismal development is highly plastic, it is capable of producing new phenotypes in response to its genetic and environmental circumstances in ways that maintain the viability of the organism. These novelties are held in place by the plasticity of development and can be recurrent and transgenerationally stable (West-Eberhard 2003). Yet, they do not require genetic change. "Adaptive phenotypic adjustments to potentially disruptive effects of the novel input exaggerate and accommodate the phenotypic change *without genetic change*." (West-Eberhard 2005: 613, emphasis in original). Mary Jane West-Eberhard speculates that plasticity is the principal cause of phenotypic novelty, even where those novelties are initiated by mutation.

> Responsive phenotype structure is the primary source of novel phenotypes. And it matters little from a developmental point of view whether the recurrent change we call a phenotypic novelty is induced by a mutation or by a factor in the environment. (West-Eberhard 2003: 503)

Plasticity does more than merely produce phenotypic novelties; it facilitates the evolution of complex adaptations through the adaptive coordination of an organism's subsystems. The evolution of complex adaptations involves the adaptive orchestration of all manner of anatomical and physiological systems. For example, the evolution of a functional wing from a generalized tetrapod forelimb precursor requires coordinated changes to bone structure, muscle insertion and origin, muscle mass, innervation, circulation, integument. The plasticity of development facilitates this integration. "In contrast to the rapid response produced by plasticity, if the production of newly favored phenotypes requires new mutations, the waiting time for such mutations can be prohibitively long and the probability of subsequent loss through drift can be high" (Pfennig et al. 2010: 459–460). It would appear, then, that contrary to Modern Synthesis orthodoxy, the production and maintenance of evolutionary novelties is largely the consequence of the plasticity inherent in organismal development. Plasticity, in turn, is a consequence of organismal purposiveness.

Adaptive Bias

Not only does organismal purposiveness innovate, it also introduces an adaptive bias to form. On the orthodox Modern Synthesis view, natural selection is the only process that biases evolutionary change. Yet, as we have seen the plasticity of development introduces and retains adaptive novelties. Plasticity also facilitates adaptive evolutionary change. It does so by a range of means. It buffers organisms against the possibly deleterious effect of mutations. At the same time, it acts as a capacitor for adaptive change by storing up genetic variation that is poised to be exploited in new circumstances (Wagner 2011). As Kirschner and Gerhart note, the robustness of development promotes adaptive evolution in three distinct ways.

> (1) it maximizes the amount of phenotypic variation for a given amount of genotypic variations; (2) it minimizes the lethality of phenotypic variation; (3) it produces phenotypic variation that is most appropriate to the environmental conditions, even conditions never before encountered in the lineage. (Kirschner and Gerhart 2010: 262)

An organism's purposive activities further promote adaptive evolution in myriad ways. Not only do organisms construct their environments in ways that direct their own evolution (Laland et al. 2015), they ameliorate the potential negative effects of their environments. For example, Sultan (2015) and Herman et al. (2016) document the way in which plants respond adaptively to stress through the establishment of environmentally induced epigenetic markers (see chapter ► "Eco-Evo-Devo"). These adaptive responses are sustained across generations. The adaptive bias of evolution is not due to selection alone (Uller et al. 2018; Hu et al. 2019). A significant portion is contributed by the purposive adaptiveness of organisms.

Constancy of Form

The Modern Synthesis theory of evolution has redefined inheritance. Whereas for Darwin and early evolutionary thinkers, inheritance was a gross pattern of similarities and differences across generations, in the Modern Synthesis it has become the transmission of replicated entities. It is one of the great empirical wagers of the Modern Synthesis that the transmission of replicators is adequate to account for the pattern of transgenerational similarity and difference required for adaptive evolution. A central motivation for this canon is the inherent stability of genes. Thanks to the stability of genes, high fidelity copies of the information required to build an organism are passed from parent to offspring.

This precept of the Modern Synthesis is the subject of heightened scrutiny. There are two forms of challenge. The first challenge points to the multiplicity of channels through which form is replicated across generations (Jablonka and Lamb 2010). The second challenge arises from the observation that genomes are not inherently stable. They are altered, maintained, and engineered by the processes of the organism and the cell (Shapiro 2013).

It is here that the purposiveness of cells and organisms contributes to the transgenerational stability of form. The activities of the genome are under the control of the cell. Cells, and entire organisms, react to their circumstances, through metabolic, endocrine, and immune responses. These responses, in turn, structure the way the cell uses the genome as a resource.

> DNA sequences do absolutely nothing until they are triggered to do so by a variety of transcription factors, which turn genes on and off by binding to their regulatory sites, and various other forms of epigenetic control, including methylation of certain cytosines and interactions with the tails of the histones that form the protein backbone of the chromosomes. All of these, and the cellular, tissue and organ processes that determine when they are produced and used, 'control' the genome. (Noble 2012: 57)

Furthermore, cells respond to genetic perturbations by mounting a cascade of DNA damage responses (Ciccia and Elledge 2010). This sort of responsive genome engineering is ubiquitous in cells.

> Cells operate under changing conditions and are continually modifying themselves by genome inscriptions. . .This cell-active view of genome change applies to all scales of DNA sequence variation, from point mutations to large-scale genome rearrangements and whole genome duplications. (Shapiro 2013: 287)

We cannot understand the way that genes, cells, whole organisms, or environments contribute to the development of an organism unless we understand how genomes react to these influences. That in turn requires the understanding of genome constancy and function as a consequence of the adaptive, responsive activities of cells and organisms.

> At the very least, new perceptions of the genome require us to rework our understanding of the relation between genes, genomes and genetics. . . . it has turned our understanding of the basic role of the genome on its head, transforming it from an executive suite of directorial instructions to an exquisitely sensitive and reactive system that enables cells to regulate gene expression in response to their immediate environment. (Keller 2014: 2425)

The reactive, adaptive dynamic control of the genome is the hallmark of a goal-directed, purposive system. Organismal purposes, then, are reflected in the dynamics of genomes.

For reasons surveyed above, there is a growing conviction among some biologists and philosophers of biology that organisms as adaptive purposive systems participate in the process of evolution (Nicholson 2014; Walsh 2015). A significant number of evolutionary processes occur because organisms are capable of bringing about changes to their form or maintaining their form, because these changes/maintenances contribute to the organism's capacities to pursue their ways of life. So, purposiveness makes a difference. There is, however, a further impediment to the widespread acceptance of explanations that appeal to organismal purposes. Teleological explanations – explanations that appeal to purposes – are generally thought to be off-limits to the natural sciences.

Teleological Explanation

There is a standard battery of objections to teleological explanation (Walsh 2015; see chapter ▶ "Explanation in Evo-Devo"). They all point to the presumptive inability of goals or purposes to explain. One common objection claims that goals or purposes are putative states of affairs with intrinsically evaluative properties. They are, so the objection goes, states of affairs that systems ought to attain. But natural states of affairs are not intrinsically normative in this way; nothing in nature happens because it ought to. So, there are no goals or purposes in nature to explain anything. Another objection charges that teleological explanations invoke intentions, the beliefs, and desires of agents. So, teleological explanations may apply to cognitive agents. But organisms, for the most part, do not have cognitive states, and so cannot take part in the pursuit of goals. Yet another common complaint is that teleological explanation posits some sort of occult form of causation by nonactual states of affairs. In a teleological explanation, goals explain the means to their attainment. Yet, at the time that the means occurs, the goal is as yet an unactualized, future event. Teleology, implausibly, requires backward causation, or causation by nonactualia.

These standard objections are easily countered. First, goals and purposes are in no way unnatural, intrinsically normative states of affairs. Being a goal is not an intrinsic property of a state of affairs at all. Rather, it is a complex relational property, the property of being a state of affairs to which a goal directed process aims. As discussed above, the goal-directedness of a process is an observable feature of its gross dynamics. Goal-directedness has a distinctively diagnostic signature. Goal-directed processes tend to attain and maintain their goals through robust, plastic, adaptive responses to their conditions, and this can be observed. Immunity, metabolism, the viability of development consist in the organism mounting adaptive responses to perturbations. Goal-directedness, as an observable phenomenon, requires no cognition. It simply requires that the system has a certain behavioral repertoire, and that it is capable of preferentially implementing those elements of its repertoire that would produce the stable endpoint across a wide range of circumstances. If goal directed behavior is observable in this way and can be achieved by noncognitive systems, then explaining the activities of a goal-directed system does not require attributing intentions to it. As Aristotle (1996) remarked "It is absurd to suppose that purpose is not present because we do not observe the agent deliberating" (*Physics II.8*) (Cooper 1987; Walsh 2006). Finally, teleological explanation requires no invocation of occult or backwards causation. Goals are not required to *cause* the occurrence of their means, merely to explain them. However, the way that goals might explain their means without causing them requires a little explication.

The simplest way to understand how goals might explain their means is by pointing to a structural similarity between the relation between means and ends on the one hand and causes and effects on the other. We may ask what properties does the relationship between causes and effects have such that we can explain effects by citing causes. The answer provided by Woodward is invariance (Woodward 2001). Invariance is a relation of counterfactual dependence.

> On my view, the key feature that a generalization must possess if it is to figure in explanations is invariance. Invariance is a kind of robustness or stability property: a generalization is invariant if and only if it would continue to hold under some range of physical changes involving intervention. (Woodward 2001: 4)

Woodward cashes this out in terms of a causal model in which *X* is the explanans term and *Y* is the explanandum. When this relation holds, we can see the way the value of the explanandum term, *Y*, counterfactually depends upon the value of the explanans, *X*. Were *X* to have taken a different value, *Y* would have also. In this way, the invariance relation answers the question "what would have been different, with respect to the effect, *Y*, if things had differed with respect to *X*?" It also tells us what would have to be the same, in counterfactual circumstances, for *Y* to occur.

Woodward's suggestion, then, is that to explain the occurrence of an event is (in part) to identify it as an instance of a robust counterfactual supporting regularity. The explanans, on this view, is not merely an event that correlates with the explanandum event. It is an event that makes a difference to whether or not the explanandum event would occur and how it would occur, across a range of possible circumstances. Causes, of course, are the type specimen of such difference makers. Causes explain their effects because effects regularly occur given their causes. Moreover, variation in causes corresponds with variations in effects. Changing background conditions, while holding other causes constant, also yields a systematic change in the occurrences of effects. In its general outline, this is now the most common approach to understanding causal explanation.

Where the event occurs as part of a purposive system's pursuit of its goals, there is an invariance relation between the event and the goal (Walsh 2015). Take, for example, thermoregulation. The objective of the endotherm thermoregulatory system is to maintain the organism at a temperature that is optimal for its metabolism and behavior. The goal state here is temperature equilibrium. Any endotherm possesses a range of activities (a repertoire) that it may call upon in order to maintain the goal of its appropriate temperature. These activities may be behavioral (seeking shade), or metabolic (sweating, panting), or physiological (producing melanin). This is a distinctive form of invariance relation. We know, for instance, that were the ambient temperature to rise, our sebaceous glands would become more active, and we would seek shade. Here the goal state – the maintenance of metabolic equilibrium – explains why the specific features of the endotherm repertoire – sweating, shade-seeking – are implemented.

More schematically, if a system's goal is e_1, and implements behavior, c_1, as a means to the attainment of the goal, then we can explain why c_1 occurred by appeal to e_1. Clearly, if the system is successful, c_1 causes e_1; e_1 counterfactually depends upon c_1. But there is another significant counterfactual supporting relation here too. As discussed above, purposive systems are capable of reliably attaining and maintaining their goals across a range of circumstances. This capacity entails two crucial features of the relation between goals and their

effects. The first is that, given the background conditions, were the system to have the same goal, e_1 in some counterfactual circumstances, it would reliably produce the same means, c_1. (An endotherm will reliably produce the same thermoregulatory response in the same conditions.) In sufficiently different background conditions, given the same goal, the system would predictably implement some *other* means, c_2. (In some circumstance, vasodilation, c_1, may be a more appropriate thermoregulatory response, and in others, shade-seeking, c_2, behavior may be.) The alternative means to the same end, c_1 and c_2, are related in a systematic way. They are both ways of attaining e, in their respective background conditions. The second feature of the relation is that were the system to have a *different* goal, e_2, then it would implement different means, c_2, to its attainment. (If an organism's set point changes, for example, during a fever, it will implement different strategies for maintaining its thermal equilibrium.) The behavior of a purposive system, then, is counterfactually dependent upon its goals. This relation between ends and means is the precise analogue of the relation between causes and effects. Just as there is a causal invariance relation between c_1 and e_1 (in the sense that e_1 counterfactually depends on c_1), there also is a purposive invariance relation between e_1 and c_1 (in the sense that c_1 counterfactually depends on e_1). Goal directed systems reliably bring about the means, c_1 to their ends, e_1.

If invariance relations underwrite explanations, then the activities of a goal-directed system should be susceptible to two different kinds of explanation. In biological systems, there is the standard causal explanation in which the occurrence of the cause (means) c_1 accounts for the occurrence of the effect (goal), e_1. There is, furthermore, a teleological explanation, in which the system's goal, e_1, accounts for the occurrence of the means, c_1. These are different explanations, of course (Walsh 2015). They tell us something quite different about the relation between c_1 and e_1. The causal explanation tells us that e_1 occurred because it was caused by c_1. But it does not tell us much about the occurrence of c_1. It does not tell us how likely or robust the occurrence of c_1 is. For its part, the teleological explanation tells us something crucial about the occurrence of c_1. It says that under the circumstances, c_1 was likely to occur, precisely because it is conducive to the production of the goal-state e_1. In effect, the causal explanation tells us how e_1 occurred, while the teleological explanation tells us why c_1 occurred. Because they tell us different things about the behaviors of a purposive system, we cannot replace the teleological explanation by the corresponding causal explanation without a loss of understanding. The upshot is that purposive systems license a special mode of explanation – teleological explanations – that do not apply to nonpurposive systems.

In this way, teleology is a perfectly natural and scientifically respectable form of explanation. It exploits the same form of invariance relations upon which causal explanations depend. Contrary to Modern Synthesis orthodoxy, teleological explanations do not require the positing of occult backward or nonactual causation. Nor are they committed to intrinsically normative states of affairs. Furthermore,

they do not attribute intentional states to noncognitive systems. There should be no impediment to the rehabilitation of teleological explanations in the natural sciences.

Conclusion

Teleological explanations have long been thought to be unacceptable in the natural sciences. The methodology of the natural sciences ushered in under the auspices of the Scientific Revolution allowed no place for explanations that appeal to purposes. And yet purposiveness is an observable phenomenon in the natural world. Organisms are the very paradigms of purposiveness. They build themselves out of the materials they themselves synthesize; they regulate their own structure and function. The significance of organismal purposiveness to evolution is particularly evident in their ontogeny. One of the signal achievements of evolutionary developmental biology and cognate fields of research is the demonstration of the difference that organismal purposive makes to the constancy of form, the direction of adaptive change in evolution, and the origin of evolutionary novelties.

Organismal purposes may make a difference to evolution, but this difference cannot be exploited in evolutionary biology if explanations that advert to purpose are scientifically unacceptable. I have argued that teleological explanation is a legitimate and wholly autonomous form of scientific explanation. In its structure, it is analogous to causal explanations, in that it exploits invariances. In a causal explanation, an invariance relation holds between the cause and effect; that is to say, effects counterfactually depend upon their causes. Analogously, in a teleological explanation, an invariance relation holds between goals and their means; that is to say, means counterfactually depend upon their goals. Purposive systems have the capacity to bring about actions (or states of affairs) in the world *because* those actions are conducive to the attainment of their goals. This capacity underwrites our ability to explain occurrences by citing the goals that they subserve.

Cross-References

- ▶ A Macroevolutionary Perspective on Developmental Constraints in Animals
- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Developmental Plasticity and Evolution
- ▶ Dispositional Properties in Evo-Devo
- ▶ Eco-Evo-Devo
- ▶ Evo-Devo's Contributions to the Extended Evolutionary Synthesis
- ▶ Explanation in Evo-Devo
- ▶ Mechanisms in Evo-Devo
- ▶ Proximate Versus Ultimate Causation and Evo-Devo

Acknowledgments I would like to thank Laura Nuño de la Rosa, Alan Love, and an anonymous referee for very helpful comments and suggestions.

References

Aristotle (1996) Physics (trans: Waterfield R). Oxford: Oxford University Press
Ciccia A, Elledge SJ (2010) The DNA repair response: making it safe to play with knives. Mol Cell 40:179–204
Cooper JM (1987) Hypothetical necessity and natural teleology. In: Gotthelf A, Lennox J (eds) Philosophical issues in Aristotle's biology. Cambridge University Press, Cambridge, pp 243–274
Descartes R (1647 [1985]) In: Cottingham J, Stoothoff R, Murdoch D (eds) Principia Philosophiae. Excerpted from selected works. Cambridge University Press, Cambridge
Herman JJ, Sultan S, Horgan-Kybelski T, Riggs C (2016) Adaptive transgenerational plasticity in an annual plant: grandparental and parental drought stress enhance performance of seedlings in dry soil. Integr Comp Biol 52:77–88
Hu Y, Linz DM, Parker ES, Schwab DB, Casasa S, Macagno ALM, Moczek AP (2019) Developmental bias in horned dung beetles and its contributions to innovation, adaptation, and resilience. Evol Dev 2019:1–16
Jablonka E, Lamb M (2010) Transgenerational epigenetic inheritance. In: Pigliucci M, Müller GB (eds) Evolution: the extended synthesis. MIT Press, Cambridge, MA, pp 137–174
Kant I (1793 [2000]) Critique of the power of judgment (trans: Guyer P, Matthew E). Cambridge University Press, Cambridge
Keller EF (2014) From gene action to reactive genomes. J Physiol 592(11):2423–2429
Kirschner M, Gerhart J (2005) The plausibility of life: resolving Darwin's dilemma. Yale University Press, New Haven
Kirschner M, Gerhart JC (2010) Facilitated variation. In: Pigliucci M, Müller GB (eds) The extended synthesis. MIT Press, Cambridge, MA, pp 253–280
Kitano H (2004) Biological robustness. Nature Rev Gene 5:826–837
Laland KN, Uller T, Feldman MW, Sterelny K, Müller GB, Moczek A, Jablonka E, Odling-Smee J (2015) The extended evolutionary synthesis: its structure, assumptions and predictions. Proc R Soci B Biol Sci. https://doi.org/10.1098/rspb.2015.1019
Lind MI, Spagopoulou F (2018) Evolutionary consequences of epigenetic inheritance. Heredity 21:205–209
Maynard Smith J (1982) Evolution and the theory of games. Cambridge University Press, Cambridge
Müller GB (2007) Evo-Devo: extending the evolutionary synthesis. Nat Rev Genet 8:943–949. https://doi.org/10.1038/Nrg2219
Nicholson DJ (2013) Organisms ≠ machines. Stud Hist Phil Biol Biomed Sci 44(4):669–678
Nicholson DJ (2014) The return of the organism as a fundamental explanatory concept in biology. Philos Compass 9(5):347–359
Noble D (2012) A theory of biological relativity: no privileged level of causation. Interf Focus 2:55–64. https://doi.org/10.1098/Rsfs.2011.0067
Pfennig DW, Wund MA, Snell-Rood EC, Cruickshank T, Schlichting CD, Moczek AP (2010) Phenotypic plasticity's impacts on diversification and speciation. Trends Ecol Evol. https://doi.org/10.1016/j.tree.2010.05.006
Riskin J (2016) The restless clock: a history of the centuries-long argument over what makes living things tick. University of Chicago Press, p 544
Shapiro JA (2013) How life changes itself: the Read-Write (RW) genome. Phys Life Rev 10(3):287–323
Sultan S (2015) Organism and environment: ecological development, niche construction, and adaptation. Oxford University Press, Oxford

Uller T, Moczek AP, Watson RA, Brakefield PM, Laland KN (2018) Developmental bias and evolution: a regulatory network perspective. Genetics 209(4). https://doi.org/10.1534/genetics.118.300995
von Bertalanffy L (1969) General systems theory. George Barziller, New York
Wagner A (2011) The origin of evolutionary innovations: a theory of transformative change in living systems. Oxford University Press, Oxford
Wallace B (1986) Can embryologists contribute to an understanding of evolutionary mechanisms? In: Bechtel W (ed) Integrating scientific disciplines. M. Nijhoff, Dordrecht, pp 149–163
Walsh DM (2006) Organisms as natural purposes: the contemporary evolutionary perspective. Stud Hist Phil Biol Biomed Sci 37:771–791
Walsh DM (2010) Two neo-Darwinisms. Hist Philos Life Sci 32:317–339
Walsh DM (2015) Organisms, agency, and evolution. Cambridge University Press, Cambridge
West-Eberhard MJ (2003) Developmental plasticity and evolution. Oxford University Press, Oxford
West-Eberhard MJ (2005) Phenotypic accommodation: adaptive innovation due to developmental plasticity. J Exp Zool (Mole Dev Evo) 304B:610–618
Woodward J (2001) Explanation and invariance in the special sciences. Br J Philos Sci 51:107–254

Part V

Evo-Devo of Basic Mechanisms

Devo-Evo of Cell Types

Günter P. Wagner

Contents

Abstract

Cell types are populations of cells that are developmentally individuated, adapted to perform a specific set of functions and form lineages of evolutionary descent. Cell types originate in evolution as a major way of how the bodies of multicellular organisms increase in complexity. The origin and evolution of cell types is thus a central issue in evolutionary developmental biology. In this chapter, the cell type concept is explained from the developmental and evolutionary perspective. Developmentally, cell type identity is determined by the activation of a core gene regulatory network which produce transcription factor proteins that form a cell type-specific core regulatory complexes. In evolution, cell types originate most likely from differentiation from an ancestral cell type. As a consequence, cell types

G. P. Wagner (✉)
Department of Ecology and Evolutionary Biology, Systems Biology Institute, Yale University, New Haven, CT, USA
e-mail: gunter.wagner@yale.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_153

form lineages that are connected by a tree to descent similar to a gene tree of genes related by gene duplication. To unravel the molecular mechanisms of cell type origination is a major current challenge of evolutionary developmental biology.

Keywords

Cell types · Cell type identity · Core regulatory network · Core regulatory complex · Sister cell type model

Introduction

The cell is the fundamental unit of life. All forms of life depend directly or indirectly on one or the other form of cellular organization. Indirect dependency on cellular organization refers to viruses, which are not cells themselves, but nevertheless depend on the cellular organization of their hosts that they parasitize. The development and evolution of complex organisms, mainly animals and plants, depend on the differentiation of cells into cell types that perform specialized functions in the body. One of the first cell type differentiations in the history of multicellular life was the differentiation between germ line cells, specialized for reproduction, and soma cells, dedicated to locomotion, acquisition of food and for defense (Buss 1987; Michod 1999; Michod and Herron 2006). The further elaboration of body plans is tightly linked to an increase in the number of different cell types (Valentine et al. 1994; Arendt 2008; Arendt et al. 2016; Wagner 2014; Erwin 2015). Humans have at least 411 morphologically recognized cell types (Vickaryous and Hall 2006), although that number is likely a gross underestimate. In contrast, the anatomically simplest free-living multicellular animal (*Trichoplax adhaerens*) has only 5–6 morphologically recognized cell types (Syed and Schierwater 2002; Smith et al. 2014), although single cell gene expression analysis suggests the existence of many more cell types in this organism, in particular different peptidergic gland cells (Varoqueaux et al. 2018; Sebé-Pedrós et al. 2018). Hence, it is clear that the evolution of animal body plans has occurred, in part, by the evolution of novel cell types. Cell type evolution is thus a central topic in the study of developmental evolution, i.e., the study of how development contributes to patterns of evolution. What is known about this process is the subject of this short summary of cell type development and evolution in animals.

The Three Dimensions of the Cell Type Concept

Traditionally, cell types have been recognized by their distinct morphology that, in many cases, reflects their specialized function: skeletal muscle cells, bone cells, neurons, etc. (Ross and Pawlina 2011). These cell types arise during development through the proliferation of undifferentiated or minimally differentiated embryonic cells that eventually undergo so-called terminal differentiation to different cell types.

The latter statement is true for most forms of development, namely those where the embryo grows from a single cell, either a fertilized egg cell or an unfertilized cell in the case of parthenogenesis. There are few exceptions to this mode of development, as for instance gemmules of sponges which are assembled by a number of cell types (Simpson 1984; Brusca et al. 2016), or body fragmentation, which is a form of asexual reproduction as seen in some flatworms and annelids (Brusca et al. 2016). Hence, cell types also need to be understood through their developmental origins, i.e., the genetic mechanisms that enable them to become specialized for different functions and to express different sets of genes. Finally, identical cell types can be identified in different species, i.e., there are homologous cell types in different species. Finding homologous cell types in different species implies that cell types are inherited during evolution and have their own evolutionary history of origination and modification. Consequently, we need to understand cell types along three dimensions: function and morphology, development, as well as evolution.

Function and Morphology

Traditionally, cell types have been recognized by their distinct morphologies (Fig. 1a). Nerve cells are in most cases dendritic, i.e., have many projections extending from their cell body. These projections can be classified into dendrites and axons, where dendrites and the cell body are the receiving end of the neuron and the axon and its pre-synaptic elements are delivering stimulation to other neurons as well as muscle and gland cells. On the other hand, skeletal muscles are long tubes of cytoplasm that develop through the fusion of myoblast cells and which have a distinct striated pattern of cytoskeletal proteins responsible for their ability to contract. The different structures of distinct cell types reflect the specialized function they are "molded" for by natural selection. Morphology and function go tightly hand in hand, at least in many easily recognizable cell types. From that fact we learn that cell types are populations of cells dedicated to different functional roles.

A purely functional/morphological classification of cell types, however, is adequate only if we focus on one or a few closely related species, say humans and primates. The situation becomes more complicated when the comparison of cellular architecture is broadened to include more distantly related animals in two ways: (1) functions that are performed by distinct cell types in one group of animals can be performed by one and the same cell type in some other animals; (2) cell types can change their function in evolution, i.e., the same (homologous) cell type can have different physiological functions in different animals. By homologous cell types we mean cell types in two species that evolved from the same cell type of the most recent common ancestor of these two species.

An example of multifunctional cell types is the so-called epitheliomuscular cell. In most animals with more complicated body plans like insects and mammals, contractility is performed by a group of cell types called muscle cells. There are different kinds of muscle cells, skeletal, smooth and heart muscles, but the point is that most routine contractile function (locomotion, blood pumping, extrusion of eggs

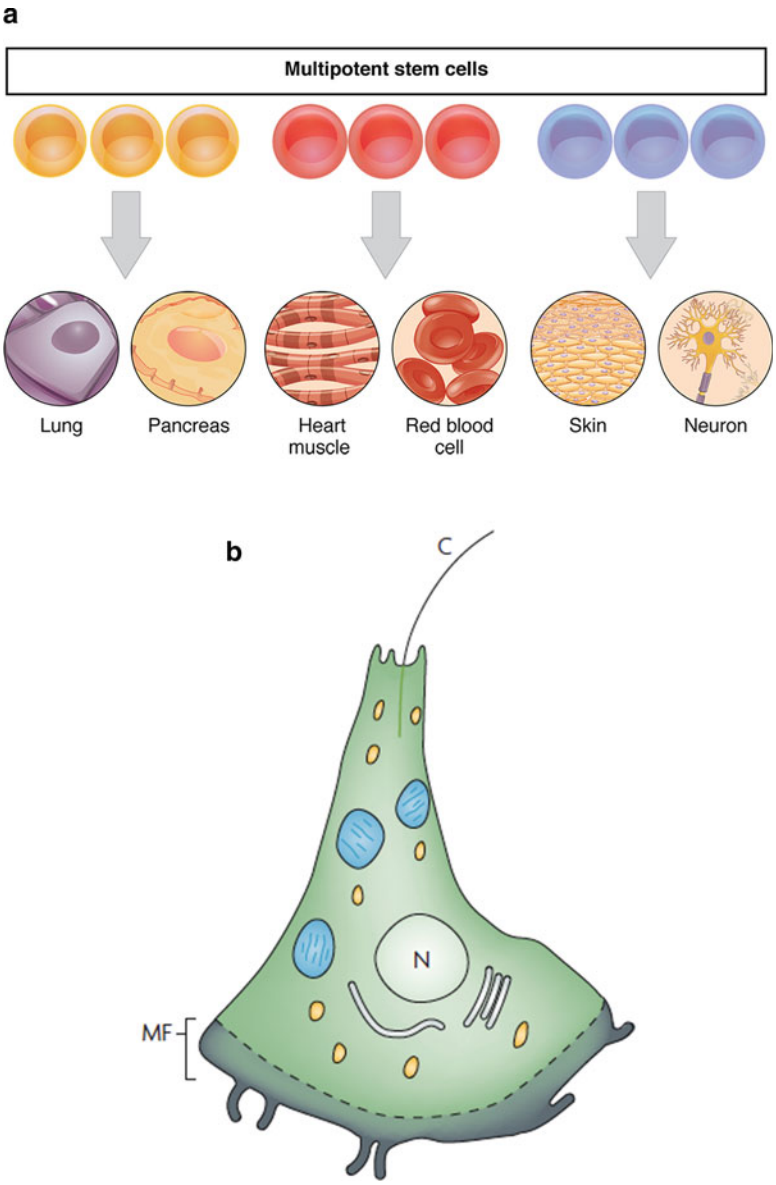


Fig. 1 Traditional notion of cell types. (**a**) Cell types have been classified on the basis of their different morphologies that largely reflect their functions. (**b**) Schematic of an epitheliomuscular cells of a cnidarian polyp. This is an example of a primitive cell type that performs several functions that, in more derived species, are performed by several specialized cell types. It is an epithelial cell, which is thus forming a barrier between the body and the environment [or between different parts of the body], but it also has contractile fibers at its base, which act like muscle cells. In addition, it can act as a secretory cell, see the yellow vesicles. The idea of the sister cell types model (Fig. 8) is that ancestrally many functions were performed by a few cell types and novel cell types arose through

or babies etc.) is performed by one or the other kind of muscle cell. Other cell types are specialized for separating spaces in the body (e.g., the walls of blood vessels) and separating the body cavity from the environment (e.g., "skin" or epidermis), or for production and secretion of digestive enzymes. These are the various kinds of epithelia. In other animals, say hydra and some other invertebrates, these functions, contraction and epithelial functions, are performed by one and the same cell type, i.e., an epithelial cell that has a part dedicated to contraction, a so-called epithelio-muscular cell (Fig. 1b).

The existence of these multifunctional cells, in particular in animals with a simpler body plan, also teaches us an important lesson about how cell types can originate in evolution. Novel cell types can originate without performing a novel function, since the functions can often be found to be performed by multifunctional cell types in other species. Rather one mode of how new cell types arise is through the segregation of functions, where the functions of the ancestral cell are segregated onto two different cell types in a more derived form. This is a form of "division of labor," from the ancestral onto distinct derived cell types. This observation leads to a model for the origin of cell types, called the sister cell type model, discussed below. Nevertheless, it is also possible that novel cell types arise through the evolution of novel functions, as suggested by the existence of cell types with functions that are not obviously also performed by ancestral cell types, such as the cnidocytes (stinging cells of jellyfish) of cnidarians. Although in these cases one has to be careful to not confuse the origin of a novel cell type with the origin of a novel cellular function. For instance, it is possible that cnidocytes are transformed secretory cells, where what is novel is the acquisition of the cnida (the stinging capsule of stinging cells) by a preexisting cell type. In this case, no new cell type identity has arisen, but a preexisting cell type acquired a novel function and shape.

The other complication is change of function. Any part of the body can change its function in the course of evolution. For instance, paired appendages in vertebrates (fins/limbs) first originated for aiding navigation by an aquatic vertebrate, a "fish" in the broad sense. Then they became modified for locomotion on land (i.e., fins were transformed into limbs), and in some groups (birds, bats, and pterodactyls), the forelimb got modified for flight (i.e., they became wings). The same is true for cell types, although the examples are not as obvious as they are for macroscopic body parts like fins, limbs, and wings. An example are some cells in the vertebrate retina.

The vertebrate retina consists of a number of neuronal cell types (Fig. 2). There are light sensitive photoreceptors, the cones and rods, and there are interneurons of various sorts that connect photoreceptors to other nerve cells: horizontal cell, ganglion cells, amacrine cells, etc. A detailed analysis of the molecular fingerprints of these cells suggests that some of the interneurons are actually modified photoreceptor cells. For instance, the so-called bipolar cell is an interneuron that connects

Fig. 1 (continued) division of labor and specialization. (Fig. 1a Modified from https://commons.wikimedia.org/wiki/File:422_Feature_Stem_Cell_new.png. Creative Commons Attribution 3.0 Unported license; Fig. 1b Reproduced from Arendt 2008, with permission from Springer Nature)

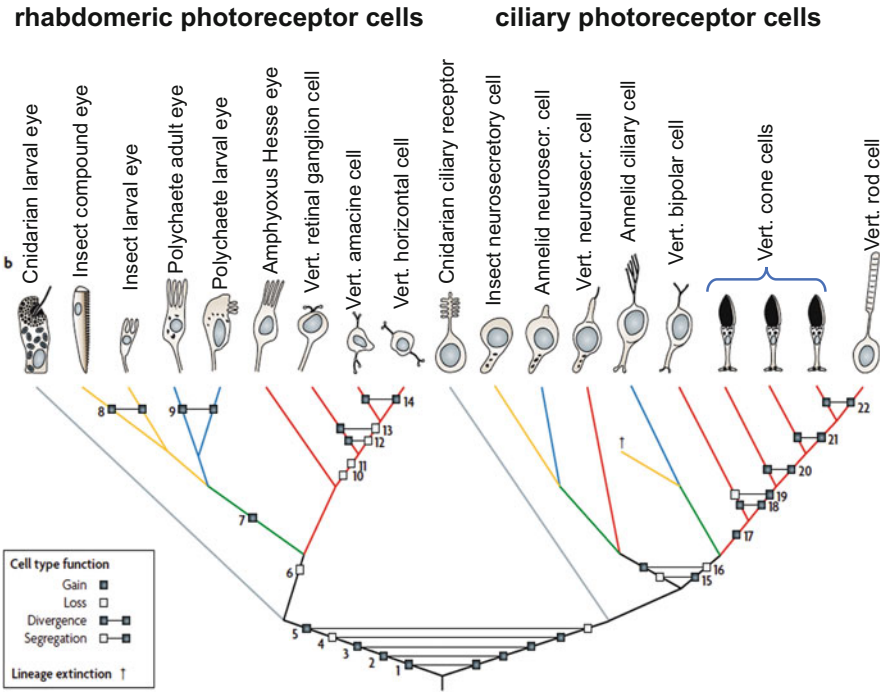


Fig. 2 Phylogenetic relationships among cells derived from the ancestral photoreceptor cells. Note that this tree includes cells from different species, as well as different cells from the same species. Even different cell types from the same species have phylogenetic relationships, similar to the case of paralogous genes in the genome of a species. It is also noteworthy that this tree of cells related to photoreceptors also includes cells that themselves are not photoreceptors in the functional sense. They are "photoreceptors" in terms of serial homology, meaning that they are more closely related to photoreceptor cells than to any other cell type in the body. (Modified from Arendt 2008, as presented in Wagner 2014, with permission from Springer Nature)

the photoreceptors with the ganglion cells, but it has the transcription factor expression profile of a photoreceptor cell and is likely a sister cell type to rods and cones (Arendt 2003, 2008). This implies that some of the interneurons in the retina are homologous with photoreceptors in an ancestral vertebrate. In other words, bipolar cells belong to the family of photoreceptor cell types, i.e., they are more closely related to photoreceptors then they are to other forms of neurons with similar interneuronal function. Bipolar cells "are" photoreceptors, even though they are not specialized for light reception, i.e., are not photoreceptors in terms of their physiology/function. This latter fact shows that the notion of a cell type in a broad comparative, evolutionary sense is "abstract," meaning that it is independent of a particular function that a cell type performs in a particular species. This is similar to the idea that the notion of a tetrapod limb is independent of whether it is used for running, swimming, flying, or piano playing. Whale flippers and bird- and bat wings are all forelimbs, regardless of their form or function.

Cell Type Development

In the previous section, we discussed that cell types exist because they are performing specialized functions necessary for the growth, survival, and reproduction of the animal or plant. To perform these functions, each cell type has to express a different, though partially overlapping, set of proteins and RNAs. For instance, muscle cells have to express actins and myosins to assemble a contractile apparatus. On the other hand, neurons need to express genes for ion channels, or for enzymes to produce neurotransmitter molecules, and for making specialized contacts with other cells, called synapses, and others (Ryan and Grant 2009). The ability to differentially regulate the expression of so-called effector genes (genes for proteins that perform physiological functions like enzymes, cytoskeletal proteins, extracellular matrix components, etc.) is the developmental basis for cell type-specific phenotypes.

During development, the origin of cell types can be conceptualized by two major phases: cell type determination and cell type differentiation. During cell type determination, a cell receives a number of signals that, more or less irreversibly, commit the cell to a particular fate. This process usually happens with little external signs of differentiation, i.e., only represents a regulatory state rather than a particular physiological phenotype. Determination can be a hierarchical, multistage process, in which a cell first becomes committed to a broad range of possible fates, e.g., becoming a cell of the central nervous system, without specifying whether it will be a glial cell or which particular neuronal cell type it will become. This initial determination can be followed by a number of further steps of refinement (neuron vs. glia, and then granular cell vs. Purkinje cell, for instance, etc.) until a specific cell type identity is reached. At this point, the so-called terminal differentiation is happening, i.e., the cell assumes its final functionally specialized phenotype and it becomes recognizable based on its gene expression profile and structure.

Cell type determination is caused either by signal molecules emitted from other cells, or even signals received from the environment, or by factors located in the cytoplasm of the cell. In the latter case, these factors are usually linked to and asymmetrically localized in the cytoskeleton of the mother cell such that one daughter cell is getting the factor and the other one not or a lesser amount. In this way, the two daughter cells acquire different cell type fates. More mechanistic details of this process of cell type determination and differentiation will be discussed in the next section.

The process of cell fate determination can be highly regular and predictable or quite stochastic and "regulatory." In this context, "regulatory" means that a developmental process is subject and responsive to extracellular influences.

The paradigm of a highly regular, deterministic process of cell type determination is the development of the nematode worm *Caenorhabditis elegans* (Fig. 3). In each embryo, each cell descends by a precisely determined sequence of cell divisions from precursor cells, and at each cell division, the two descendent cells will have precisely predictable fates. This highly regular mode of cell type development is typical for small animals with a low and species-specific number of cells overall. Examples are nematodes, rotifers, and similar small invertebrates. That is to say that

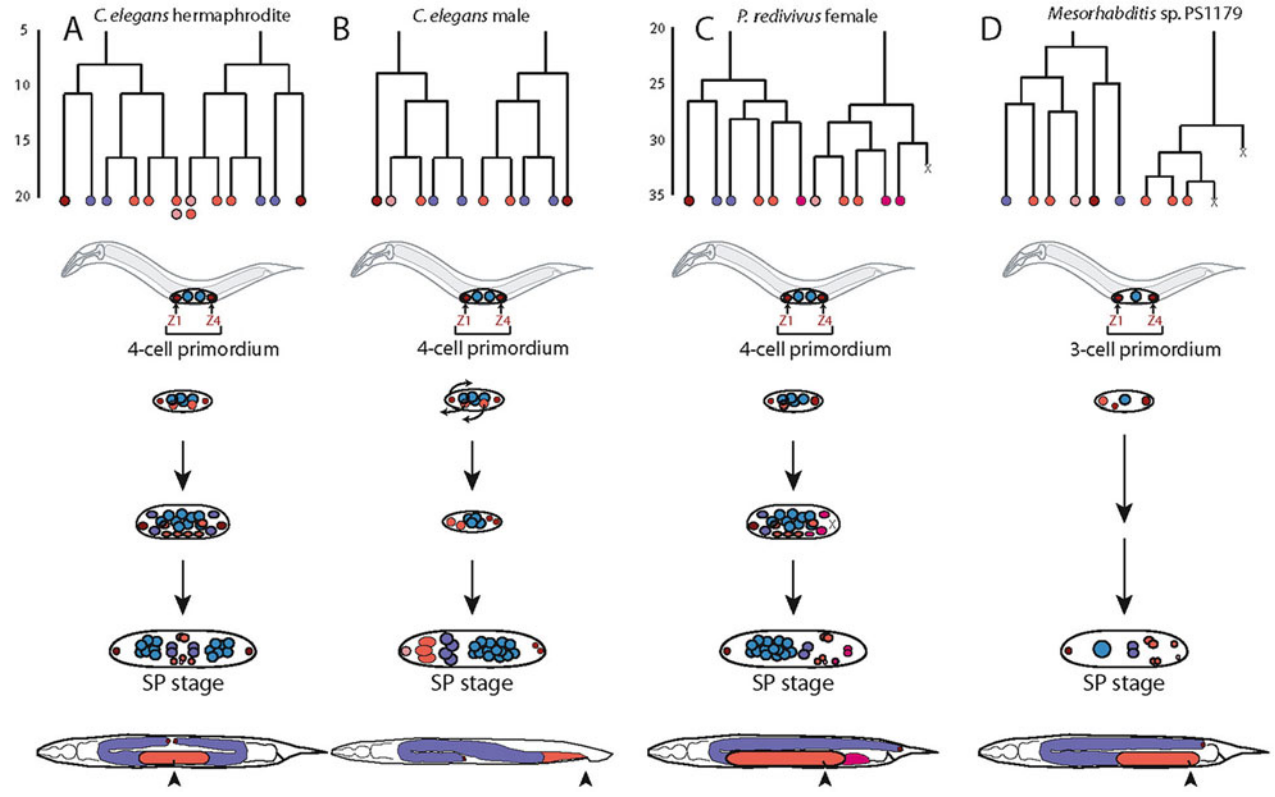


Fig. 3 Development of the cells of the reproductive system in four species of nematodes. For each species, a tree of developmental relatedness is presented. Note that corresponding cell types arise from slightly different cell lineages, showing that developmental cell lineage derivation is not rigidly linked to cell type identity. (Reprinted from Developmental Biology, Volume 264, Issue 1, David Rudel and Ralf J Sommer, The evolution of developmental mechanisms, pp. 15–37, 2003, with permission from Elsevier)

developmental cell lineages are strongly conserved across individuals of the same species. Interestingly, this mode of cell type development, so deterministic and regular within a species, is only partially conserved when we look across species.

In contrast, animals with a large and variable number of cells, like vertebrates and even insects, tend to have a much less regular mode of cell type development. Individual cell lineages are not as predictive of the eventual fate of a cell, although some very broad patterns of cell type development do exist. For instance, the cells of the gut epithelium and its appended organs (e.g., liver, pancreas, lung etc.) tend to derive from a population of cells that are set aside early in development, called the endoderm, while the skeletal muscles and the skeletal cells come from an embryonic cells population called the mesoderm. In addition, the sequence of cell states an individual cell goes through in reaching a particular cell type identity can be variable, even in normal development. For instance, the stromal fibroblast cells of the uterine lining (the endometrium) have been reported to originate from mesenchymal stem cells in the bone marrow, the pericytes of blood vessels in the endometrium, or from stem cells in the uterus itself. Another example are tissue fibroblasts which also can come from mesenchymal stem cells in the bone marrow, but also from epithelial cells through a process called EMT, epithelial-mesenchymal transition, as well as from monocytes, a kind of immune cell (Filer and Buckley 2010). All these examples show that cell types represent a certain gene regulatory state regardless of how this state is reached during development or during a pathological process. Cell type identity is an intrinsic gene regulatory state and not rigidly associated with a particular developmental origin.

Cell Type Evolution

Even at the most superficial level, the same cell types can often be recognized in different species, i.e., they are inherited, together with other traits, from the common ancestors of the species compared and remain recognizable across generations and across speciation events. Technically, this fact is called homology: the same cell type in different species, regardless of form and function. Of course, not all animals have all the same cell types. This fact shows us that cell types have to originate in evolution at some point in time, since they are not shared among all animals. The same conclusion is reached when contemplating the variation in the number of recognizable cell types in different species, as mentioned in the introduction to this chapter. Anatomically more complex organisms, and those that represent body plans that originated later in phylogeny, tend to have more cell types than anatomically simpler animals. A more detailed discussion of the process of cell type origination is given in the section on evolutionary origin of cell types below.

Cell types are not only functionally and developmentally distinct but also evolutionarily individualized, i.e., they can be found in different species and each has its own evolutionary history. Function, developmental differentiation, and evolutionary individuation are the three aspects of the same phenomenon, the biology of cell types.

Molecular Mechanisms of Cell Type Individuation

The development and differentiation of cell types has been studied in various ways since at least 200 years. In the last 20 years, however, a convergent consensus emerged, where research groups working on different systems have proposed very similar models of how cell type identity is realized at the molecular level. Examples are the core gene regulatory network model of Graf and Enver (2009), the terminal selector gene model by Hobert (2011), the kernel model by Davidson and Erwin (2006), and the character identity network model (Wagner 2007). In brief, the gene regulatory network that leads to cell type differentiation can be abstracted into a model with three levels: inductive signals, a core gene regulatory network, and a layer of effector gene regulation and expression (Fig. 4).

As mentioned above, cells either receive signals from other cells or inherit signaling products in their cytoplasm from their mother cell. In either case, these signals "tell" the cell what fate they should assume in order to integrate into the fully formed organism and contribute their functional role. These signals usually act transiently, i.e., they are critically important during a limited time during development. These signals are most often polypeptides, like TGF (transforming growth factor) or sonic hedgehog, or small molecule signals, like steroid hormones or prostaglandins. These signals then act directly or indirectly to activate the core regulatory network genes. An example of a more or less direct signaling is steroid hormones that bind to so-called nuclear receptors, i.e., transcription factor proteins that become transcriptionally active once they bind their ligand. Indirect signaling often involves binding of the signal to a membrane bound receptor, which in turn can activate a more or less complicated cascade of intracellular signaling, ending in the modification of a transcription factor protein that is then either activated and transported or retained in the nucleus, or degraded and prevented from acting. Examples are the so-called hedgehog signaling molecules which bind to a receptor called patched, which then set into motion an intracellular signaling cascade that ends in preventing the proteolytic splitting of a transcription factor protein.

Let us call this layer of regulation the layer of transient input signals (Fig. 4). Cell type determination is usually caused by multiple signals that either act in tandem or in combination to specify the cell type identity. These signals can act to some degree redundantly, which has important consequences for the evolution of cell type determination mechanisms (see below).

The biological role of transient input signals is to activate a set of transcription factor genes which constitute the core gene regulatory network (Fig. 5). Three features characterize the core network.

First they are thought to form a positive feedback loop, which leads to the stabilization of the expression of the members of the core network so that the core network can remain active even when the inductive signals have been turned off. This positive feedback also explains why, experimentally, the cell fate of a cell can be induced by the forced expression of one or a few of the members of the core network. The other members of the network are getting activated because they are activated in a positive feedback loop which can be triggered by the induction of (almost) any part of the network.

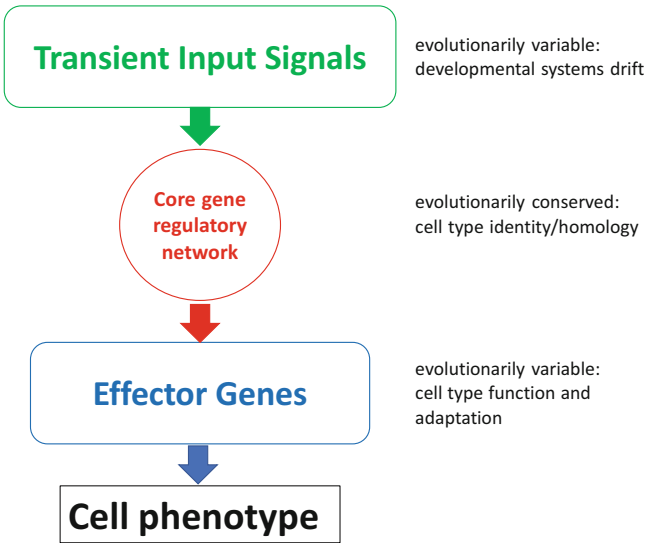


Fig. 4 Schematic structure of mechanisms of cell type identity. Cell type identity is triggered in development by a number of transient input signals which activate a core gene regulatory network. The core network consists mostly of transcription factor genes. The products of the core network genes then regulate so-called effector genes, which have products that perform physiological work, like enzymes and extracellular matrix proteins, etc., which in turn produce the functional and morphological phenotype of the cell. The three layers of this network model have different degrees of evolutionary conservation. The input signals can be surprisingly labile, so that homologous cell types can be induced by different signaling molecules in different species. In contrast, the core regulatory network tends to be the most conserved part of the gene regulatory network that produces a cell of a particular type. The layer of the effector genes evolve to meet the adaptive needs of the cell and the organism

Second, the transcription factors produced by the genes of the core network usually work cooperatively. That means that they form a physical complex of transcription factor proteins, a so-called core regulatory complex (Arendt et al. 2016) that is cell type-specific. An example is the core regulatory complex of motor neurons of the spinal cord, and the so-called V2 interneurons (Fig. 6).

Finally, the core regulatory complex performs two functions, namely activating the differentiation batteries of effector genes which make the physiological phenotype of the cell, and second directly or indirectly suppressing genes related to an alternative cell type identity. The set of effector genes activated by the core network and their detailed regulation is then the third layer of the gene regulatory network model of cell type identity.

The structure of the "layer cake model" of cell type identity (Fig. 4) has important consequences for the developmental evolution of cell types. The first is that the three layers of the model have very different levels of evolvability.

The top layer of the model, the transient input signals, evolve at a higher rate than the core network layer. That is probably due to the fact that the input signals are partially redundant, which means that they can replace each other, which they do

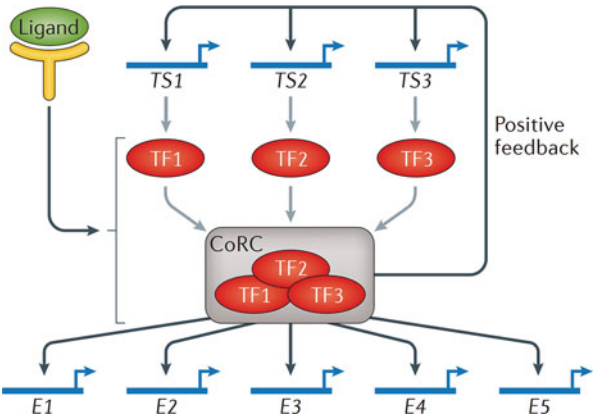


Fig. 5 The core regulatory complex (CoRC). The products of the genes in the core regulatory network are proteins that physically and functionally interact to form the core regulatory complex or CoRC. According to this model, the CoRC is the actual molecular agent that regulates the effector genes and is cell type-specific ensuring the expression of a cell type-specific set of effector genes. (Reproduced from Arendt et al. 2016, with permission from Springer Nature)

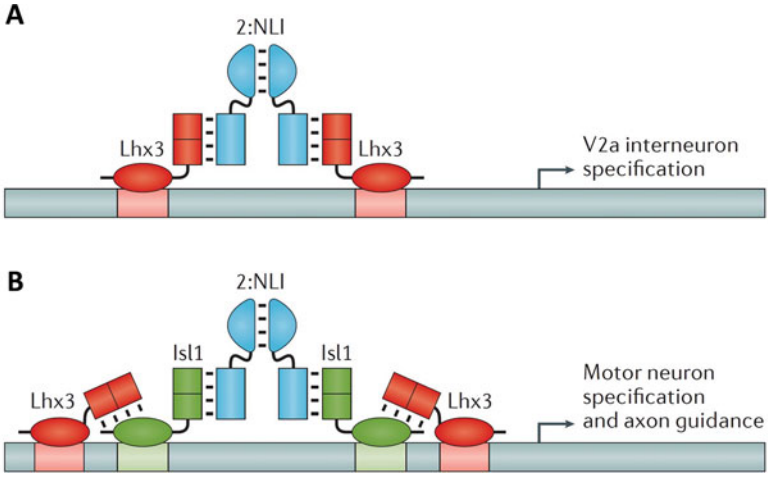


Fig. 6 Examples of core regulatory complexes (CoRCs). (**a**) The CoRC of the so-called V2 interneurons in the spinal cord of the chicken consists of four transcription factor proteins, two copies of LNI and Lhx3 each. (**b**) The CoRC of a motor neuron consists of six transcription factor proteins, two copies of LNI, Lhx3, and Isl1. (Reproduced from Arendt et al. 2016, with permission from Springer Nature)

over the course of evolutionary time. Many of the examples of developmental systems drift (Weiss and Fullterton 2000; True 2001), where the same cell type in different species develop in response to different input signals, are due to changes at that level of the gene regulatory network.

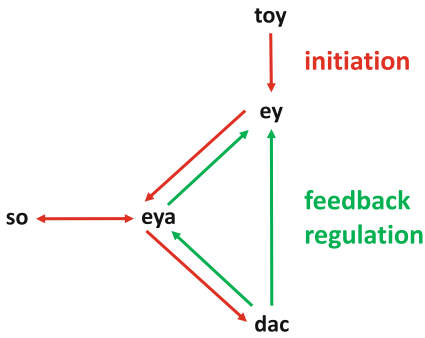


Fig. 7 Example of a conserved core gene regulatory network, the network that gives eye cell identity in insect development

The second layer, the core network, is usually much more conserved and is strongly linked with cell type identity. Some of the most impressive examples of gene regulatory network conservation are core networks, like the Pax/eyeless network of photoreceptor development (Fig. 7). The mechanistic reason for the evolutionary stability of the core network part of the layer cake model is the need for cooperativity of the members of the core regulatory complex. Molecular cooperativity requires evolutionary co-adaptation among the members of the core regulatory complex. Functional cooperativity requires the presence and participation of all members of the core regulatory complex and the positive feedback necessary to stabilize the expression of the core network also are incompatible with losing individual members of the network. Conceptually, the core network and the core regulatory complex are the material manifestation of the abstract concept of cell type identity. They enable the differential expression of effector genes and thus allow distinct cell types to have different phenotypes and functions. But it needs to be mentioned as well, that the core network is not fully determining the phenotype of the cell and thus different phenotypes can be realized by the same cell type in different species (see above about evolutionary change of function of the same cell type).

Finally, the third layer of the gene regulatory network model is made up by the effector genes and their immediate regulators. Whether a gene is included in this tier of the gene regulatory network depends on the cis-regulatory elements (CRE) of the gene. Only genes that have a CRE responding to the core regulatory complex will be expressed in the corresponding cell type. While this is an obvious truth from a mechanistic point of view, this fact has important consequences for the developmental evolution of cell types. Since the expression of the effector genes depends on their CREs, the same core network can regulate different sets of effector genes in different species. For example, the gut muscles of most animals are smooth muscle cells, with the notable exception of insects. In insects, the gut cells are striated like skeletal muscle cells but nevertheless are regulated by the same set of transcription factors as the gut muscle cells of other animals (Brunet et al. 2016). Hence the module for building the striated arrangement of contractile proteins came under the control of the core regulatory complex that determines the identity of gut muscle cells.

The set of target genes can evolve through changes in the CRE of effector genes without any need to change the core network. In abstract terms, this mechanistic fact means that cell type identity, represented by the core network and core regulatory complex, can be decoupled from the cell phenotype, represented by the set of target effector genes activated by the core network. Cell type identity and cell phenotype are evolutionarily independent variables. This, for instance, explains how "the same cell" can perform different functions in different species, as explained above in the section on "▶ Form and Function in Evo-Devo."

The "layer cake" model outlined above explains the process of determination and terminal differentiation of a cell type. In complex organisms, however, this state of development is often reached through a hierarchical process of narrower and narrower cell fate determination, as explained in the section "▶ Devo-Evo of Cell Types." It is now thought that epigenetic modification of the DNA and/or histones associated with the DNA is responsible for the narrowing competence of developing cells. DNA methylation and histone methylation cause parts of the genome to be inaccessible for gene expression. A consequence of this fact is that the effect of signaling molecules on the cell fate determination depends on both, the signals present, and the history of the cells that are exposed to these signals. The history of the cell is inscribed in the epigenetic modifications of the genome and the chromatin (Feng et al. 2010).

Evolutionary Origin of Cell Types

As mentioned in the introduction to this chapter, the number of cell types has increased during the evolution of animals and plants largely correlated with the complexity of the body plans. The process responsible for this increase in cell type number are quite incompletely known, but the most widely supported model is the so-called sister cell type model (Arendt 2008). This model is inspired by the fact that in many anatomically simpler organisms, cells tend to be multifunctional, like the epithelium-muscle cells discussed above. The basic idea is that novel cell types originate from the differentiation of a multifunctional ancestral cell type by giving rise to two new cells. Hence, according to this model, cell types form a bifurcating tree of evolutionary relationships (Fig. 2). A consequence of this model is that different cell types in the same body are serial homologues with different degrees of relatedness, similar to different genes in a genome that originated by gene duplication (Wagner 2014; Musser and Wagner 2015).

The sister cell type model can be visualized as a set of nested evolutionary trees, as shown in Fig. 8a. There is a tree-like relationship among species, in this example shown for three species. Embedded in this species tree are the trees of origination and descent of different cell types, in the same way as gene trees among duplicated genes can be thought of as being embedded in the species tree. When we pull out the cell type tree from the species tree (Fig. 8b), we obtain a tree reflecting the evolutionary relationships among the cell types in different species as well as within each species. This is called the cell type tree.

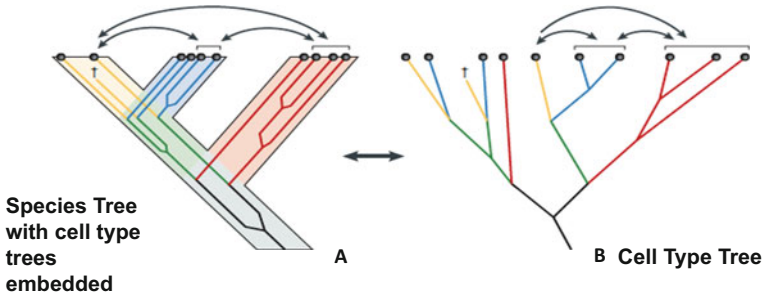


Fig. 8 The sister cell type model for the origin of novel cell types. (**a**) A hypothetical species tree of three species and embedded in the species tree is the evolutionary history of the cell types. Note that this is a tree in a tree, i.e., assuming that novel cell types arise through a process of cell type splitting, where an ancestral cell types gives rise to two novel sister cell types. (**b**) The same cell type tree as in A, only pulled out from the species tree, showing the phylogenetic relationships among the cells and cell types from different species as well as from the same species. This representation is similar to that of a gene tree of genes from genomes of different species. (Reproduced from Arendt 2008, with permission from Springer Nature)

The sister cell type model is a plausible scenario of cell type origination and can explain, for instance, the high degree of hierarchical similarities in gene expression patterns (Liang et al. 2015). It is, however, not the only thinkable mode of cell type origination. One could also conceive of a scenario where a novel cell type arises from combining gene regulatory network modules from different ancestral cell types (Arendt et al. 2016). There is currently no strong evidence supporting this mode of evolution, but researchers in developmental evolution need to be aware of this alternative model.

A frequent source of confusion is the relationship between developmental lineage trees and cell type evolution trees. Developmental lineage trees represent the developmental relatedness of different cells during the life of an individual. The best documented examples are the cell lineages in nematode worms (Fig. 3) and the cleavage divisions during the early development of so-called spiralian animals, like mollusks. On the other hand, cell type trees, like those depicted in Fig. 2, represent the evolutionary process of cell type origination. For instance, the three main types of muscle cells in bilaterians, skeletal-, gut-, and heart-muscles have an evolutionary relationship where gut and heart muscles are more closely related to each other than either is to skeletal muscles (Brunet et al. 2016). This is indicated by the different embryological origins as well as the core transcription factor complexes, CoRC, determining cell type identities. In chordates, the skeletal muscles arise from the somites, while gut and heart muscles are derived from the unsegmented ventral mesoderm. Skeletal muscle cells have a CoRC that includes MyoD, E12, MASTR, and SRF/MEF2, while the CoRC of gut and heart cells contains Myocardin and paralogs of the FOX, Nkx, and GATA families of transcription factors (Brunet et al. 2016).

The difficulty arises because ontogenetic and phylogenetic relationships can be congruent, i.e., form the same tree pattern, but they do not have to be the same and

are conceptually distinct. There are several lines of evidence which demonstrate the decoupling between ontogenetic and phylogenetic cell type trees. One comes from the fact that the same set of cell types can arise along different ontogenetic cell lineage trees. This is best documented in different species of nematode worms already mentioned above and illustrated in Fig. 3 (Rudel and Summer 2003; Sommer 2005). Unless we assume that the same cells have evolved independently in different nematode lineages, the ontogenetic cell lineages cannot be congruent with the evolutionary cell type tree, at least not all of them. Another fact is that even within the same species, the same cell type can differentiate from different precursor cells, as explained above, and thus not all of these lineage relationships can also reflect the evolutionary history of these cells. Another example is the development of cone and amacrine cells in the retina. Cones are functional photoreceptor cells, and amacrine cells are interneurons. Both are developmentally derived from a common precursor cell, i.e., are developmentally sister cells, but have little else in common. Amacrine cells are more like other GABA-ergic inhibitory neurons and evolutionarily may be more related to mechanoreceptors than with photoreceptors and bipolar cells.

Overall, the evolutionary history of cell types is much less well known than, say, the history of gene families or the evolutionary relationships among anatomical body parts, which have been investigated since the beginnings of evolutionary comparative anatomy. The reason is mostly technical. Without sophisticated genomic, transcriptomic, and immunobiological techniques (revealing the "molecular fingerprints" of the cell types), it is very hard to reliably distinguish different cell types, and identify them in different species, where they can have evolved different functions and thus different morphologies. In addition, the comparative biology of cell types requires an understanding of the molecular mechanisms underlying cell type identity and how cell type phenotypes can be decoupled from cell type identity. With these two advances in hand, the developmental evolution of cell types is a field ready to advance.

Gene Expression Evolution of Cell Types

Comparing gene expression across cell types and species reveals that evolutionary individualization, i.e., the ability of one cell type to evolve a different phenotype than other cell types of the same species, is not associated with complete independence of gene expression evolution (Musser and Wagner 2015). When the gene expression profiles of two homologous cell types are compared between two species, then the two cell types of the same species tend to be more similar than each of them are to their corresponding cell type in the other species. This is unexpected, because the two cell types are phylogenetically older than the two species compares (that is the case since we assume that the two cell types are homologous among the two species). Since the cell types have a longer independent history than the corresponding cells in the two species one would expect that the two cell types in the same species are more divergent than the corresponding cell types among the species. This is often not the case. This is explained by the observation that

expression of genes in different cell types is co-evolving. Coevolution of gene expression is most likely caused by the fact that some part of the gene regulatory networks of these two cells is shared and thus some mutations that affect gene expression in one cell type can have a similar effect in the other cell type (Liang et al. 2018).

Cross-References

- ▶ Developmental Homology
- ▶ Developmental System Drift
- ▶ Typology and Natural Kinds in Evo-Devo

References

Arendt D (2003) Evolution of eyes and photoreceptor cell types. Int J Dev Biol 47:563–571

Arendt D (2008) The evolution of cell types in animals: emerging principles from molecular studies. Nat Rev Genet 9:868–882

Arendt D, Musser JM, Baker CVH, Bergman A, Cepko C, Erwin DH, Pavlicev M, Schlosser G, Widder S, Laubichler MD, Wagner GP (2016) The origin and evolution of cell types. Nat Rev Genet 17(12):744–757. https://doi.org/10.1038/nrg.2016.127

Brunet T, Fischer AH, Steinmetz PR, Lauri A, Bertucci P, Arendt D (2016) The evolutionary origin of bilaterian smooth and striated myocytes. elife 5. https://doi.org/10.7554/eLife.19607

Brusca RC, Moore W, Shuster SM (2016) Invertebrates, 3rd edn. Sinauer Associates, Sunderland

Buss LW (1987) The evolution of individuality. Columbia University Press, New York

Davidson EH, Erwin DH (2006) Gene regulatory networks and the evolution of animal body plans. Science 311:796–800

Erwin DH (2015) Novelty and innovation in the history of life. Curr Biol 25(19):R930–R940. https://doi.org/10.1016/j.cub.2015.08.019

Feng S, Jacobsen SE, Reik W (2010) Epigenetic reprogramming in plant and animal development. Science 330(6004):622–627. https://doi.org/10.1126/science.1190614

Filer A, Buckley CD (2010) Fibroblasts and stromal cells. In: Serhan CN, Ward PA, Gilroy DW (eds) Fundamentals of inflammation. Cambridge University Press, Cambridge, pp 126–140

Graf T, Enver T (2009) Forcing cells to change lineages. Nature 462:587–594

Hobert O (2011) Regulation of terminal differentiation programs in the nervous system. Annu Rev Cell Dev Biol 27:681–696. https://doi.org/10.1146/annurev-cellbio-092910-154226

Liang C, Forrest AR, Wagner GP (2015) The statistical geometry of transcriptome divergence in cell-type evolution and cancer. Nat Commun 6:60–66. https://doi.org/10.1038/ncomms7066

Liang C, Musser JM, Cloutier A, Prum RO, Wagner GP (2018) Pervasive correlated evolution in gene expression shapes cell and tissue type transcriptomes. Genome Biol Evol 10(2):538–552. https://doi.org/10.1093/gbe/evy016

Michod RE (1999) Darwinian dynamics: evolutionary transitions in fitness and individuality. Princeton University Press, Princeton

Michod RE, Herron MD (2006) Cooperation and conflict during evolutionary transitions in individuality. J Evol Biol 19(5):1406–1409; discussion 1426–1436. https://doi.org/10.1111/j.1420-9101.2006.01142.x

Musser JM, Wagner GP (2015) Character trees from transcriptome data: origin and individuation of morphological characters and the so-called “species signal”. J Exp Zool B Mol Dev Evol 324 (7):588–604. https://doi.org/10.1002/jez.b.22636

Ross MH, Pawlina W (2011) Histology: a text and atlas, 6th edn. Wolters Kluwer, Philadelphia
Rudel D, Sommer RJ (2003) The evolution of developmental mechanisms. Dev Biol 264:15–37
Ryan TJ, Grant SG (2009) The origin and evolution of synapses. Nat Rev Neurosci 10 (10):701–712. https://doi.org/10.1038/nrn2717
Sebé-Pedrós A, Chomsky E, Pang K, Lara-Astiaso D, Gaiti F, Mukamel Z, Amit I, Hejnol A, Degnan BM, Tanay A (2018) Early metazoan cell type diversity and the evolution of multicellular gene regulation. Nat Ecol Evol 2:1176–1188
Simpson TL (1984) The cell biology of sponges. Springer, New York
Smith CL, Varoqueaux F, Kittelmann M, Azzam RN, Cooper B, Winters CA, Eitel M, Fasshauer D, Reese TS (2014) Novel cell types, neurosecretory cells, and body plan of the early-diverging metazoan *Trichoplax adhaerens*. Curr Biol 24(14):1565–1572. https://doi.org/10.1016/j.cub.2014.05.046
Sommer RJ (2005) Evolution of development in nematodes related to *C. elegans*. WormBook, ed. The *C. elegans* Research Community, WormBook. http://www.wormbook.org. https://doi.org/10.1895/wormbook.1.46.1
Syed T, Schierwater B (2002) *Trichoplax adhaerens*: discovered as a missing link, forgotten as a hydrozoan, re-discovered as a key to metazoan evolution. Vie Milieu 52(4):177–187
True JR (2001) Developmental system drift and flexibility in evolutionary trajectories. Evol Dev 3:109–119
Valentine JW, Collins AG, Meyer CP (1994) Morphological complexity increase in metazoans. Paleobiology 20:131–142
Varoqueaux F, Williams EA, Grandemange S, Truscello L, Kamm K, Schierwater B, Jekely G, Fasshauer D (2018) High cell diversity and complex peptidergic signaling underlie placozoan behavior. Curr Biol 28(21):3495–3501 e3492. https://doi.org/10.1016/j.cub.2018.08.067
Vickaryous MK, Hall BK (2006) Human cell type diversity, evolution, development, and classification with special reference to cells derived from the neural crest. Biol Rev Camb Philos Soc 81 (3):425–455. https://doi.org/10.1017/S1464793106007068
Wagner GP (2007) The developmental genetics of homology. Nat Rev Genet 8:473–479
Wagner GP (2014) Homology, genes and evolutionary innovation. Princeton University Press, Princeton
Weiss KM, Fullterton SM (2000) Phenotypic drift and the evolution of genotype-phenotype relationships. Theor Pop Biol 57:187–195

The Evolution of Cleavage in Metazoans

Miguel Brun-Usan and Isaac Salazar-Ciudad

Contents

Abstract

Cleavage is the earliest developmental stage. During this stage, the fertilized oocyte gives rise to a cluster of smaller cells (blastomeres) with a particular spatial

M. Brun-Usan
Genomics, Bioinformatics and Evolution, Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona, Barcelona, Spain
e-mail: m.brunusan@gmail.com

I. Salazar-Ciudad (✉)
Genomics, Bioinformatics and Evolution, Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona, Barcelona, Spain

Evo-Devo Helsinki Community, Center of Excellence in Experimental and Computational Biology, Institute of Biotechnology, University of Helsinki, Helsinki, Finland
e-mail: isaac.salazar@helsinki.fi

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_50

pattern (a cleavage pattern). Different metazoan species have different cleavage patterns, but most of them fit into a small set of basic types.

The relationship between the phylogeny of a given species and its cleavage pattern is far from direct, but most taxa seem to use the same basic cell processes (such as directed cell division or cell adhesion) to build their cleavage patterns. We assess which are those mechanisms in the first section of this chapter. In a second section, we explore how the combined action of these mechanisms can account for the emergence of particular cleavage patterns in different metazoan taxa and the evolutionary transitions between them.

Keywords

Early development · Cleavage pattern · Cell processes · Evo-devo

Introduction

The fertilized oocyte contains all the information to, given the appropriate environmental conditions, build a functional adult organism by means of a complex process called development.

The first stage of development is called cleavage. Cleavage starts from a single cell, the oocyte (or egg), that despite being more or less spheric is far from being homogeneous. Normally, oocytes have internal gradients (maternally inherited) that are oriented along one axis called animal-vegetal axis (Gilbert and Raunio 1997). During cleavage, a series of fast cell divisions partition the oocyte into a set of smaller cells called blastomeres. The spatial distribution of blastomeres observed when cleavage finishes (at the onset of gastrulation) is what we call a "cleavage pattern" (Gilbert and Raunio 1997). As a general rule, cleavage proceeds without an overall growth of the embryo (the volume of the embryo is roughly equal to that of the oocyte). In many organisms, this is because the oocyte is surrounded by a protective eggshell (also involved in selective metabolite exchange) that limits the space available for the developing embryo).

A major driver of the diversity observed in metazoan cleavage patterns is yolk: a nutritive substance normally concentrated in the oocyte's vegetal pole. Yolk interferes with the cytoskeletal processes involved in cell division, and therefore blastomeres in the vegetal part of the embryo tend to divide more slowly than blastomeres in the animal part of the embryo (Gilbert and Raunio 1997). Moreover, if yolk is dense enough, it cannot be pierced by the cleavage furrow when blastomeres divide, often resulting in incomplete cell divisions in which blastomeres are not totally separated by the cytoplasmic membrane. This type of cleavage is called meroblastic. According to the distribution of yolk within the blastula, meroblastic cleavage occurs in eggs that are either telolecithal (yolk is distributed throughout most of the blastula) or centrolecithal (yolk is located in the center of the blastula). Blastulae with a low or moderate amount of yolk display holoblastic cleavage (the furrows of cell divisions traverse the whole blastula, whereby blastomeres get individualized).

In addition, many cleavage patterns exhibit geometrical regularities in their blastomere arrangement, which allows for a general classification into a few major cleavage types.

Distantly related taxa can exhibit similar cleavage patterns, while species belonging to the same taxon can have different cleavage types (Valentine 1997). These discrepancies between the cleavage patterns and the phylogentic position of metazoan taxa are difficult to explain and seem very counterintuitive unless we gain more knowledge on how the different blastomere arrangements can be generated during early development. This means how the spatio-temporal combination of different cell processes can generate the different cleavage patterns. The aim of this chapter is to gain an overview of such cell processes and to review how they are combined in the early development of the major metazoan groups. For the taxa with enough available data, evolutionary transitions between different cleavage patterns are also addressed.

Cell Processes Involved in Cleavage

Compared to later developmental stages, only a single kind of relatively undifferentiated cells exists during cleavage, and the number of cell processes that they can display is relatively small. These cell processes are:

Cell Division

Cell division can occur in a specific direction (the plane of cell division can be oriented in different ways in space), at a specific moment in time, and with a specific degree of size asymmetry between daughter cells (Gillies and Cabernand 2011).

Direction of cell division: Cells can be polarized and this polarization can determine the direction in which cells divide. Ultimately, cell polarization results when one or several sources of spatial information are translated into a spatially asymmetric distribution of some specific molecules within the cell. In many cells under division, this asymmetry promotes a differential attachment of the astral microtubules to the part of the cortex with the highest concentration of these molecules, thus tilting the mitotic apparatus and biasing cell divisions to occur perpendicularly to the direction of cell polarization. The sources of spatial information can be located either within the cell itself (autonomous mechanism) or in the cell's surroundings (inductive mechanisms).

The autonomous mechanisms do not require any physical or chemical interaction with other cell(s) to determine the direction of cell division. That means that cells use asymmetries that are already present in the intracellular environment to polarize themselves. These asymmetries are usually inherited and usually consist in the heterogeneous distribution of some factor(s) in the cytoplasm (e.g., mRNAs). In other cases, this heterogeneity is attained when some factors (if dense enough) are

attracted by gravity to the lower part of the embryo, creating new spatial information. These factors can interact with the mitotic spindle in such a way that the spindle always tends to point towards the place where the factors are most abundant.

When spatial cues are absent, the cell shape itself (specifically, its longest axis) is able to determine the direction of cell division. This is commonly referred as Hertwig's rule, and it occurs because of the differential tension in astral microtubules, which depends on the contact angle between the microtubule tips and the cell's surface. By simple geometry, this angle is smallest at the cell boundary at the most distant points of the cell, causing that the astral microtubules attached there exert a stronger tension than the microtubules attached elsewhere, thus leading to the alignment of the mitotic spindle along the longest axis of the cell (and then to cell division to occur perpendicular to that axis).

Finally, it exists a phenomenological rule (Sachs' rule) by which the direction in which a cell divides tends to be perpendicular to the direction of the division that gave rise to it (that is its mother cell division). This has been proposed to arise from the stereotypic (90°) duplication of the centrioles between cell divisions that in turn biases the position of the mitotic spindle towards perpendicularity.

Cues in the cell's surroundings can also provide external spatial information by means of short-range or long-range diffusible signals between neighboring cells (inductive mechanisms). A special case of "short range" signals is the physical contact between cells. In this case, cells tend to divide towards (or against in some cases) the part of the cell making contact to adjacent cells. This has been suggested to occur because physical contact in a cell region would modify the underlying cell cortex so that the astral microtubules are stabilized in this region, increasing the local traction of the mitotic spindle during cell division.

Differential growth: During cleavage, cells in a blastula can divide at the same time (synchronous cell divisions) or not (asynchronous cell divisions). Synchronous cell divisions give rise to different cleavage patterns than asynchronous cell divisions. In general, the resulting cleavage patterns depend on the relative rates of cell divisions between the different regions of the blastula. Assuming that some factors can trigger (or inhibit) cell division, this asynchrony can be achieved by a heterogeneous distribution of those factors in different regions of the blastula.

One of these factors is yolk (which usually forms an animal-vegetal gradient) that is known to delay (or even prevent) cell division. As a consequence, cells close to the vegetal pole divide at a slower pace and remain bigger than those close to the animal pole. In some groups, cell division is inhibited just in a single specific blastomere, which becomes larger than the others.

Specification of daughter cells' size: In general, when a cell divides, the resulting daughter cells are equally sized (symmetric cell division), but in many embryos some cell divisions are asymmetric: one daughter cell (macromere) is significantly larger than the other (micromere). Different mechanisms can result in asymmetric cell division. In some cases, the relative size of daughter cells is regulated by the asymmetric concentration of intracellular factors (e.g., PAR proteins in *C. elegans* embryos). The microtubules of the mitotic spindle get more stabilized in the regions of the cell where these factors are more abundant, generating asymmetric pulling

forces during cytokinesis. Since the concentration of these factors often varies along the animal-vegetal axis, cell size gradually increases along the animal-vegetal axis.

Alternatively, asymmetric cell division can result from an inherently asymmetric spindle. In these cases (e.g., *Tubifex* worms), one centrosome is inactivated, so as only a half of the mitotic spindle is plenty developed and exerts a greater traction force than the other, degenerated, half of the spindle, thus displacing the cleavage plane to one side of the cell.

Asymmetric mitosis may play a role in generating variation between cleavage patterns. This is because when the cells are tightly packed (e.g., by increased cell adhesion), the resulting blastomere arrangement may depend on the relative size between blastomeres (e.g., small blastomeres may occupy the furrows between big ones). Cell division can also be asymmetric if the mother cell has some kind of internal polarity (e.g., an mRNA gradient), which the two daughter cells inherit in a differential manner, even if they are equally sized. That way one daughter cell can incorporate different molecules than the other, which may cause differential gene expression between sister cells.

Cell Processes Not Related to Cell Division

Cell adhesion: During cleavage, cell adhesion keeps the blastomeres together, thus maintaining the physical integrity of the blastula as a whole. Moreover, cell adhesion increases the contact surface between adjacent cells, which can lead to cell shape changes that may affect the relative position and contacts between neighboring blastomeres (Lecuit and Lenne 2007) and even the direction of the cell divisions if Hertwig's rule applies. If adhesion molecules are expressed nonuniformly on the surfaces of individual blastomeres, complex spatial arrangements, such as embryonic cavities or cell chains can be formed. In some taxa, adhesion strength is not constant over cleavage time, but cells suffer cycles of increased cell adhesion coupled to cell division cycles.

Local variations in cell adhesion (and in the surface of contact between blastomeres) are important when the cell fate determination is controlled by inductive mechanisms. In these cases, cells are not induced below a certain area of contact, but are only induced above this area.

Cortical rotation: During the first cell divisions in certain taxa (e.g., *Xenopus*, snails), blastomeres rotate over themselves just after cell division around the rotation axis that links the two cells (Meshcheryakov and Beloussov 1975). Around this axis, rotation occurs in the same sense in all blastomeres (e.g., all counterclockwise respect to their sister blastomere). Whereas in some taxa this rotation does not seem to have any morphogenetic effect in the blastomere arrangement (as in *Xenopus*), it has been suggested that this rotation produces relevant changes in cell relative positions in other taxa (Brun-Usan et al. 2017; see section "Nematoda + Nematomorpha"). The molecular mechanics of this rotation remains unclear, but it seems to be related with the chiral structure of the F-actin, a protein present in the cell cortex.

Packing constraints: When cleavage proceeds inside an eggshell, a compressive effect may be exerted by the limitation of the available physical space for the blastomeres (Kajita et al. 2003). Due to geometrical considerations, when a set of spheres (blastomeres) is packed within a limited three dimensional space, there are only a small number of optimal cell spatial arrangements.

Notice that many of the previously described mechanisms are not directly encoded genetically (Newman 2011). Rather, they arise from the complex dynamics of the cell cytoskeleton and from purely physical processes like membrane surface tension, volume displacement, gravity, and molecular diffusion. It is worth mentioning that some of these processes also apply to inanimate matter. Because of that, some nonliving systems such as soap-bubbles or mineral aggregates share many geometric regularities with cleaving embryos. The "cleavage patterns" in these inorganic systems inform, thus, of which are these "default" cleavage patterns. These are the cleavage patterns that require less precise regulation and that are, thus, more likely to arise in evolution (since they require less mutational changes).

Moreover, most of the cellular processes described in here, as well as their molecular basis are not specific of blastomeres, but are also found in unicellular organisms, meaning that they were already present before the origin of multicellularity (Newman et al. 2003; Sebé-Pedrós et al. 2010). Thus, these easy-to-arise patterns may have represented the raw material upon which evolutionary forces may have acted in order to build more complicated patterns later on (by using more cell processes and regulating their spatial and temporal location finely).

Selective forces need also be considered to understand why some of these patterns are evolutionary conserved while others are not. The adaptive significance of these conserved patterns may rely on two (not exclusive) facts. First, if cell fates are specified by cell-cell interactions between specific blastomeres, a constant relative position between these blastomeres is crucial for the appearance of functional adult organs. In these cases, variations in the blastomere positions within the blastula should be maladaptive and selectively suppressed. This is often referred as "internal selection" (Riegler 2008). Second, adaptive modifications of some aspects of early development (e.g., an increase in the amount of yolk in order to nourish the embryo, or a hardening of the eggshell to provide it physical and biological protection) may, in turn, have an effect on the shape and arrangement of the blastomeres (Wray 2000).

Evolution of Cleavage in Metazoans

In order to see how the described phenomena can account for the different cleavage patterns and their evolutionary transitions, we present an overview of the cleavage patterns among the extant metazoans (See Fig. 1):

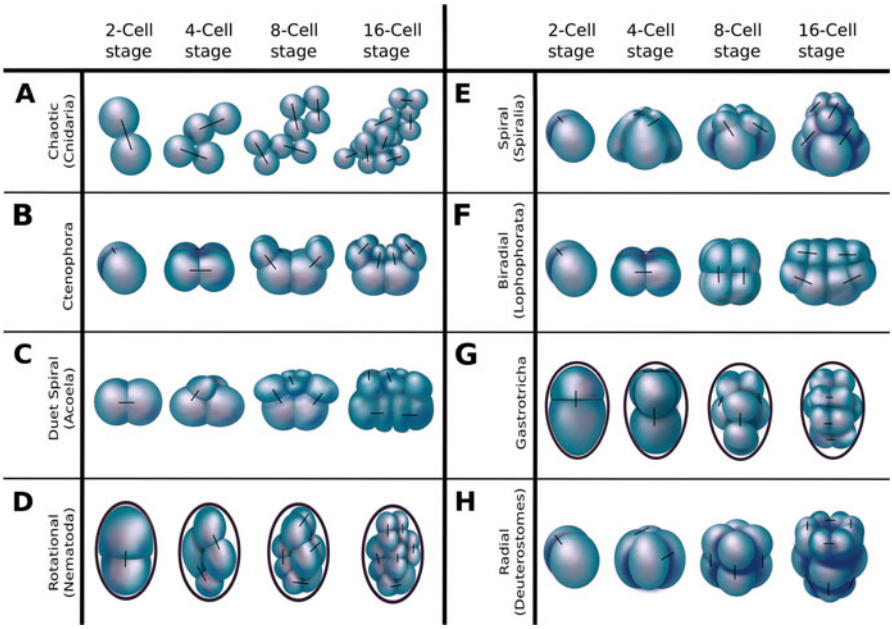


Fig. 1 Example (holoblastic) cleavage patterns found in metazoans. Most of them may be explained by means of the combination of a few conserved processes (see text, section "Cell Processes Involved in Cleavage"). All blastulae are displayed in lateral view with the animal pole on the top, and the small straight lines link sister blastomeres when both of them are visible. (A) Chaotic (=anarchic) cleavage pattern characteristic of nonbilaterian taxa such as Cnidarians. (B) The cleavage pattern of Ctenophores. (C) Duet spiral cleavage pattern of Acoel flatworms. (D) Rotational cleavage pattern of *C. elegans* (a model species representative of Nematoda). (E) Quartet spiral cleavage pattern as displayed by many Spiralian taxa (the "pseudospiral" cleavage pattern found in other nonspiralian taxa is similar to this one until the 8-cell stage). (F) Biradial pattern of some Lophophorates. (G) Lost of spiralian features in the cleavage of Gastrotricha (class Macrodasyoida). (H) Radial cleavage pattern of a Deuterostome (sea urchin)

Nonbilaterians

Despite their apparent morphological simplicity, the cell processes and the cleavage patterns deployed during the early development of nonbilaterian groups (Poriferans, Placozoans, Cnidarians, and Ctenophores) are extremely diverse (Adamska et al. 2011). In general, they show holoblastic cleavage patterns (even though some species have abundant yolk) that are characterized by their irregularity. Cell divisions are often asynchronous and random in direction, resulting in amorphous blastulae with low cohesion and no recognizable geometrical regularities (anarchical or chaotic cleavage). In many species, especially among cnidarians, the cleavage pattern is also variable between individuals (involving even transient syncytial stages by random fusion between blastomeres or by anomalous cytokinesis).

Interestingly, during these disordered cell divisions, some nonbilaterian taxa show transitory ordered patterns resembling those found in spiralians and deuterostomes (see sections "Nematoda + Nematomorpha" and "Arthropoda"). These are called, respectively, pseudospiral and radial-like patterns, but they are restricted to the very early stages (before 8-cell stage) and appear only in some individuals within a species. This suggests that they are likely to be produced by the mechanical stability of cell adhesion between blastomeres (best packing configurations).

The great spatio-temporal variability of the chaotic cleavage pattern prevents the determination of the cell fates during early cleavage: the cell fate of each blastomere cannot be unequivocally determined by its embryological context, since the relative position and identity of its surrounding blastomeres is far from constant. Some nonbilaterians also exhibit truly nonchaotic patterns. In some sponges, cell divisions are perpendicular to the cell surface (polyaxial cleavage) or to the animal-vegetal axis (incurvational cleavage).

On the contrary, Ctenophores display a regular cleavage pattern in which the four nearly identical quadrants organized around the animal-vegetal axis correspond with those found in the adult organism. This pattern does not resemble any other one found in metazoans, which does not help to clarify the phylogenetic position of this controversial taxon.

Xenacoeloelomorpha + Chaetognatha

Xenacoelomorpha (Acoela, Nemertodermatida, and Xenoturbellida) are thought to branch from the rest of the bilateria very early on. In general, their cleavage is holoblastic and shares some characteristics with the spiral one (Wanninger 2015). In Acoela, this cleavage pattern is called duet spiral. Mechanistically, it seems that the main difference to the quartet spiral cleavage (see section "Nematoda + Nematomorpha") relates to the timing in which the cell processes are deployed: in xenacoelomorpha, the "spiralizing" events leading to oblique cell divisions start one cell-cycle earlier (in the 2-cell stage) and the synchrony between cell divisions is lost earlier than in Spiralia. The mechanisms specifying the clockwise-counterclockwise alternation, as well as the cell fates and modes of cell fate determination differ substantially between spiralia and xenacoelomorpha. Thus, it is likely that both quartet and duet spiral cleavage patterns have evolved independently from an ancestral radial-like cleavage pattern.

In Chaetognatha, another bilateral phylum whose phylogenetic position is very controversial, the cleavage is holoblastic with equally sized and tightly packed blastomeres, making difficult to attribute their pattern to radial or spiral. However, some features like the left-right alternation of cell divisions respect to the AV axis and their cell fates suggest that their development is more similar to protostomes than to deuterostomes (Shimotori and Goto 2001).

Scalidophora

In Priapulids (the only scalidophoran taxon whose early development has been accurately described), the cleavage pattern is holoblastic, synchronous, and subequal (slightly different sizes between micro- and macromeres) (Wennberg et al. 2008). Up to gastrulation, cell divisions tend to occur at right angles to each other, generating a symmetric pattern that resembles the radial one. After the 16-cell stage, the blastula is so compact (either by increased cell adhesion or compression from the eggshell) that the visible face of each blastomere acquires a polygonal shape. The directions of further cell divisions seem to depend on these shapes: cell divisions take place along the longest axis of the visible face of each blastomere (this is specially clear for "rectangular" blastomeres). This may imply that a Hertwig-like rule restricted to the outer faces of blastomeres is the main driver of priapulid cleavage.

Nematoda + Nematomorpha

Nematode development is in general holoblastic and shows relatively high variation, especially within the subclass *Enoplia*). Cell fates are specified very early in development through a variety of mechanisms (Gilbert and Raunio 1997; Goldstein 2001). In their predominant mode of cleavage (holoblastic rotational), the longest axis of the ellipsoidal egg corresponds to the future antero-posterior (AP) axis. The polarity of this axis (which part of it will become the anterior part of the body) is determined either by entry point of the sperm in the oocyte or by the relative position of the egg within the uterus (the mechanism is taxon-specific).

Several mechanisms, including cell adhesion, cortical rotation, and specific cell-cell contacts controlling the direction of cell division, determine the blastomere arrangement. Inter-specific variations in this arrangement can be explained by variations in the strength and timing of these mechanisms.

In the model species *C. elegans*, the first cell division is asymmetric along the AP axis, producing a large anterior blastomere and a small posterior one (the germline precursor). Just after the first cell division, cells divide by default along successive orthogonal axes, suggesting that Sachs' rule applies in this system. However, cells belonging to the posterior half of the embryo always divide along the same AP axis because just after cell division and spindle positioning, the centrosome and nucleus rotate as a unit 90°, counteracting the "orthogonalizing" effect of Sachs' rule and leaving the mitotic apparatus oriented in the same axis as the preceding cell division. The compressive effect of the eggshell has also a pivotal role in the blastomere arrangement of nematoda. This is supported by the way the eggshell shape correlates with different blastomere configuration in different nematode taxa.

In Nematomorpha, a phylogenetically related phyla, cleavage is also holoblastic and all cell divisions are symmetric. Their cleavage pattern is highly variable, presenting transitory pseudospiral appearances that vanish after the 8-cell stage.

Because of this high variation, the cell fate of each blastomere is not specified until later stages (Malakhov and Spiridonov 1984).

Arthropoda

The early development of arthropods (including the two related phyla Onychophora and Tardigrada) is very diverse, but always results in a similar segmented body pattern (the phylotypic stage). In general, the geometry of blastomere arrangement is fairly irregular and homologous structures cannot be unambiguously derived from individual blastomeres (Scholtz and Wolff 2013). In very general terms, arthropod eggs have maternally provided asymmetries both in the antero-posterior and dorso-ventral axis that are essential for further development. These eggs are very yolky and display meroblastic cleavage. In many species, noncellularized nuclei (energids) start dividing deep within the yolk and then get displaced to the periphery forming a monolayer around the egg called blastoderm (intralecithal cleavage). After this migration, energids get cellularized and the yolk remains in central position (centrolecithal cleavage).

In other cases, the cytokineses are almost complete but the yolk and the blastomeres start dividing in one (2D) side of the embryo (discoidal or superficial cleavage). In this case, and due to Sachs' rule, transient regular (squares of 2 or 4 cells in each edge) configurations are often visible.

Holoblastic or yolk-poor cleavage patterns appear in some arthropod lineages that are viviparous or have planktotrophic larvae. In general, these holoblastic cleavage patterns display mixed features and thus cannot be classified in the main categories. For instance, a number of crustacean groups exhibit a cleavage, called modified spiral, which loosely resembles the canonical spiral pattern (See next section). Their cell fate map, however, and the cell processes involved in the direction of cell division (cell contacts) are different from the one observed in spiralians, suggesting that the crustacean cleavage is not homologous to the spiralian one. Probably, these early claims of spiral-arrangement in crustaceans was a misconception driven by the goal of finding embryological characters supporting the taxon *Articulata* (Annelids + Arthropods), nowadays rejected. Rather the contrary, the similarities between the crustacean cleavage and those found in nematomorphs and scalidophorans points to a radial-like holoblastic cleavage (which can arise from basic cell processes) as the ancestral mode for Ecdysozoans.

Spiralia

Spiralia comprise almost half of the animal phyla. Most of them (Mollusca, Annelida, Nemertea, Platyhelminthes, Entoprocta, and Gnathostomulida) exhibit a very conserved cleavage pattern called spiral or "quartet spiral" (Hejnol 2010). The quartet spiralian cleavage begins with two meridional cell divisions giving rise to four large macromeres. These macromeres then divide towards the animal pole but at

an oblique angle relative to the animal-vegetal axis, giving rise to four, normally smaller, animal micromeres that are all displaced to the right (or all to the left depending on the organism) of its sister macromere. This tilt between macro- and micromeres can be explained by oriented cell division (the mitotic spindles are tilted prior to cell division) and/or by cortical rotation after cell division. The direction of this tilt is determined by maternally inherited factors and often correlates with the symmetry of adults (e.g., snails with a tilt to the right have a dextrally coiled shell). The ensuing cell divisions follow a right-left alternation (the reverse alternation applies if the third division is to the left), making that, when viewed from the animal pole, the new micromeres seem to spin clockwise or counterclockwise when they arise. Sachs' rule has been proposed to be the driver of this alternation (Brun-Usan et al. 2017).

When cell fates are compared between different spiralians, it is often observed that the same adult or larval organs in different species arise from the same blastomeres (defined by lineage and relative position in the blastula). However, the mode of cell fate determination differs between quartet spiralian cleavers. Specifically, the so-called "D-blastomere" (a mesodermal precursor) can be specified either by cell–cell interactions after the fifth cell division or by asymmetrical segregation of cytoplasmic determinants, which in turn is caused by asymmetric cell division at the 4-cell stage.

In some Spiralians, the quartet spiral pattern is total or partially lost by different causes. For instance, in lophophorates (Brachiopoda, Bryozoa, and Phoronida), all cell divisions are symmetric, so that the distinction between macro- and micromeres does not hold as in canonical spiralians. In addition, their blastomeres are loosely attached, and consequently the mechanical interactions between them are weak. Under these conditions, the mechanism of cortical rotation lacks efficiency and is unable to produce any net cell displacement towards spirality (this mechanism requires enhanced cell-cell adhesion, see section "Introduction"). Thus, the first cell divisions proceed perpendicularity (Sachs' rule) until the 8-cell stage, which exhibits a radial-like pattern. After the 8-cell stage, the cleavage pattern of the different phyla of lophophorates becomes less predictable and more idiosyncratic: in Brachiopoda the pattern becomes irregular mainly due to asynchronous cell divisions, whereas in Ectoprocta and Phoronida both spiral-like and radial-like cleavages have been reported (Pennerstorfer and Scholtz 2012). This latter pattern (biradial pattern) leaves successively four and eight tiers of 4 blastomeres in line, but its symmetry axes are not always related to the larval body axes. Intraspecific variations of the cleavage patterns of lophophorates have also been reported, including the coexistence of radial-like and spiral patterns within the same population. This fact, at least in this group, can be understood by considering that small differences in cell mechanics (e.g., population-level variation in cell adhesion) can lead to drastic effects in the resulting blastula configuration.

The remaining spiralian phyla display a variety of highly derived forms of spiral cleavage. Many of these deviations from the quartet spiral pattern involve the compressive effect of very elongated eggshells, which produces drastic blastomere

rearrangements (Wanninger 2015). This, in turn, prevents the relative twist between macro- and micromeres (Rotifera) and/or even the formation of quartets, thus deleting any spiral appearance (Acanthocephala, Gastrotricha). Finally, a massive amount of yolk correlates with the loss of the spiral pattern (and a switch to a specific meroblastic cleavage) in cephalopod mollusks.

Deuterostomes

Deuterostome cleavage is holoblastic, with loosely attached blastomeres and typically radial. In radial cleavage, early cell divisions follow Sachs' rule: they are either parallel or perpendicular to the animal-vegetal axis, depending on their relative position along the animal-vegetal axis. Thus, along this axis blastomeres are always located one on the top of each other, not in oblique positions as in spiralia. In addition, some of these cell divisions are often asymmetric, yielding groups of cells of different size sorted along the animal-vegetal axis. Deuterostome taxa exhibit slightly different radial patterns, which arise from changes in the cell adhesion and in the timing and location of asymmetric cell divisions.

In ascidians (Urochordata), radial cleavage is replaced by bilateral cleavage, a remarkably conserved pattern, even between distantly related species. In it, the furrow of the first cell division establishes a plane of symmetry that separates the future right and left halves of the embryo. During most part of the cleavage, this plane keeps the right half of the embryo as the mirror image of the left one. First cell divisions proceed perpendicularly one to another (Sachs' rule), but other, more complex, forms of oriented cell division appear very early on, causing the blastula to depart more and more from radial cleavage. The evolutionary transition to this bilateral cleavage from the radial one is related to the presence of the centrosome-attracting body (CAB) in ascidians. The CAB is an actin-rich organelle that anchors the centrosomes of some neighboring blastomeres, so that they remain attached one to another. This in turn has a double effect: on the one hand it reduces the ways in which blastomeres can move, and on the other hand it enables the asymmetric segregation of maternal determinants in one of the two daughter cells via asymmetric cell division (Munro et al. 2006). The asymmetric distribution of these determinants, combined with inductive signals between neighboring cells within the constant cleavage geometry, pave the way for a very early cell-fate determination. This allows ascidians to develop quickly a functional tadpole larva with a small number of cells.

Other important departures from radial cleavage are found within vertebrates, and many of them are driven by a great amount of yolk in the vegetal pole. In amphibians, this causes the equatorial cell divisions to be displaced towards the yolk-free animal pole (displaced radial cleavage). If the yolk is distributed over all the egg, as in fishes, reptiles, and birds, only a meroblastic cleavage restricted to the surface of the animal pole can happen. In this case, first cell divisions still follow Sachs' rule as in radial cleavage, but the stereotypic pattern disappears soon. This kind of cleavage

is called discoidal and presents morphological commonalities with the one found in some Arthropoda.

In placental mammals, the early embryo develops inside the mother's body in a close metabolic dependence. Their eggs are consequently yolk-free, and the cleavage is holoblastic. The second round of cell division is not only perpendicular to the previous one, but also perpendicular between the two blastomeres (one is meridional, the other equatorial). Because of the resulting tetrahedral configuration, it is called rotational cleavage (notice the resemblance with the rotational cleavage of nematoda is restricted to the 4-cell stage, and the mechanisms involved differ). After that, cell divisions become asynchronous with cycles of increased cell adhesion, and their directions are determined by Hertwig's rule and the contacts between adjacent blastomeres, losing any radial (or even regular) appearance.

Conclusions

Despite their diversity, most cleavage patterns seem to have been built by means of the combination of a handful of similar and evolutionary old cell processes (Salazar-Ciudad et al. 2003). Among the many developmentally available patterns, most metazoans seem to use those exhibiting both mechanical stability and basic symmetry axes. These invariant cleavage patterns, in which the timing, orientation, and symmetry of cell divisions are precisely defined for all cells, allow the early use of inductive mechanisms for cell fate and body axes determination. Conversely, inductive mechanisms can affect the timing and orientation of cell division, thus determining subsequent fate decisions in a dynamic interplay.

More irregular and variable cleavage patterns are also displayed by many taxa (especially early divergent groups). Many of these groups with a loose control of cell processes during cleavage exhibit similarities in the blastomere arrangement in the very early stages, which seems to be due to mere packing principles. In these cases, the variability in blastomere arrangement prevents the early cell fate determination via inductive cellular interactions (Salazar-Ciudad 2010). Overall, our analysis suggests that the genetic control over early developmental events is not necessary for non-trivial cleavage patterns to arise, but in order to make these patterns more robust, repeatable and heritable (Hagolani et al. 2019).

Evolutionary transitions between different cleavage patterns have happened many times. Some of them may be explained by adaptive changes in their underlying cell processes (e.g., changes in cell adhesion, in the amount of yolk or the acquisition of a more rigid eggshell), but the developmental bases of these transitions are poorly understood (Brun-Usan et al. 2017). Much work remains to be done (involving experiments in model and nonmodel species, and computational approaches) in order to disentangle how the interplay between developmental and selective forces has sculpted the geometry of metazoan cleavage patterns.

Cross-References

- ▶ A Macroevolutionary Perspective on Developmental Constraints in Animals
- ▶ Mechanisms in Evo-Devo
- ▶ Mechanisms of Pattern Formation, Morphogenesis, and Evolution
- ▶ Modeling Evolution of Developmental Gene Regulatory Networks
- ▶ The Developmental Hourglass in the Evolution of Embryogenesis
- ▶ The Evolution and Development of Segmented Body Plans
- ▶ Twisted Shells, Spiral Cells, and Asymmetries: Evo-Devo Lessons Learned from Gastropods

References

Adamska M, Degnan BM, Green K, Zwafink C (2011) What sponges can tell us about the evolution of developmental processes. Zoology 114:1–10

Brun-Usan M, Marín-Riera M, Grande C, Truchado-Garcia M (2017) A set of simple cell processes are sufficient to model spiral cleavage. Development 144:54–62

Gilbert SF, Raunio AM (1997) Embryology: constructing the organism. Sinauer Associates Inc, Sunderland

Gillies TE, Cabernard C (2011) Cell division orientation in animals. Curr Biol 21:599–609

Goldstein B (2001) On the evolution of early development in the Nematoda. Phil Trans Roy Soc B: Biol Sci 356:1521–1531

Hagolani PF, Zimm R, Marin-Riera M, Salazar-Ciudad I (2019) Cell signaling stabilizes morphogenesis against noise. Development 146(20)

Hejnol A (2010) A twist in time-the evolution of spiral cleavage in the light of animal phylogeny. Int Comp Biol 50:695–706

Kajita A, Yamamura M, Kohara Y (2003) Computer simulation of the cellular arrangement using physical model in early cleavage of the nematode Caenorhabditis elegans. Bioinformatics 19:704–716

Lecuit T, Lenne PF (2007) Cell surface mechanics and the control of cell shape, tissue patterns and morphogenesis. Nat Rev Mol Cell Biol 8:633–644

Malakhov VV, Spiridonov S (1984) The embryogenesis of *Gordius* sp. from Turkmenia, with special reference to the position of the Nematomorpha in the animal kingdom. Zool Zh 63:1285–1296

Meshcheryakov VN, Beloussov LV (1975) Asymmetrical rotations of blastomeres in early cleavage of gastropoda. Roux Arch Dev Biol 177:193–203

Munro E, Robin F, Lemaire P (2006) Cellular morphogenesis in ascidians: how to shape a simple tadpole. Curr Op Gen Dev 16:399–405

Newman SA (2011) Animal egg as evolutionary innovation: a solution to the embryonic horglass puzzle. J Exp Biol B Mol Dev Evol 316:467–483

Newman SA, Forgacs G, Muller GB (2003) Before programs: the physical origination of multicellular forms. Int J Dev Biol 50:289–299

Pennerstorfer M, Scholtz G (2012) Early cleavage in *Phoronis muelleri* (Phoronida) displays spiral features. Evol Dev 14:484–500

Riegler A (2008) Natural or internal selection? The case of canalization in complex evolutionary systems. Artif Life 14:345–362

Salazar-Ciudad I (2010) Morphological evolution and embryonic developmental diversity in metazoa. Development 137:531–539

Salazar-Ciudad I, Jernvall J, Newman SA (2003) Mechanisms of pattern formation in development and evolution. Development 130:2027–2037

Scholtz G, Wolff C (2013) Arthropod embryology: cleavage and germ band development. In: Minelli A, Boxshall G, Fusco G (eds) Arthropod biology and evolution. Springer, Berlin/Heidelberg, pp 63–89

Sebé-Pedrós A, Roger AJ, Lang FB, King N, Ruiz-Trillo I (2010) Ancient origin of the integrin-mediated adhesion and signaling machinery. Proc Natl Acad Sci 107:10142–10147

Shimotori T, Goto T (2001) Developmental fates of the first four blastomeres of the chaetognath *Paraspadella gotoi*: relationship to protostomes. Develop Growth Differ 43:371–382

Valentine JW (1997) Cleavage patterns and the topology of the metazoan tree of life. Proc Natl Acad Sci U S A 94:8001–8005

Wanninger A (2015) Evolutionary developmental biology of invertebrates. Springer, Wien

Wennberg SA, Janssen R, Budd GE (2008) Early embryonic development of the priapulid worm *Priapulus caudatus*. Evol Dev 10:326–338

Wray GA (2000) The evolution of embryonic patterning mechanisms in animals. Semin Cell Dev Biol 11:385–393

The Evolution and Development of Segmented Body Plans

Ariel D. Chipman

Contents

Abstract

Segmentation is both a morphological phenomenon and a developmental process occurring in bilaterally symmetrical animals. A segmented body plan is one in which repeated body units are arranged along the anterior–posterior axis, each unit containing elements from a number of organ systems. Segmentation is found in three phyla: the arthropods, annelids, and chordates. There is some debate over whether the segmented body plan in these three phyla is homologous. However, despite many similarities, when using multiple data sources, the bulk of the evidence points towards a convergent evolution of the segmented body plan

A. D. Chipman (✉)
The Department of Ecology, Evolution and Behavior, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel
e-mail: ariel.chipman@mail.huji.ac.il

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_136

and the segmentation process in these three phyla. Segmentation probably arose first as an efficient mode for repeating units of different organ systems along the body axis. It then provided an improved mode of locomotion. Once segmentation became fixed in several lineages, these lineages diversified dramatically because of the enhanced evolvability and modularity that is thought to be conferred by the segmented body plan and its development.

Keywords

Segments · Somites · Annelida · Chordata · Arthropoda

Introduction

The evolution of segmentation has been a central theme in evolutionary developmental biology since the 1990s. Indeed, some of the first attempts at molecular evo-devo involved analyses of genes known from the *Drosophila* segmentation cascade in other arthropods (e.g., Patel et al. 1989). The term "segmentation" is used to denote two separate concepts, which are not always clearly differentiated (Minelli and Fusco 2004). One is the morphological phenomenon of the segmented body plan, i.e., the existence of segments. The other is the process generating this body plan, i.e., the generation of segments. Given the strong conceptual link between the process and the phenomenon, it is not surprising that segmentation provides a model for linking a morphological phenomenon and its evolution with the developmental process behind it and its evolution. The segmented body plan is the result of the segmentation process, and the evolution of the segmented body plan is through the evolution of the segmentation process.

Segmentation as Morphology

Presented most simply, segmentation is the organization of the body in a series of repeated units arranged along the anterior–posterior axis of the organism – segments. A stricter and more common definition of segmentation requires these repeated units to include components of several different, typically noncentralized, organ systems, arranged in register with each other. To illustrate, a segmented organism could be organized in segments each including, e.g., a skeletal unit, a neural ganglion, a nephronic unit, and a segmental muscle. This type of narrowly defined segmentation is found in only three animal phyla: Arthropoda, Annelida, and Chordata.

There are many additional cases wherein there is serial morphological repetition that does not conform to this narrow definition of segmentation (Minelli and Fusco 2004). This could be through an external annulation of the body that is not reflected in the internal organization of different organs, as is the case in onychophorans. This could also be through a reiterated organization of specific organ systems, without these being in register with repeats of other organs systems, as is the case in the

excretory system in some mollusks. In these cases, the organism is sometimes said to be "metameric" rather than "segmented" (Couso 2009). Alternatively, one can speak of metamerism as a property of a given organ system, and segmentation as a property of a whole organism (contra Budd 2001). The diversity of examples of partial segmentation can provide insights into how segmentation may have evolved.

Even within segmented organisms, as strictly defined above, not all segments conform to the platonic ideal of a segment. There are many examples of segment fusion, loss of metamerism in specific organ systems, mismatch in the segmental register of some systems relative to others, etc. Nonetheless, the general character of a segmented body plan can almost invariably be identified in embryonic development. It is during embryonic development, in the process of segment generation, that the true importance of the segmented body plan becomes obvious. For it is the process of the reiterated generation of individuated units, which then differentiate to give rise to all of the metameric organ systems within each individual segment, that is the foundation of the segmented body plan. It is here that segmented animals can be most clearly differentiated from nonsegmented ones.

The Segmented Phyla

There are three phyla that contain segmented organisms according to the strict definition: Arthropoda, Annelida, and Chordata.

Arthropoda

In the arthropods, all species have a clear overt segmentation of the body, with a very small number of unusual exceptions (mostly parasitic forms). Segmentation is most clearly visible in the exoskeleton, which is composed of serially repeated cuticular plates. Segmental longitudinal muscles connect the exoskeletal segments, allowing movement between the segments. Most (or all) segments have segmental appendages. The nervous system is arranged in segmental ganglia (which are sometimes fused), and segmental organization is also evident in respiratory organs, in some sensory organs, and in some lineages also in excretory organs.

Annelida

The annelids form the second major segmented phylum. Although segmentation is a synapomorphy (shared derived character) of the phylum, it has been lost several times in specific lineages (Chipman 2008a). A typical annelid segment includes both external annulation and internal septa, separating adjacent segments (though these have been lost in some taxa). As in arthropods, there are segmental muscles connecting to the body wall, segmental appendages, and a ladder-like segmentally

arranged nervous system. The excretory system is also arranged segmentally with metanephridia spanning the septum between two adjacent segments.

Chordata

Segmentation in chordates is very different from the previous two phyla. It is most obvious in the vertebrates, with partial segmentation also visible in the cephalochordates. There is usually no external evidence of segmentation (but some early fossil vertebrates do have segmentally arranged scales). Segmentation is most evident in the skeletal system, with segmental vertebrae and ribs. The trunk muscles are segmental and connect adjacent skeletal segments. The nervous system is only partially segmental, with most segmental structures belonging to the peripheral nervous system. The excretory system is primitively segmented, but this is lost in terrestrial vertebrates. The circulatory system also has segmental components. An interesting aspect of vertebrate segmentation is that in addition to trunk segmentation, vertebrates have two other minor forms of segmentation or metamery. These are the segmental arrangement of the branchial arches, with associated gills, gill slits and hematic arches (found also in cephalochordates), and the segmentation of the hindbrain, with connected cranial nerves.

A Common Origin for Segmentation?

The similarities in the segmental organization of annelids and arthropods, most notably external annulation, paired metanephridia, paired appendages, segmental muscles, segmental ganglia, and segmental coelomic spaces (Scholtz 2002), led traditional morphologists to unite these two phyla under the taxon Articulata (see chapter ▶ "Evo-Devo and Phylogenetics"). Current phylogenies reject this grouping, but the question remains whether these similarities represent a complex convergence event (see chapter ▶ "Convergence"), or whether they indicate the existence of a homologous set of characters reaching back to the common ancestor of these two phyla, at the base of Protostomia. Some authors have even suggested that the segmentation of vertebrates, despite many differences, is also homologous, pushing back the origin of the segmented body plan to the base of Bilateria; an idea best framed by the question "was Urbilateria segmented?" (Kimmel 1996). This question has been debated extensively in the literature, with some authors (Kimmel 1996; Couso 2009) suggesting an ancient origin of segmentation. Such a suggestion is based on perceived similarities in the embryonic development of the different segmented taxa, and the involvement of specific signaling pathway, such as Notch signaling, in the segmentation process in all three phyla (see section "Why Did Segmentation Evolve" below). Other authors argue for a convergent origin, based on the inherent differences in segmental structure and in the segmentation process and based on reconstruction of ancestral character states using both

fossil and extant organisms (Seaver 2003; Chipman 2010; Richmond and Oates 2012).

A thorough analysis of the origins of segmentation as a morphological phenomenon must take multiple sources of data into consideration and incorporate different analytical approaches. This has been done to a certain extent (Couso 2009; Chipman 2010; Ten Tusscher 2013; Vroomans et al. 2016), but there is still no consensus among all authors. The fossil record does not support a common origin for segmentation, since there is evidence for gradual attainment of segmentation, at least in arthropods (Budd 2001; Chipman 2010). However, the existence of clear overt annulations in *Dickinsonia*, which is suggested to be an upper stem-group bilaterian (Gold et al. 2015), raises the possibility that maybe some form of reiterated structures was present very early in bilaterian history. Conversely, theoretical considerations as well as analysis of gene network structure suggest that there may be common design principles in the generation of a segmented body, and the networks underlying these common principles could have evolved de novo several times in unrelated lineages (Chipman 2010; Richmond and Oates 2012; Vroomans et al. 2016; see chapter ► "Convergence").

Segmentation as a Developmental Process

The main approach to reconstructing the history of the segmented body plan, and the one most relevant from an evo-devo perspective, is through reconstructing the evolution of the segmentation developmental process, by comparing the process in the three segmented phyla, and in multiple species in each phylum, in order to trace the changes in the process within and between phyla.

Chordata

The process of segment generation has been studied in most detail within chordates, and specifically in the main vertebrate model systems, namely, zebrafish, chick, *Xenopus,* and mouse. Somitogenesis is the process in which an unsegmented mesodermal tissue, the presomitic mesoderm or PSM, is patterned to give rise to distinct blocks of tissue, the somites (Holley and Takeda 2002). The somites will later differentiate to give rise to most of the segmentally repeated structures. Migrating embryonic neural crest and ganglia from the developing neural tube align with somite derived structures to give rise to segmental components of the peripheral nervous system. The conceptual model that explains the function of the somitogenesis process is known as the "clock and wavefront model" (Cooke and Zeeman 1976). According to this model, cellular oscillations (the segmentation clock) generate a traveling wave, which moves across cells anteriorly from the PSM. The traveling wave meets a wavefront or morphogen gradient that decreases from anterior to posterior. At a certain level of the morphogen, the oscillating pattern is frozen, thus translating a variable pattern in time to a fixed pattern in space. Work

in the last few decades has demonstrated that somitogenesis is indeed driven by an oscillating genetic network in the PSM (Palmeirim et al. 1997; Oates et al. 2012). At the core of this network is a negative feedback loop possibly based on members of the transcriptional repressor family HES (hairy/enhancer-of-split), which by repressing their own expression with a certain delay give rise to oscillatory expression dynamics. The gradients of the conceptual model presumably include opposing gradients of members of the FGF and WNT signaling families on the one hand and retinoic acid on the other. Synchronization among oscillating cells in the PSM is through Delta-Notch signaling (Liao and Oates 2017). While the number and specific identity of the genes involved in this process vary among the different vertebrate models studied, it is fundamentally similar in all cases involving members of the FGF, Wnt and Notch signaling pathways.

Arthropoda

The segmentation process has also been studied extensively in arthropods. It is worth pointing out that the main model for segmentation in arthropods has traditionally been the fruitfly *Drosophila melanogaster.* In *Drosophila,* segmentation occurs through a cascade of interacting transcription factors comprising (in sequence) maternal factors, gap genes, pair-rule genes, and segment-polarity genes. In *Drosophila*, segments are generated approximately simultaneously rather than sequentially, and individual segments are specified in a unique rather than iterative manner. It is now clear that this species presents a derived and unusual mode of segmentation, and it will thus not be discussed further here. The common and ancestral mode of segment generation in arthropods takes place in a posterior growth zone, wherein segments are generated sequentially in an anterior–posterior progression (Auman and Chipman 2017; Williams and Nagy 2017). While there are less functional data for arthropods than there are for vertebrates, expression data suggest that arthropod segmentation is also driven by an oscillating mechanism. This mechanism generates a traveling wave, which moves across cells and is fixed at a certain point in space, giving rise to a reiterated pattern of segmental gene expression that ultimately generates segmental borders. The oscillating mechanism is thought to have ancestrally been based on Notch-Delta signaling (Chipman 2008b), although in the flour beetle, *Tribolium castaneum*, the oscillating mechanism is a negative feedback loop based on the interaction of three transcription factors: *evenskipped, oddskipped,* and *runt* (Choe et al. 2006). The transition between these two putative oscillators and their exact phylogenetic distribution remain unclear (Williams and Nagy 2017).

An added complication to the story of arthropod segmentation is the fact that in insects there are two distinct modes of segmentation. The first is the sequential segmentation process described in detail above. The second is a derived mode of simultaneous segmentation, first identified in *Drosophila* and found mostly in holometabolous insects, but also to a certain extent in hemimetabolous insects. The two segmentation modes are sometimes found together in the same embryo,

with anterior segments generated simultaneously and posterior segments generated sequentially. In other cases, all segments are patterned simultaneously (as in *Drosophila*) or all sequentially (as in *Tribolium*). The number of segments patterned through sequential or simultaneous segmentation is variable among taxa and the phylogenetic distribution of the two modes is complex (Stahi and Chipman 2016). The terms "long germ," "short germ," and "intermediate germ" are sometimes used to refer to simultaneous segmentation, sequential segmentation, and a combination of both, respectively, although this is an over-simplification. The terms originally referred to the number of segments patterned prior to gastrulation (Sander 1976; Davis and Patel 2002). Recent work has shown that the connection between germ type and segmentation mode is not as strong as previously believed (Stahi and Chipman 2016).

Annelida

Much less is known about the mode of segment generation in annelids. The best studied system is the leech embryo, but as in arthropods, it turns out that this model system is not representative for its clade. In leeches, segments are generated through the activity of posterior stem cells known as teloblasts. Each division cycle of these stem cells gives rise to the precursors of a single segment. Teloblast-based segmentation is thought to be ancestral to annelids, although the leech case is deemed to be an extreme example, whereas many polychaetes have lost the stem cells (Balavoine 2014). Studying segmentation in annelids is complicated by the fact that most of the segmentation process takes place during larval development and not in the embryo (with the exception of clitellates), and larval stages are more difficult to access. The variable rate of segmentation during larval development argues against a segmentation clock as such. There is also no evidence of any oscillating gene expression patterns or traveling waves (Balavoine 2014). There have been some reports of genes known from segmentation in arthropods or vertebrates being involved in segmentation in annelids, most notably Notch signaling (Rivera and Weisblat 2009), and some of the segment polarity and pair-rule genes known from the *Drosophila* cascade (Balavoine 2014). However, these data have been called into question by other researchers (Seaver 2003).

Regardless of the details, most segment generating mechanisms share a number of important commonalities (Scholtz 2002). Segmentation proceeds, with few exceptions, from the anterior to the posterior, with segments being added in a subterminal domain. The process is usually a reiterative process, with a single set of events repeating itself in the formation of each segment. The nascent segment includes the precursors of all or most of the organ systems that will be contained in the mature segment. Finally, the segmentation process is coupled with a process of posterior axis elongation (Jacobs et al. 2005; Chipman 2008b, 2010; Balavoine 2014). A corollary of the common anterior to posterior progression of segmentation coupled with axial growth is that in a segmenting embryo, the anterior–posterior axis also represents a time axis, with posterior segments being younger and displaying

earlier stages in the segment formation process, and anterior segments being more mature and displaying later stages. This facilitates the analysis of the mode of segment generation, by providing a window into a sequence of events within a single developing embryo.

Why Did Segmentation Evolve?

Although there is still no consensus, the bulk of the evidence from comparative embryology and from the fossil record points towards segmentation being a morphological phenomenon that evolved several times in different lineages, through convergent evolution of the developmental process. This convergence is based on common design principles and originated from a common bilaterian ancestor with posterior elongation and an existing suite of gene regulatory networks (Chipman 2010; Vroomans et al. 2016). These may have already included a network for generating repeated structures in one or more organ systems (possibly the nervous system). Hence, the many similarities in the developmental process in the different segmented taxa, despite their independent phylogenetic origin. If segmentation is indeed convergent, the question arises, why did segmented body plans arise numerous times, and why is the variety of segmented animals so large?

There are in fact two separate questions here: what was the selective force for the appearance of the segmented body plan? and what about the segmented body plan allowed lineages that possessed it to diversify? The answer to the first question has been debated surprisingly little (Budd 2001) and has yet to be resolved. Reiterated structures may have first arisen as a response to an increase in the length of the body and the need to distribute various organ systems (e.g., sensory organs and their processing, excretory organs) along the extended body axis. Once several organ systems evolved to be distributed along the main axis, there was an advantage to coordinating their distribution by merging their development into a single process. This developmental process would have been the precursor to the segmentation process seen today. Thus, according to this scenario, the selective advantage to segmentation would have been improved efficiency in distributing organ systems along an elongated animal. Extending this coordination to skeletal elements, appendages and muscular elements would provide an improved mode of locomotion (Budd 2001).

The three fully segmented phyla – arthropods, annelids, and vertebrates – are all highly successful from an evolutionary point of view. Arthropods are by far the most diverse phylum on earth, by any metric chosen. Annelids hold third place in terms of species number, but represent a very broad ecological range. Vertebrates are not as diverse as many other groups, but dominate most eco-systems where they are present. Is this success directly linked to their segmented body plan? Once the mode of segmentation was established, it provided numerous advantages beyond the original selective pressures that led to it (see chapter ▶ "Developmental Exaptation"). The segmental organization is extremely modular and thus highly evolvable (see chapter ▶ "Evolvability"). It is obvious from looking at the diversity of arthropods (and to a lesser extent that of annelids) that they are all variations on a theme.

The basic underlying segmental body plan is common to the phylum, but it is the variability of segment number and type and of the specific specialization of individual segments in terms of shapes, sizes, and appendage types that are responsible for the outstanding diversity of arthropods. It is reasonable to state that the evolutionary success of the segmented phyla is due to the long-term advantages provided by segmentation (both in the morphological and the developmental sense) and that these are not the same as the selective advantages that led to its first appearance and consolidation (see chapter ▶ "Developmental Innovation and Phenotypic Novelty").

Cross-References

- ▶ Convergence
- ▶ Developmental Exaptation
- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Evo-Devo and Phylogenetics
- ▶ Evolvability
- ▶ History and Current Theories of the Vertebrate Head Segmentation
- ▶ Inherency

References

Auman T, Chipman AD (2017) The evolution of gene regulatory networks that define the arthropod body plan. Integr Comp Biol 57:523–532

Balavoine G (2014) Segment formation in annelids: patterns, processes and evolution. Int J Dev Biol 58(6–8):469–483

Budd GE (2001) Why are arthropods segmented? Evol Dev 3(5):332–342

Chipman AD (2008a) Annelids step forward. Evol Dev 10(2):141–142

Chipman AD (2008b) Thoughts and speculations on the ancestral arthropod segmentation pathway. In: Minelli A, Fusco G (eds) Evolving pathways: key themes in evolutionary developmental biology. Cambridge University Press, Cambridge, pp 339–355

Chipman AD (2010) Parallel evolution of segmentation by co-option of ancestral gene regulatory networks. BioEssays 32(1):60–70

Choe CP, Miller SC, Brown SJ (2006) A pair-rule gene circuit defines segments sequentially in the short-germ insect *Tribolium castaneum*. Proc Natl Acad Sci U S A 103(17):6560–6564

Cooke J, Zeeman EC (1976) Clock and wavefront model for control of number of repeated structures during animal morphogenesis. J Theor Biol 58(2):455–476

Couso JP (2009) Segmentation, metamerism and the Cambrian explosion. Int J Dev Biol 53:1305–1316

Davis GK, Patel NH (2002) Short, long, and beyond: molecular and embryological approaches to insect segmentation. Annu Rev Entomol 47:669–699

Gold DA, Runnegar B, Gehling JG, Jacobs DK (2015) Ancestral state reconstruction of ontogeny supports a bilaterian affinity for Dickinsonia. Evol Dev 17(6):315–324

Holley SA, Takeda H (2002) Catching a wave: the oscillator and wavefront that create the zebrafish somite. Semin Cell Dev Biol 13(6):481–488

Jacobs DK, Hughes NC, Fitz-Gibbon ST, Winchell CJ (2005) Terminal addition, the Cambrian radiation and the Phanerozoic evolution of bilaterian form. Evol Dev 7(6):498–514

Kimmel CB (1996) Was Urbilateria segmented? Trends Genet 12(9):329–331
Liao B-K, Oates AC (2017) Delta-Notch signalling in segmentation. Arthropod Struct Dev 46 (3):429–447
Minelli A, Fusco G (2004) Evo-devo perspectives on segmentation: model organisms, and beyond. Trends Ecol Evol 19(8):423–429
Oates AC, Morelli LG, Ares S (2012) Patterning embryos with oscillations: structure, function and dynamics of the vertebrate segmentation clock. Development 139(4):625–639
Palmeirim I, Henrique D, Ish-Horowicz D, Pourquié O (1997) Avian hairy gene expression identifies a molecular clock linked to vertebrate segmentation and somitogenesis. Cell 91 (5):639–648
Patel NH, Kornberg TB, Goodman CS (1989) Expression of engrailed during segmentation in grasshopper and crayfish. Development 107:201–212
Richmond DL, Oates AC (2012) The segmentation clock: inherited trait or universal design principle? Curr Opin Genet Dev 22(6):600–606
Rivera AS, Weisblat DA (2009) And Lophotrochozoa makes three: Notch/Hes signaling in annelid segmentation. Dev Genes Evol 219(1):37–43
Sander K (1976) Specification of the basic body pattern in insect embryogenesis. Adv Insect Physiol 12:125–238
Scholtz G (2002) The Articulata hypothesis – or what is a segment? Org Divers Evol 2(3):197–215
Seaver EC (2003) Segmentation: mono- or polyphyletic? Int J Dev Biol 47(7–8):583–595
Stahi R, Chipman AD (2016) Blastoderm segmentation in *Oncopeltus fasciatus* and the evolution of arthropod segmentation mechanisms. Proc R Soc Lond B 283:20161745
Ten Tusscher KH (2013) Mechanisms and constraints shaping the evolution of body plan segmentation. Eur Phys J E Soft Matter 36(5):54
Vroomans RM, Hogeweg P, Ten Tusscher KH (2016) In silico evo-devo: reconstructing stages in the evolution of animal segmentation. EvoDevo 7:14
Williams TA, Nagy LM (2017) Linking gene regulation to cell behaviors in the posterior growth zone of sequentially segmenting arthropods. Arthropod Struct Dev 46:380–394

Mechanisms of Pattern Formation, Morphogenesis, and Evolution

Isaac Salazar-Ciudad

Contents

Abstract

This chapter introduces the diversity of ways in which developmental mechanisms lead to pattern formation and morphogenesis. Developmental mechanisms are described as gene networks in which at least one of the genes affects some cell behavior (cell division, cell adhesion, apoptosis, cell contraction, cell growth, signal and extracellular matrix secretion, etc.). These mechanisms mediate one of the most important processes in development: the transformation of specific distributions of

I. Salazar-Ciudad (✉)
Genomics, Bioinformatics and Evolution, Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona, Barcelona, Spain

Evo-Devo Helsinki Community, Center of Excellence in Experimental and Computational Biology, Institute of Biotechnology, University of Helsinki, Helsinki, Finland
e-mail: isaac.salazar@helsinki.fi; Isaac.Salazar@uab.cat

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_51

cell types in space (starting with the zygote) into other, often more complex, spatial distributions of cell types (later developmental stages ending up in the adult). This chapter explains in detail why genes alone are unable to account for pattern formation and why they require cell behaviors and epigenetic factors. Three main types of developmental mechanisms are described in this respect: autonomous, inductive, and morphogenetic. This chapter also explains how these three types of mechanisms are combined in animal development and how these different combinations lead to different kinds of phenotypic variation and morphological evolution. It is concluded that understanding the mechanisms of development is crucial to have a more complete evolutionary theory in which extant phenotypes can be explained based not only on natural selection but also on what phenotypic variation can be produced by development in each generation.

Keywords

Evo-devo · Developmental mechanisms · Pattern formation · Morphogenesis

Introduction

Evolution can be understood as change over generations on a lineage of reproducing organisms. Development, on the other hand, can be understood as the process of change over an organism's life. Although it is common to refer colloquially to inheriting some phenotypic trait (such as eye color) from a relative, the phenotype is not inherited as such, only the gametes are. Thus any organism's phenotype, and any difference between organisms in successive generations, has to be built de novo from those gametes in each generation. The building of the organism is the process of development. In that sense, the range of phenotypic changes that can arise through the process of development determines the range of possible changes in evolution (Alberch 1982). This chapter gives a general overview of the repertoire of mechanisms that operate in development to build the phenotype and its variation.

The changes in development that lead to changes in the phenotype arise, ultimately, from changes in the environment (what is usually called phenotypic plasticity) and changes in the DNA. The identification of these causes was central to twentieth-century biology. This identification, although very important, does not by itself allow us to understand or predict how the phenotype will change. In that respect, genetics tells us that the inheritance of phenotypic characters is largely due to the inheritance of the DNA, and that the changes in these characters imply changes at the level of the DNA (if those changes are heritable). It is currently not understood, however, why or how some specific genetic changes lead to some phenotypic changes, and not to others, and what is the range of phenotypic changes that can arise from genetic changes (in a given generation and population). In a large number of cases, there are descriptions of which genetic changes are associated with which phenotypic changes, but this, by itself, does not entail an understanding of why these genetic changes lead to those specific phenotypic changes.

Understanding the relationship between genetic variation and phenotypic variation, also called the genotype-phenotype map, is probably one of the greatest challenges in twenty-first-century biology. This is currently the main limiting factor for the advancement of evolutionary biology, medicine, and biology in general. In fact, the rise of evo-devo in the late twentieth century and early twenty-first century is largely related to the realization of the importance of the genotype-phenotype map and of its developmental bases in evolution (Alberch 1982; Müller 2007).

The genotype-phenotype map is generally acknowledged to be quite complex. Usually, this complexity is understood to arise from the fact that the phenotype results from the interaction among a large number of genes and between those genes and epigenetic factors of several kinds. This is especially the case when the phenotype considered is morphology. Morphology, understood here as the 3D spatial distribution of cells and cell types in an organism, arises through the process of development. It is important to understand, however, that it is not the case that without these genetic and epigenetic interactions it would be possible to produce realistic phenotypes with a simple genotype-phenotype map (Salazar-Ciudad 2006b, 2007). Without those interactions, the phenotype would simply not exist beyond single isolated cells. It is precisely because in development cells interact through extracellular signaling and mechanical interactions that a phenotype made of something more than a single cell or small blob of disorganized cells can be built.

This fact has not always been obvious to everybody. That probably has to do with the way in which genetics was formulated in the nineteenth and early twentieth century. Genetics developed before the discovery of DNA, its structure, and its role in inheritance. Genetics is the study of inheritance, and as such, experimental genetics was at first based on crossing organisms with different phenotypes and observing the phenotypes of their offspring. Since phenotypes are complex, those studies predominantly focused on easily identifiable and mostly discrete phenotypic characters (e.g., yellow vs. green peas). At some point, it was discovered that the patterns of inheritance of some of these characters could be explained by the transmission of some discrete physical particles in the cell's nucleus. Even later it was discovered that these particles are made of DNA, and that some DNA sequences code for different sorts of RNA and proteins. From that it may seem possible to say that genes are "coding" for discrete phenotypic characters, such as the greenness or the yellowness of peas, or more generally for characters of all sorts. It is currently known, however, that the information necessary for the color of the pea, or the information for any phenotypic character for that matter, is rarely contained within the gene "coding" for that character (especially when morphological characters are considered). It is normally impossible to guess the phenotypic effect of a change in a gene product from its DNA sequence, or even from its protein structure. This is not because of a limitation in our knowledge, but simply because the information is not there. Single genes do not build phenotypic characters and their variation on their own. Genes affect the phenotype because they are embedded in networks of genetic and epigenetic interactions. Due to these networks of interactions, variation in these genes leads to variation in some phenotypes. In a way, the information for greenness and yellowness is contained, and distributed, in the whole network of genetic and

epigenetic interactions involved in each trait development. How those networks work and how they lead to phenotypes, and their variation, are the main questions of this chapter. To understand that better it is informative to consider in more detail what gene products and cells can do.

What Gene Products and Cells Can Do

What Gene Products Can Do

Sequence variation in a gene can have multiple and complex effects on the phenotype. Gene products, however, can only do a limited number of things.

First, they can bind to each other or to other molecules. Binding refers to nonchemical bonding, the close apposition of molecules due to hydrogen bridges, van der Waals forces, and other weak interactions. The specificity of that binding largely depends on the structure of gene products: their constituent atoms, their spatial distribution, and their chemical covalent bonds. Noncovalent binding to other molecules can alter the spatial distribution of the atoms in a gene product (a conformational change) and then affect to which other molecules a gene product can bind. Binding can occur with stable molecules or with molecules that are at the transitional unstable state between two other molecules and, as a result, catalyze different chemical reactions (as in enzymes).

Second, gene products can move passively, by the physical process of diffusion; or actively, through conformational changes mediated by cycles of bonding – for example, to ATP and ADP molecules as in the case of myosin. Gene products, like other molecules, can also be moved passively due to binding to a structure that moves (e.g., to an organelle that is being moved by the gene product kinesin).

Third, gene products can undergo changes of state. This is simply a change in what a gene product is "doing" (including the actions described above). These changes are alterations in either the conformation or composition of a gene product (e.g., as a result of binding or chemical reaction) that change its binding specificity and, possibly, how it moves. Usually this does not occur at random. A given gene product can have a relatively small number of possible conformational states and react to a small number of modified forms (e.g., by phosphorylation or proteolysis). In that respect, many gene products are like small computational devices. They undergo a number of transitions between states, or outputs, depending on the set of molecules that interact with them in each moment (or input). These computations tend to be relatively simple. Many transcriptional factors, for example, can only bind to the DNA if phosphorylated. In these cases, the gene product has only two states, the nonphosphorylated and the phosphorylated. In other, more complex cases, a given conformational change only occurs if a gene product binds to (or is modified by) two different molecules (thus implementing an "AND" logic gate), if it binds to either of them (an "OR" logic gate), or it binds to only one of them (an "XOR" logic gate). What determines the outputs a gene product gives to each set of inputs is basically its structure. In general, however, it is quite difficult to predict the structure,

possible conformational changes, and binding specificity of a gene product from its sequence. All these questions have been extensively researched, and it is nowadays widely acknowledged that at this molecular level there is already a rather complex genotype-phenotype map (the genotype would be the DNA sequence, and the phenotype the protein structure; Fontana and Schuster 1998). The above discussion applies also to the cis-regulatory elements in DNA.

If gene products are limited in what they can do, how can they build complex multicellular organisms such as us? It seems they cannot do it on their own. To be involved in the building of the body, gene products need to affect what cells do. Again, cells can do a small number of things, but it is important to realize that those things are not reducible to what gene products can do. Many of the things cells can do (dividing, binding, etc.) are not determined by gene products as such but are intrinsic to cells. In fact, lipid micelles and liposomes are, under some conditions, able to divide, fuse, "die," and bind to each other (Schrum et al. 2010). Environmental factors, noise, and some properties of the liposomes and micelles (such as size) are what determine when they will divide, fuse, adhere, or disintegrate. There are theories suggesting that cells may have originated before and independently from gene products and DNA (Segré et al. 2001).

Since organisms are mostly made of cells (and some extracellular matrix; ECM), it is clear that development involves the regulation of what cells do (dividing, binding, moving, etc.) over time and space in the embryo. In that sense, development could be reduced to the description of what cells do in each moment and place. Thus, irrespective of how complex gene products are, they influence development to the extent that they affect, directly or indirectly, the things cells do.

What Cells Can Do

Cells can also do a limited number of things (cell behaviors). They can divide. If the mother cell has some internal spatial polarization, the two daughter cells can be of different sizes and inherit a different set of molecules from the mother. Cells can also fuse with each other, and they routinely do so in the development of some organs (such as muscle). Cells can grow or shrink in size. They can bind to each other or to the ECM. This is often mediated by adhesion gene products expressed in the membrane that show some binding specificity for molecules present in the membrane of other cells or in the ECM. In spatially polarized cells, different parts of the membrane may express different adhesion proteins and, thus, bind to different types of cells. Cells can also die. Cell death may be elicited by signals from other cells as part of the healthy normal development of an organism. Cells can contract part of their body. As a result of contraction and adhesion, cells can change their shape. Cells can move in a passive or active way. Passive movement occurs as a result of cells being bound to others that move actively or contract actively. Active cell movement is a result of coordinated adhesion and contraction. Cells can also secrete ECM and extracellular diffusible molecular signals that may then bind to specific receptors in other cells. All these things that cells can do and their mechanical

properties have been suggested as being crucial not only to understand current development but also its origins (see chapter ▶ "Inherency"). According to these authors, early multicellular animals' morphology was determined mostly independently of gene networks (Newman et al. 2006). Later, gene networks would have been recruited to make development less sensitive to changes in the environment and to noise.

Cells can also change their state. These are changes in what a cell is doing, that is, which cell behaviors are active in each moment and how much. As in the case of molecules, cells can be considered as computational devices that elicit a different response or output (the cell behaviors activated and by how much), depending on the inputs received. The inputs are the extracellular signals a cell is receiving (typically diffusible gene products), their concentration, the intensity and direction of the mechanical forces being received, and whether they are stretching or compressing the cell. In each cell, which output arises from which specific set of inputs is determined by the network of gene products and other molecules expressed in the cell and by some cell-level properties such as the metabolic state, cell size, and cell shape. It is important to realize that, although this cell-level computation seems complex, the outputs themselves are relatively simple – the activation of one or several of these cell behaviors with different intensities. In addition, in most organisms' development, cells give relatively fast and short-lived responses to inputs. Development proceeds further simply because these responses often involve the secretion of signals and mechanical forces that are themselves inputs for other cells. In that sense, cells are constantly receiving signals and changing their behaviors. Thus, the complexity of development does not arise from how cells respond to signals, or from the signals themselves, but from the emergent spatio-temporal pattern of signals and responses in whole cell collectives.

Developmental Mechanisms

If gene products and cells can do only a limited number of things, the question then is how these behaviors are organized during development to build the body. Here, a *developmental mechanism* is defined as any network of interacting gene products in which at least some gene regulates some cell behavior so as to lead to pattern formation (Salazar-Ciudad et al. 2003). In that sense, each developmental mechanism is a different way of arranging what cells and gene products can do, or, in other terms, a different network topology. This definition pays special attention to the gene network topology and not to the network of cell interactions. Although this is mostly for convenience and it could be done in some other way (see Newman 1994), this definition has the advantage that there is much more empirical information about gene network topologies than about which cells interact with which.

This definition of developmental mechanism considers only gene networks involved in pattern formation. Pattern formation is understood here as the transformation of one spatial distribution of cell types (a developmental pattern) into another. The process of development can then be described as a sequence of pattern transformations, starting from the zygote, between different developmental patterns

over time. Note that according to this definition, mere changes in morphology (such as in morphogenesis) are also considered pattern formation (see Fig. 1). Note also that gene networks with the same topology and affecting the same cell behaviors but in which genes bind to, or regulate, each other with different intensities are considered to belong to the same developmental mechanism.

There are three things to stress here about pattern formation with regards to developmental mechanisms. First, all pattern formation events start from an initial pattern. It is totally arbitrary and up to the researcher from which initial developmental pattern to which later pattern to focus attention but there is always an initial pattern, at least the zygote, and it is almost always spatially nonhomogeneous. Thus, in the same way that there is no such thing as a gene to make a leg (or green and yellow peas), neither is there a single developmental mechanism to make a leg. There are, instead, developmental mechanisms that can build a leg from some specific initial conditions (see Fig. 1). The set of different final patterns arising by a developmental mechanism from different initial patterns, different environments, or different genetic mutations (as long as those do not change the gene network topology) are what is here called the *variational properties* of that developmental mechanism.

Second, the process of development implies the creation of new spatial information that is not present in the genome or in the structure of the gametes. Historically, it has sometimes been suggested that information is not created but rather simply transformed or unfolded, that in some way the information to build the body is present in the genome. The concept of information in biology is complex and elusive, but in the case of development there is a change in the spatial information over time: cells get into different places within the embryo in a specific pattern. This information cannot be said to be present in the genes. Rather, it arises from the interaction between those genes and the previously existing developmental patterns, starting from the spatial structure of the oocyte. In this sense, new epigenetic information (the developmental patterns themselves) gets generated from previous epigenetic information (previous developmental patterns), genetic information (the gene networks), and some other epigenetic information (such as the mechanical properties of cells, cell behavior, and other biophysical processes).

Third, the concept of developmental mechanisms and their variational properties supersedes the concepts of developmental constraints (Salazar-Ciudad 2006b) and evolvability (Salazar-Ciudad 2007). The developmental constraint concept arose in contrast to the view held in the neo-Darwinian approach that all phenotypic traits have genetic additive variation and that, thus, variation never limits the action of natural selection. Although this roughly holds true for single traits, it is not true when combinations of traits are considered. In fact, it is well known that many traits tend to correlate; in other words, they do not vary independently from each other and, thus, cannot take all possible combinations of trait values. The developmental constraint concept was used to present the idea that because there are complex genetic and cellular interactions during development, the relationship between genotype and phenotype is complex and not all conceivable phenotypes are possible. The problem with this view is that, in fact, it is precisely because of these complex genetic and cellular interactions that the phenotype and its variation are possible at all. Without

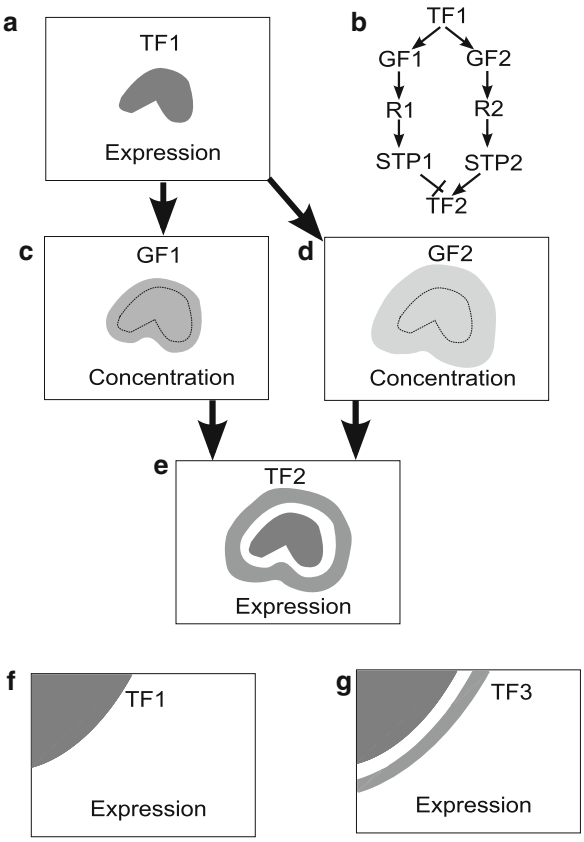


Fig. 1 Simple example of pattern formation. The initial developmental pattern in *A* gives rise to the developmental pattern in *E*. *B* shows a possible developmental mechanism, inductive hierarchic, able to produce this pattern formation. In *A* and *E*, the *square* represents a lattice of epithelial cells seen from above. In *A*, the area in *dark gray* marks the spatial distribution of the cells expressing a transcriptional factor TF1. The same *dark gray* territory is present in *E*, but in addition there is a new territory, in *medium gray*, expressing a transcriptional factor, TF2. In *B*, GF1 stands for growth factor 1, whose expression gets activated by TF1, while GF2 stands for growth factor 2, whose expression is also activated by TF1. R1 stands for the membrane receptor of GF1 and R2 for that of GF2. These are assumed to be expressed in the whole epithelium. STP1 stands for the signal transduction pathway of GF1 and STP2 for that of GF2. For simplicity, molecules are not represented. The pattern shown in E arises from the diffusion of GF1 and GF2. These are expressed in the same cells as TF1, since their expression is activated by it, but are secreted in the extracellular space and diffuse. Both GF1 and GF2 get degraded over time, but GF2 is smaller and then effectively diffuses at larger distances than GF1. *C* shows the spatial distribution of GF1 and *D* that of GF2 (for simplicity the different concentrations of these molecules are not represented, only where the molecules are present in the extracellular space). It is in the two regions marked in *C* and *D* that the concentration of those molecules is large enough to activate their receptors and the signal transduction pathways. STP1 inhibits the expression of TF2, while STP1 activates it. As a result, TF2 is expressed where GF2 is present, but GF1 is not. This is a ring at a certain distance from GF1. Notice that the territory of expression of TF2 resembles a ring with a shape similar to that of territory TF1 centered around it. The *broken line* indicates transcriptional inhibition. *G* shows the developmental pattern resulting from the same developmental mechanism in *B* acting on the initial developmental pattern in *F*

these interactions, we simply would not have complex phenotypes and variation but rather simple unicellular organisms. In that sense, the concept of developmental mechanisms and variational properties better reflects the fact that morphological variation arises because of development (not in spite of development), and that development can work in different ways in different organisms and, thus, lead to different morphological variation (different variational properties). A similar situation occurs with evolvability. Evolvability is a concept used in different disciplines without necessarily being clearly or compatibly defined (see chapter ► "Evolvability"). In its most widespread version (Wagner and Altenberg 1996), evolvability is understood to be the capacity of something (a genotype, a species, a part of development) to produce adaptive variation. The problem is that whether some variation is going to be adaptive or not depends on the environment, and, thus, one cannot tell simply from the genetics or development (as in evolvability studies) whether this will be the case or not. Thus, it is more useful and less confusing to have a concept for the kind of variation that is produced (i.e., the variational properties concept) and, then, others for which variation is adaptive (independently from how such variation is produced).

Autonomous Mechanisms

Autonomous mechanisms are developmental mechanisms in which pattern transformation occurs without cells interacting with each other. Instead, the non-homogeneous spatial structure of a cell, typically a zygote, is translated into a multicellular context by cell division. Thus, zygotes, insofar as they inherit a spatial structure, can by mere cell division such as in cleavage, arrive at a new pattern in which the different spatial regions of the zygote end up within different cells but with the same relative spatial arrangement.

Inductive Mechanisms

Inductive mechanisms are developmental mechanisms in which pattern formation is attained by having cells in different places express different genes because of molecular signaling between cells. These are by far the most-studied and best-understood developmental mechanisms. The inductive developmental mechanisms that have been proposed in the literature can be classified into two main types: Hierarchic and emergent inductive mechanisms.

Hierarchic Inductive Mechanisms

In hierarchic inductive mechanisms, a group of cells secrete a diffusible signal (or expresses a membrane-bound signal) that then diffuses in the extracellular space (or binds to a receptor on a cell in close contact to it) and binds to extracellular receptors on other cells. That binding activates a signal transduction pathway that

leads to gene expression changes in the receiving cells. The receiving cells may respond by secreting some other extracellular signals that may reach the original group of cells sending the first signal. In hierarchic mechanisms, the response of cells to incoming signals does not affect how these incoming signals are being secreted at the source cells (i.e., at the cells that originally sent the signal; see Fig. 1). As a result of this signaling, a new pattern arises that in the simplest case contains two groups of cells or territories: the original group of cells sending the signal and the set of cells that receive that signal at a concentration high enough for the activation of its receptor. The latter group has a distribution in space, or territory shape, that resembles that of the former with some variation in width, depending on how much the signal diffuses, and some blurring due to the averaging nature of the diffusion process itself (Salazar-Ciudad 2006a).

In more complicated cases, different concentrations of the original signal can lead to the differentiation of different types of cells, as in the French flag model, but even in this case the shape of the newly induced groups of cells would resemble that of the source cells, namely, concentric rings around the cells sending the signal (see Fig. 1). In even more complicated examples, signals coming from different sources (see Fig. 2) can combine in space – for example, using logical operations – to lead to slightly more complex patterns. In a 2D lattice of cells, maximal complexity patterns (where each cell has a different gene expression pattern) can be attained from two

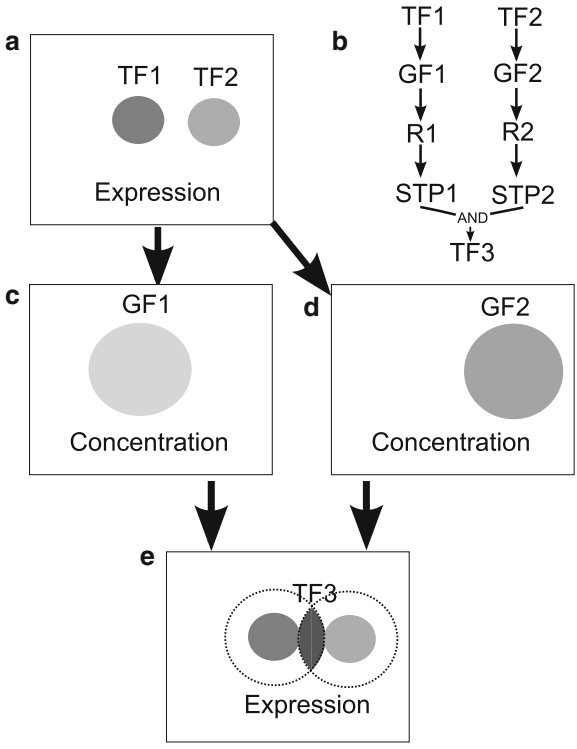


Fig. 2 As in Fig. 1, but here the developmental pattern in *E* has a new territory where transcriptional factor TF3 is expressed. This gene is expressed where both GF1 and GF2 are present at enough concentration to activate their respective receptors. The *discontinuous lines* in *E* mark where the two growth factors are present (as also shown in *C* and *D*)

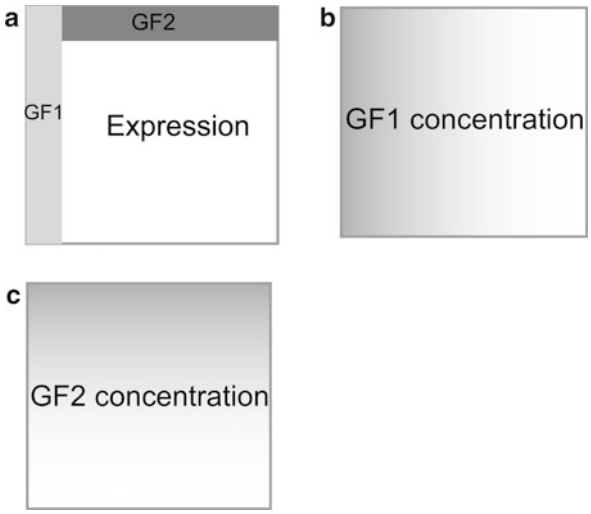


Fig. 3 Initial pattern in which two territories each express a different growth factor (GF1 and GF2). These are secreted in the extracellular space and produce two perpendicular gradients along a lattice of epithelial cells. As a result, each cell receives a unique combination of concentrations of those two growth factors, and thus each cell can be said to receive distinct, unique epigenetic information. For that to translate into a real developmental pattern in which each cell expresses a particular combination of genes a very complex gene network is required (too complex to be shown here). In addition, this network would be different for lattices with different numbers of cells

signals producing perpendicular gradients (see Fig. 3). This, however, requires a rather complicated network in which very small differences in each signal concentration can activate completely different gene expression patterns downstream.

Emergent Hierarchic Networks

In emergent networks, the response of cells to incomings signals involves the secretion of secondary signals that affect the rate at which the first signals are secreted. As a result, patterns arise where the spatial distribution of cells usually does not resemble that of the cells sending the first signals. In the simplest cases, the secondary signals activate the secretion of the first, and these then make a positive loop by which all cells end up expressing both signals. The most interesting case, however, is when one signal, usually called an activator, promotes its own secretion and that of another signal, the inhibitor, which when received by cells inhibits the secretion of the activator. Depending on the diffusibility of these signals and on how much they affect each other, rather complex patterns can arise from these networks. These mostly consist of stripes or spots of high activator concentration. In some cases, very similar patterns arise from different initial conditions, while in others, depending on the exact network, stripes and spots arise as concentric rings around the signals in which the activator was expressed in the initial conditions. This type of

mechanism is usually called a "reaction-diffusion" or "Turing-like" mechanism (Meinhardt 1982).

Morphogenetic Mechanisms

From the above discussion, it seems clear that not many developmental patterns can be achieved from autonomous and inductive mechanisms alone. The former only translate spatial asymmetries within the zygote into groups of cells, while the latter only produce stripes, spots, and distorted copies of the shapes of the groups of cells sending a signal. More complex patterns can be attained by combining signals coming from groups of cells in different spatial locations. However, the location and shape of the groups of cells sending a signal are not able to change in any conceivable way. If the territories are themselves produced by inductive mechanisms, their shapes are modifications of those of other previous territories and, ultimately, only of those spatial regions present in the zygote. In the zygote, there are normally only two or three more or less perpendicular axes defining two or three spatial regions: the animal-vegetal axis, an anterior-posterior axis, and in some species a left-right axis. Moreover, none of these mechanisms can change the location of cells in space (they only change the location of cell types or gene expression within groups of cells).

It is by the use of morphogenetic developmental mechanisms that the spatial distribution of cells can be changed. Changes in cell spatial location are due to mechanical interactions between cells. There is a quite large diversity of morphogenetic mechanisms (see Salazar-Ciudad et al. 2003 for a review), but in general, in here, the gene network involved is not so important as the diversity of cell behaviors used.

Mechanical forces are generated by cells and transmitted through cells and the ECM by means of four cell behaviors: cell contraction, cell growth, cell division, and ECM secretion (Lecuit et al. 2011). Cell division probably generates forces in a secondary way since it involves contraction and growth of the membrane. Adhesion by itself may not actively generate forces, but it mechanically couples cells so that the forces in one cell pull others.

From simple initial conditions, morphogenetic mechanisms can lead to rods, tubes, invaginations, cavities, and extensions in one dimension, while others contract (Newman 1994; Newman et al. 2006; Salazar-Ciudad 2006a). From more complex initial conditions, much more complex morphologies can arise, up to those observed in the most complex animals.

Composite Developmental Mechanisms

Morphogenetic mechanisms can do more than change cells' spatial location. If inductive and morphogenetic mechanisms act either at interspersed moments or at around the same time, then the locations and shapes of the territories sending signals are not reducible to the spatial regions within the zygote and their combinations by

logical operations (as with inductive mechanisms). In morphodynamic mechanisms, morphogenetic mechanisms act on the groups of cells sending signals, and these signals then diffuse from a larger repertoire of territory shapes. In morphostatic mechanisms, inductive mechanisms act first in development to establish different cell types in a field of cells, and then different morphogenetic mechanisms act in each cell type or combination.

By combining inductive and morphogenetic mechanisms in a morphostatic way, one can produce all the territory shapes possible from inductive mechanisms (mostly concentric rings), as well as those patterns resulting from their deformation by morphogenetic mechanisms. Combining inductive and morphogenetic mechanisms in a morphodynamic way additionally allows for the territory shapes that arise from diffusible signals secreted from the territory shapes of morphogenetic mechanisms. This clearly enables many more and more complex shapes. This difference is supported by computational models of development, and it has been suggested to have wide implications for the evolution of morphology and development (Salazar-Ciudad and Jernvall 2004; Salazar-Ciudad 2005).

Evolution by Morphostatic and Morphodynamic Mechanisms

In line with the above discussion and simulation studies, researchers have suggested that when combining the same set of inductive and morphogenetic mechanisms (or for the set of all possible combinations of inductive and morphogenetic mechanisms), morphodynamic combinations would generally lead to more complex genotype-phenotype maps and to more complex and disparate morphological variation than morphostatic combinations (see Fig. 4). By more disparate, it is meant that patterns arising from a given composite developmental mechanism (e.g., from changes in the initial patterns or mutations in the mechanisms not affecting its gene network topology) would show greater differences from each other in the case of morphodynamic rather than morphostatic mechanisms. This has lead to propose that, due to the larger disparity of patterns possible by those mechanisms, the evolution of major changes in morphology (e.g., novelty) is more likely to arise from morphodynamic mechanisms than from morphostatic mechanisms. This is simply due to the larger disparity of morphologies or patterns possible by those mechanisms. This would especially be the case for complex morphologies, since morphodynamic mechanisms are much more likely than morphostatic ones to produce complex patterns.

In contrast, the relatively high nonlinearity imposed by the interdependent interactions between inductive and morphogenetic mechanisms means that morphodynamic mechanisms are less likely to produce gradual variation. That is, a higher proportion of genetic mutations in a morphodynamic mechanism will lead to qualitatively different new final patterns or to no change in the produced pattern at all. In those situations in evolution when a very optimal morphology has been reached, either globally or for a specific organ, it may be more advantageous not to change that morphology or to change it only slightly in a gradual way. This is

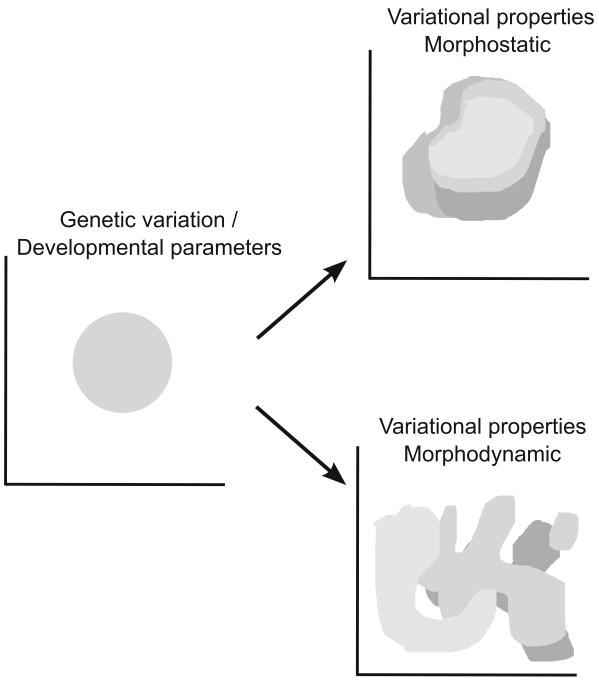


Fig. 4 This figure illustrates that the relationship between genotype and phenotype (parameter space and morphospace) is more complex in morphodynamic than in morphostatic mechanisms. The figure shows in different shades of *gray* the genetic variation or developmental parameter variation in a given population and time (*left*) and the resulting morphological variation by morphostatic (*upper right*) and morphodynamic mechanisms (*bottom right*). Each shade of gray represents the distribution of produced phenotypes by a different developmental mechanism. Notice the wider spread of produced morphologies in the case of morphodynamic mechanisms

more likely to be possible if this morphology is produced by a morphostatic mechanism. Thus, while in many cases morphodynamic mechanisms would be the mechanisms that would first arise in evolution to produce a specific morphology, there may often be a selective pressure to replace these morphodynamic mechanisms with morphostatic ones to produce the same phenotypes or small variations of them. This replacement, however, would be less likely for complex morphologies since morphostatic mechanisms are less likely to produce complex pattern transformations (for the reasons discussed in the previous section).

Note that the distinction between morphostatic and morphodynamic mechanisms does not imply a difference in the number of genes involved or in genetic complexity. Genetic complexity comes from the specific mechanisms being combined, rather than from whether those mechanisms are combined in a morphostatic or mophodynamic way. In that respect, a mechanism can change from being morphostatic to being morphodynamic, or vice versa, by a mere change in the relative timing of the activation of its different composing mechanisms, leading, in most cases, to dramatic changes in the pattern transformations produced. Simulation studies show that one can expect

morphostatic mechanisms to require, in general, more genetic interactions, and the combination of more inductive and morphogenetic mechanisms, to produce a complex pattern than morphodynamic mechanisms. In that respect, it is interesting to note that there seem to be substantial differences between animal groups at the level of how morphostatic or morphodynamic their whole development is (Salazar-Ciudad 2010). Thus, vertebrates, which are generally perceived to be the most complex animals, are also found to be the most morphodynamic.

Although mechanisms can change from morphostatic to morphodynamic by mutations affecting the relative timing of their composing mechanisms, having morphostatic or morphodynamic mechanisms in the development of morphology, either of the whole body or a body part, can have major consequences on evolution. Thus, lineages making extensive use of morphodynamic mechanisms, such as vertebrates, should be expected to have more complex and less gradual variation compared to the variation expectable in lineages using a more morphostatic development, such as diptera, for example. These differences would also be expected to be found between organs in the same organism that are morphodynamic to different extents and should lead to different ways of evolving (i.e., more gradual, simple changes vs. more complex and disparate changes).

In summary, this chapter examines how considering in more detail the nature and dynamics of developmental mechanisms allows predictions to be made about how development evolves and how it affects morphological evolution. These predictions are not possible from classical evolutionary approaches centered on genomes, genes, alleles, and their replacement over time.

Cross-References

- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Evolution of Complexity
- ▶ Evolvability
- ▶ Inherency
- ▶ Mechanisms in Evo-Devo

References

Alberch P (1982) Developmental constraints in evolutionary processes. In: Bonner JT (ed) Evolution and development. Dahlem Konferenzen. Springer, Heidelberg, pp 313–332

Fontana W, Schuster P (1998) Shaping space: the possible and the attainable in RNA genotype-phenotype mapping. J Theor Biol 194:491–515

Lecuit T, Lenne PF, Munro E (2011) Force generation, transmission, and integration during cell and tissue morphogenesis. Annu Rev Cell Dev Biol 27:157–184

Meinhardt H (1982) Models of biological pattern formation. Academic, London

Müller GB (2007) Evo-devo: extending the evolutionary synthesis. Nat Rev Genet 8:943–949

Newman SA (1994) Generic physical mechanisms of tissue genesis: a common basis for development and evolution. J Evol Biol 7:467–488

Newman SA, Forgacs G, Muller GB (2006) Before programs: the physical origination of multicellular forms. Int J Dev Biol 50:289–299

Salazar-Ciudad I (2005) Graduality and innovation in the evolution of complex phenotypes: insights from development. J Exp Zool B Mol Dev Evol 304:619–631

Salazar-Ciudad I (2006a) On the origins of morphological disparity and its diverse developmental bases. BioEssays 11:1112–1122

Salazar-Ciudad I (2006b) Developmental constraints vs. variational properties: how pattern formation can help to understand evolution and development. J Exp Zool B Mol Dev Evol 2:107–125

Salazar-Ciudad I (2007) On the origins of morphological variation, canalization, robustness, and evolvability. Integr Comp Biol 47:390–400

Salazar-Ciudad I (2010) Morphological evolution and embryonic developmental diversity in metazoa. Development 137:531–539

Salazar-Ciudad I, Jernvall J (2004) How different types of pattern formation mechanisms affect the evolution of form and development. Evol Dev 6:6–16

Salazar-Ciudad I, Jernvall J, Newman SA (2003) Mechanisms of pattern formation in development and evolution. Development 130:2027–2037

Schrum JP, Zhu TF, Szostak JW (2010) The origins of cellular life. Cold Spring Harb Perspect Biol 9:2212

Segré D, Ben-Eli D, Deamer DW, Lancet D (2001) The lipid world. Orig Life Evol Biosph 31:119–145

Wagner GP, Altenberg L (1996) Complex adaptations and the evolution of evolvability. Evolution 50:967–976

Part VI

Plant Evo-Devo

Structural Fingerprints of Development at the Intersection of Evo-Devo and the Fossil Record

Gar W. Rothwell and Alexandru M. F. Tomescu

Contents

Abstract

The plant body preserves diagnostic structural features that develop as the result of specific regulatory genes and growth regulators. When recognized in extinct species, those features serve as structural *fingerprints* for the regulatory programs by which they were produced. We review the contributions of the fossil record to

G. W. Rothwell
Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR, USA

Department of Environmental and Plant Biology, Ohio University, Athens, OH, USA
e-mail: rothwelg@science.oregonstate.edu; rothwell@ohio.edu

A. M. F. Tomescu (✉)
Department of Biological Sciences, Humboldt State University, Arcata, CA, USA
e-mail: mihai@humboldt.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_169

understanding the evolution of plant development in a temporal (geologic time) and a structural perspective (morphology, anatomy), and we highlight major topics in plant evolution in which integration of data from fossil and living plants has yielded significant resolution. Up to the present, the most ubiquitous growth regulator, auxin, has been documented as essential to the regulation of secondary growth and wood formation not only in seed plants, but also in several other major groups in which living species are no longer characterized by secondary growth. Additional fingerprints of growth regulation reveal the occurrence of gravitropic responses in fossils that extend back in time 400 million years and explain the evolution of equisetacean reproductive morphologies, living and extinct, by the interaction of modular regulatory programs. Still other fingerprints document parallel evolution of stem/leaf organography in several clades of living plants (e.g., ferns, *Equisetum*, and seed plants) and of substantial rooting systems that facilitated evolution of giant trees in extinct lycophytes and seed plants. Future application of techniques for identifying and interpreting the significance of structural fingerprints to a much broader spectrum of developmental processes holds tremendous potential for the paleontological record to substantially illuminate and enhance understanding of systematics and evolution within the context of plant development.

Keywords

Anatomy · Auxin · Body plan · Developmental regulation · Fossil · Leaf · Morphology · Paleo-evo-devo · Phytomer · Rhizomorph · Root · Secondary growth · Strobilus · Structural fingerprint

Introduction

Paleontology has a long history of illuminating patterns of evolution, but not the processes that underpin evolution. Until relatively recently, evolutionary processes have been investigated primarily within the realm of classical and population genetic theory. Nevertheless, our understanding of such processes has remained frustratingly incomplete. This situation has begun to change with the rise of molecular biology (ca. 1980s), which is providing a platform for a rapidly increasing number of techniques by which a deeper understanding of gene regulatory processes is being forged. The relatively new discipline of developmental molecular biology, in particular, presents exciting potential for the rapid advancement of knowledge on the processes that underpin evolution at the organismal level.

Developmental molecular studies characterize evolution within the context of differential developmental trajectories under the control of gene regulation, including the activities of developmental gene networks and growth regulators. This fruitful approach also provides, for the first time, an opportunity for ontogenetic studies of extinct plants to begin to contribute to our growing understanding of evolutionary processes (Rothwell et al. 2014; Spencer et al. 2015; Tomescu et al.

2017). The rationale that underlies such paleontological studies is simple. In plants there are ontogenetically diagnostic structural features that result from the activity of specific regulators of development (genes, hormones), and such features can be regarded as *fingerprints* for the specific regulatory pathways by which they have developed (Rothwell et al. 2014). Furthermore, by mapping on phylogenetic trees of living plants the earliest occurrences of genetic regulatory pathways that produce such fingerprints, the tempo of evolution of structural innovations can be documented and correlated with the evolution of gene regulation (e.g., Langdale 2008; Harrison 2016). As is also true for the emerging discipline of paleogenomics, when employed as reciprocal hypothesis tests, these combined approaches comprise powerful methodologies for integrating pattern and process in plant evolution.

The purpose of this contribution is to characterize plant paleo-developmental evolutionary biology, to explain the rationale for and scope of such studies, to highlight studies that integrate patterns of plant evolution and the fossil record with rapidly developing understanding of the role of regulatory genetics in organismal ontogeny, and thereby to illuminate the developmental foundations of plant evolution in an updated perspective of F.O. Bower's and W.N. Stewart's upward outlook.

Beyond Principles: What Has the Inclusion of Data from the Fossil Record Contributed to Evo-Devo Plant Biology

Fossils provide direct evidence for the process of evolution. As bearers of morphological and anatomical characters, fossils are best integrated into evolutionary studies within an evo-devo framework. Inclusion of fossils in evolutionary hypotheses pre-dated and foreshadowed the modern evo-devo paradigm. Classic transformational series, such as those proposed for the evolution of the conifer bract-scale complex or the sphenopsid sporangiophore, were elaborated based on fossils long before the rise of evo-devo molecular biology. Such paleontological data illustrate morphological (and, implicitly, developmental) change through time, the very agenda of evo-devo.

The types of data contributed by fossils range from basic observations on the shape or position of organs, to interpretations of plant development, and to comparative datasets including complex anatomical or morphological relationships between plant parts, tissues, or cells. Crucial for the latter are anatomical and morphological fingerprints that allow for the recognition of developmental and physiological processes in extinct plants and, thus, can bridge the gap between molecular biology and hundred-million-year old fossils. These different types of data illuminate diverse aspects of the evolution of plant features including growth patterns and dynamics (topology, tempo, and modes of meristematic growth; developmental domain partitioning; tissue-level positional patterning of cells and cell types); mechanisms of growth regulation and growth responses; organization of the plant body; and reproductive biology. In turn, these diverse plant features and their temporal (stratigraphic), taxonomic, and phylogenetic context address several categories of

knowledge relevant to the evo-devo agenda: tempo and mode of evolution (minimum ages for the evolution of specific features, processes, or regulators; sequence of character evolution), evidence for homology, and phylogenetic relationships.

Growth Patterns and Dynamics

The precisely structured anatomy of plants is the result of spatially and temporally coordinated sequences of cell division, growth, and differentiation. One aspect of such developmental sequences is the early partitioning of meristematic tissues into domains with distinct developmental trajectories, i.e., developmental domain partitioning, such as the specification of protoderm versus ground meristem versus procambium in apical meristems. In the root apical meristem, another aspect of developmental domain partitioning involves the early establishment of the Körper (body) and Kappe (cap) domains, characterized by distinct patterns of cell division. The two domains cover different extents of the root apical meristem and give rise to different tissues of the root in different plant lineages; therefore, this partitioning bears a phylogenetic signal. Importantly, because they are identified based on patterns of cell division, the Körper and Kappe domains can be recognized in fossils with anatomical preservation, and not just in live, developing plants. This has allowed for recognition of a type of gymnospermous Körper-Kappe organization in a Carboniferous (ca. 320 Ma) root apical meristem (Fig. 1) that is different from those of all extant gymnosperms (Hetherington et al. 2016a) and, thus, reveals structural diversity previously unaccounted for, that could be used in phylogenetic inference.

Plant reproductive structures are often produced as a result of expression of a reproductive regulatory module in meristems otherwise responsible for vegetative growth. Reproductive regulatory modules likely conserved across embryophytes involve *LEAFY* genes, the AP2 gene subfamily, MIKC MADS-box genes, and Polycomb group genes (Tomescu et al. 2017). In all known cases, the reproductive growth mode is activated in apical meristems. However, Paleozoic and Mesozoic sphenopsid fossils have recently been shown to exhibit patterns of size and positioning of reproductive structures (sporangiophores) consistent with activation of a reproductive regulatory module in intercalary meristems. This has implications for the topology and mode of meristematic growth, suggesting that growth in reproductive mode can be effected not only by apical meristems, but also by intercalary meristems. This is the first example of reproductive growth arising from intercalary meristems, a mode of growth that could not have been predicted from the modern flora alone, and which has deep implications for the homology and evolution of sphenopsid reproductive structures (see section "The *Equisetum* strobilus: A Case of Reciprocal Illumination").

Also associated with intercalary meristematic growth, rapid internode elongation that exceeds the tensional capacity of mature protoxylem cells generates rhexigenous protoxylem lacunae. Such lacunae found in *Equisetum* and grasses (Fig. 2) indicate that rapid growth from intercalary meristems evolved independently in distant plant lineages. The lacunae also provide a fingerprint for this topology (position) and tempo of meristematic growth that can be identified in the fossil record. If the

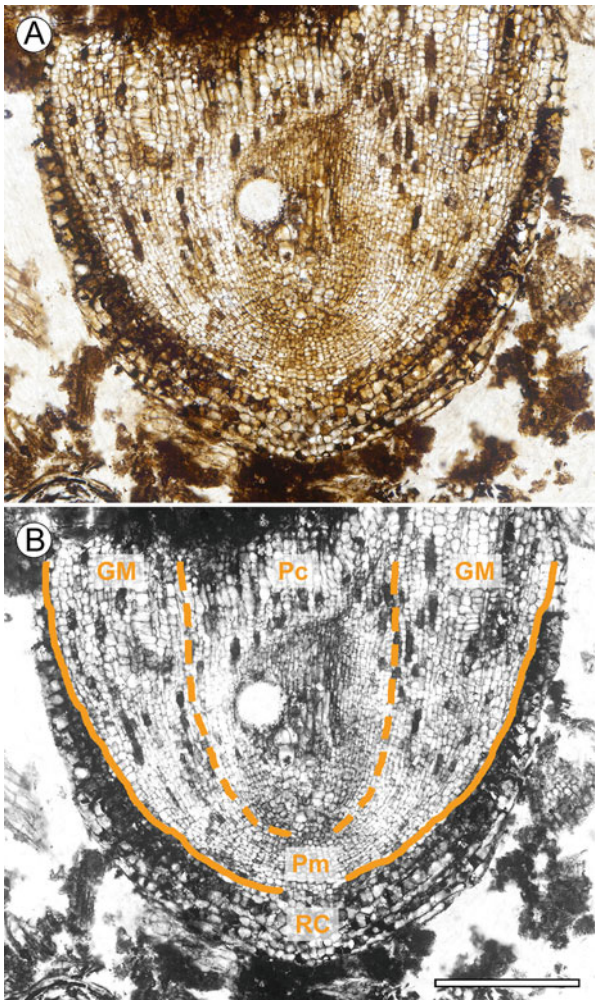


Fig. 1 (**a**). Apical meristem of a Carboniferous (ca. 320 Ma) gymnosperm root. (**b**). Same image as in a, with the root cap (RC), promeristem (Pm), and primary meristems (GM – ground meristem; Pc – procambium) indicated. Solid line separates the Kappe domain (represented in this root by the root cap) and Körper domain (everything else); dashed line separating procambium from the rest of root corresponds to Körper/Kappe boundary in extant gymnosperm roots. Scale = 400 μm. Image courtesy of Alexander Hetherington

Givetian (ca. 385 Ma) plant *Ibyka* (Fig. 3) does indeed include rhexigenous protoxylem lacunae (as opposed to areas of incomplete preservation of protoxylem parenchyma), such rapid intercalary meristematic growth may have evolved as early as the Middle Devonian.

Alveolar megagametophyte cellularization, a type of tissue-level positional patterning of cells, is the result of a specific sequence of cell wall construction relative to position in the gametophyte, which leaves a conspicuous anatomical fingerprint (Fig. 4a). This fingerprint can be used to infer homology of process and regulatory mechanisms that can be traced into the fossil record. Seed plant megagametophytes that exhibit alveolar cellularization (Fig. 4b, c) extend back to the Famennian (Late Devonian, ca. 160 Ma), indicating that this feature shared by living and extinct seed plants represents a synapomorphy for the clade.

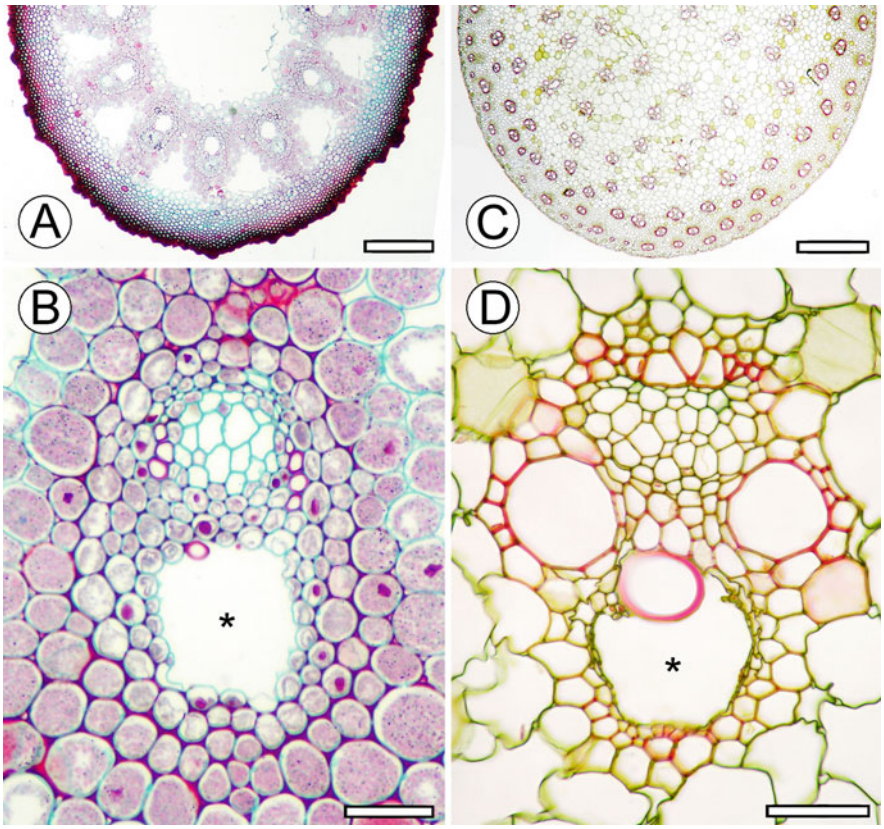


Fig. 2 Rhexigenous protoxylem lacunae (asterisks) produced by rapid internode elongation due to growth from intercalary meristem located at base of internode. (**a**, **b**). *Equisetum*. (**c**, **d**) *Zea*. Cauline vascular bundles in B and D shown with phloem at top and xylem at bottom. Scales = 500 μm (**a**); 75 μm (**b**); 750 μm (**c**); 50 μm (**d**)

In a different type of approach, the morphology and anatomy of fossil plants have inspired modeling studies of growth dynamics whose implications can be used to generate hypotheses about genetic regulatory mechanisms. Dynamics of apical meristematic growth in terms of growth rates and frequency of branching, taxis and angle of branching, meristem size, and growth determinacy, as illustrated by Silurian and Devonian tracheophytes, have been modeled by Niklas (1997 and references therein) and Stein and Boyer (2006). These studies have shown that variations in only a small set of parameters can generate a wide diversity of plant branching architectures. They also indicate that a shared set of underlying developmental regulators may be responsible for all this diversity, and point to specific developmental processes and domains to be studied by molecular biology in order to identify these regulators.

In another modeling approach, Stein (1993) used data from living plants – concerning the role of auxin in shaping the vascular system – to model features of

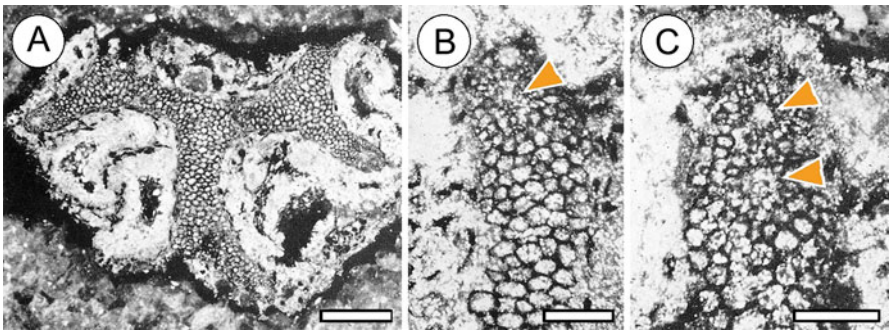


Fig. 3 Protoxylem lacunae of possible rhexigenous origin in the Givetian (ca. 385 Ma) plant *Ibyka amphykoma*. (**a**). Cross section of main axis with deeply lobed xylem; lacunae are small light areas close to tips of xylem lobes. (**b**, **c**) Details of xylem lobes with protoxylem lacunae at tips (arrowheads); protoxylem starting to divide (**b**) and already divided (**c**) radial direction showing divergence of lateral trace. Scales = 500 μm (**a**); 200 μm (**b**, **c**). Published by permission of Botanical Society of America (American Journal of Botany 60(4)/1973, p.375)

extinct plants, namely, stelar architecture, another feature reflecting developmental domain partitioning (vascular vs. ground tissues) and tissue-level positional patterning of cells (protoxylem vs. metaxylem). Comparisons between stelar configurations predicted by this model and the xylem architecture of fossil plants can be used, like in the case of branching architecture, to identify the model parameters responsible for xylem architecture of those plants. In turn, these parameters can be used to infer the functions of putative developmental regulators responsible for xylem architecture and formulate testable hypotheses for studies of the evolution of stelar architecture. Interestingly, whereas Stein's model was successful in predicting stelar architecture in aneurophytalean progymnosperms and aneurophyte-like plants (Fig. 5), it was less successful in generating stelar architectures comparable to those of cladoxylopsids. This result could be indicating fundamental differences between cladoxylopsids and progymnosperms in terms of regulatory programs controlling vascular architecture, consistent with the view that the two groups represent phylogenetically distinct lineages.

Fundamental Plant Growth Responses

Much of plant response to external stimuli consists of modulation of the location, direction, and rate of growth. Gravitropic growth represents a fundamental and conspicuous plant growth response. The fossil record provides a minimum age for gravitropic responses, demonstrating positive gravitropism in below-ground plant parts and negative gravitropism in above-ground parts no later than the Early Devonian (Lochkovian), ca. 415 Ma ago (Matsunaga and Tomescu 2017). These Early Devonian plants exhibit organs which grow downward into the substrate or in directions opposite those of the parts bearing reproductive structures or leaves.

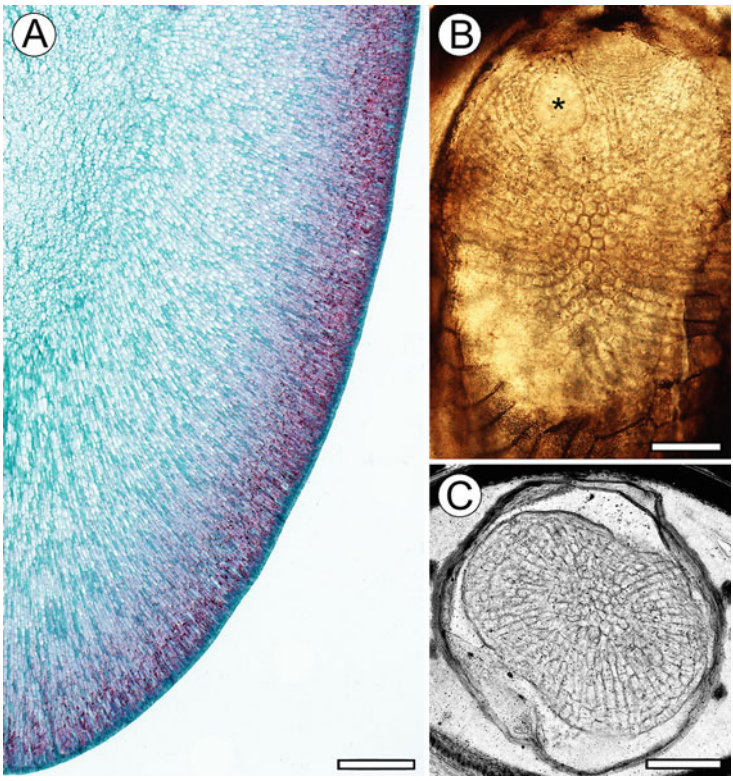


Fig. 4 Alveolar megagametophyte cellularization in extant and fossil seed plants. (**a**) Longitudinal half of chalazal portion of extant *Ginkgo* megagametophyte; alveolar cellularization recognized by radial cell files oriented perpendicular to megagametophyte surface. (**b**, **c**) Longitudinal and transverse sections (respectively) of megagametophytes of Late Carboniferous (ca. 305 Ma) pteridosperm (seed fern) *Gnetopsis elliptica*, displaying alveolar cellularization (asterisk marks archegonium). Scales = 1 mm (**a**); 200 μm (**b**); 250 μm (**c**). (**b** and **c** courtesy of Jean Galtier)

Furthermore, rhizomatous axes and cormose bases of some Early Devonian plants only developed rhizoids on portions that were in contact with the substrate (e.g., the rhizoids of *Nothia aphylla*; see section "Gravitropism"). Like in the downward growing organs, this polarization of rhizoid positioning implies the presence of gravity signal transduction mechanisms.

Fossils also provide information on the tempo and mode of evolution of positive gravitropism (see section "Sequence of Character Evolution"). Most of the Early Devonian plants exhibiting positively gravitropic responses do not have stem-leaf-root organization, which indicates that positive gravitropism pre-dates the evolution of roots. Additionally, fossils illustrate positively gravitropic organs that are not roots, such as undifferentiated axes of Early Devonian polysporangiophytes or rooting organs with stem homology. These indicate that gravitropic responses and root identity are not necessarily coupled (Matsunaga and Tomescu 2017).

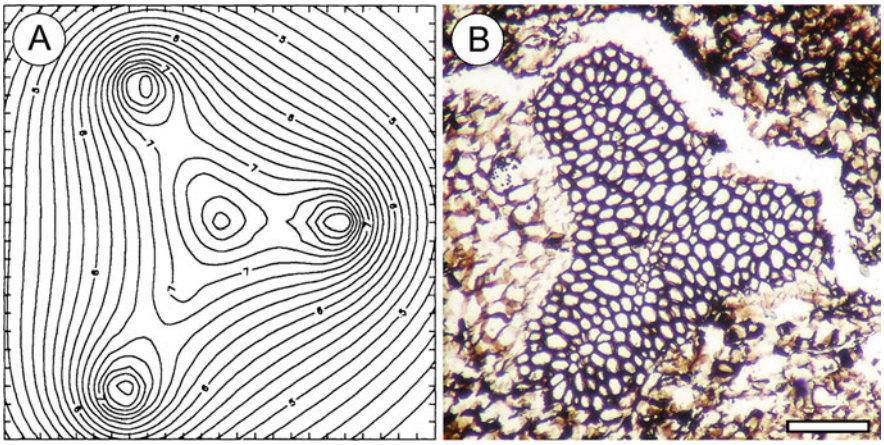


Fig. 5 Model based on small number of parameters controlling auxin dynamics at apical meristem and responsiveness of target tissue to auxin concentrations (**a**) predicts stele anatomy of Early Devonian (ca. 400 Ma) euphyllophyte probably related to progymnospermous lignophytes (**b**). Scale = 150 μm. (a) published by permission of University of Chicago Press (International Journal of Plant Sciences 154(2)/1983, p. 247)

Underground interactions between plants are another type of growth response elicitors. These interactions include kin recognition that directs growth toward minimizing interference between the roots of conspecifics, closely related or clonal individuals, to avoid competition and maximize resource exploitation. The fossil record illustrates kin recognition-driven growth responses, as shown by roots that curve away from one another or follow parallel trajectories, such as the Early Permian (ca. 280 Ma) in situ roots *Pinnatiramosus*. Similar patterns of growth orientation in root-bearing axes of the Early Devonian *Sengelia* suggest that underground kin recognition had evolved to direct rooting system development in lycophytes as early as 410 Ma ago.

Homology and Sporophyte Body Plans

Allowing access to the rich extinct diversity within plant clades, the fossil record includes morphologies and anatomical features that are absent among modern plants. Such features can be crucial in understanding the homology of plant structures or the origin of body plans, especially in lineages whose living representatives are isolated at the tips of long phylogenetic branches. The extant lycophyte *Isoetes* has a highly derived morphology that was understood, in terms of homologies, only through studies of the extensive fossil record of its clade, the rhizomorphic lycophytes. The studies emphasizing comparative anatomy, morphology, and embryogeny assembled a body of evidence that supports deep shoot homology of the lower corm of *Isoetes* and leaf homology of the "rootlets" attached to it, along with severely

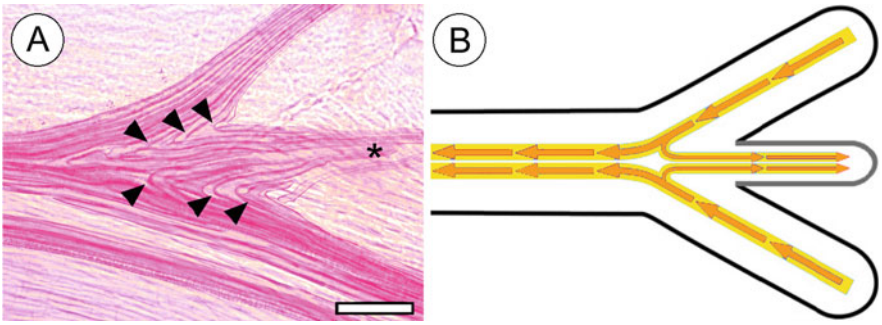


Fig. 6 *Selaginella*. Arching (U-shaped) tracheids (arrowheads) that connect steles of main stem (bottom right), side branch (top right), and rhizophore (asterisk) in (**a**) represent anatomical fingerprint for reversal of polar auxin transport associated with rhizophore development: from basipetal transport in stem and branch, to acropetal in rhizophore (**b**); yellow strips = vascular tissue; orange arrows = polar auxin transport. Scale = 200 μm

diminished elongation growth and branching capacities of the main axes, compared to extinct relatives (e.g., Lepidodendrales; see section "Lepidodendralean Rooting Structures").

In the same realm of body plan and organ homologies of the lycophyte sporophyte, a recent study has taken a comparative anatomy approach to address hypothesized homology relationships between the rhizophore of *Selaginella* and rooting structures associated with branching points in fossil early lycophytes and zosterophylls (Matsunaga et al. 2017). That study revealed an anatomical fingerprint for a reversal of polar auxin transport associated with rhizophore development: basipetal polar auxin transport in shoots to acropetal in the rhizophore. This fingerprint, in the form of arching (U-shaped) tracheids (Fig. 6), could be sought for in fossils, to test for presence of similar polar auxin transport patterns and to illuminate the homologies of early lycophyte rooting structures.

Another classic puzzle of plant morphology involves the origin and homologies of the *Equisetum* strobilus and sporangiophore. In an example of reciprocal illumination, understanding of vegetative meristematic growth in *Equisetum* can be used to formulate evo-devo hypotheses on reproductive development that can be tested based on information from fossil sphenopsids. This leads to a generalized model explaining variations in sphenopsid reproductive morphology, which, combined with information on reproductive developmental anatomy in extant *Equisetum*, provides an explanation for the origin of the strobilus and a hypothesis of sporangiophore homology (Tomescu et al. 2017; see section "The *Equisetum* Strobilus: A Case of Reciprocal Illumination").

The typical sporophyte organization in modern tracheophytes comprises three basic types of vegetative organs: stems, leaves, and roots (i.e., stem-leaf-root organography). Leaves and roots each share minimal sets of defining features that render them comparable across the entire breadth of tracheophyte diversity. An outlook on tracheophyte morphological evolution within a phylogenetic context

that excludes the fossil record can easily take these features and their ubiquity in extant plants as indicating that leaves and roots are each homologous across all vascular plants. Conversely, inclusion of the fossil record in such a broad outlook plays a crucial role in resolving major aspects of the evolution of this basic body plan and the homologies of leaves and roots. Specifically, Late Silurian and Devonian tracheophytes characterized by simple body plans (undifferentiated branching axes bearing sporangia) form paraphyletic grades at the base of major branches of tracheophyte phylogeny, demonstrating that stem-leaf-root organography evolved independently in different lineages (Rothwell et al. 2014). This implies that neither leaves nor roots are homologous across different lineages. Furthermore, several lines of evidence reveal that leaves and roots almost certainly evolved independently more than twice (Boyce and Knoll 2002; Tomescu 2009; see section "Euphyllophyte Leaf Evolution").

Sequence of Character Evolution

Plant phylogenies can be used to infer the mode of morphological evolution. Character distribution on phylogenetic trees can be and has been used to infer sequences of character evolution and ancestral character states. However, because phylogenetic trees represent hypotheses of relationships, sequences of character evolution predicted based on them are just as hypothetical. This is particularly evident in systematic trees that exclude extinct taxa (Rothwell and Nixon 2006). Within this context, fossils provide the only direct means for testing sequences of character evolution. Presence or absence of structures and anatomical features in fossils of different ages within a lineage provide direct evidence for the order of appearance of those features. An example is the sequential evolution of characters in organs we call leaves, during their independent parallel evolution in ferns and seed plants. Fossils demonstrate that whereas seed plants evolved determinate growth and broad pinnules before adaxial-abaxial polarity in the leaves, in filicalean fern leaves evolution of adaxial-abaxial polarity preceded broad pinnules and determinacy (Sanders et al. 2009; see section "Euphyllophyte Leaf Evolution").

Lycophyte rooting structures are diverse and so are their homologies, some of which are not fully resolved (Rothwell and Erwin 1985; Tomescu 2011; Matsunaga et al. 2017). The oldest unequivocal lycophyte roots were described in the Early Devonian plant *Sengelia*, which produced roots on specialized axes of the branching system that are stem homologs. *Sengelia* rooting systems consist of horizontal or downward-growing root-bearing axes with laterally diverging roots. In all cases, the roots expand in a horizontal plane, irrespective of the orientation of subtending root-bearing axes. These observations indicate that, in lycophytes, root identity was uncoupled from positive gravitropism, a feature fundamentally associated with modern plant roots – in *Sengelia*, the organs that exhibit a gravitropic response are the root-bearing axes and not the roots. The roots of *Sengelia* also provide evidence for the sequence of character evolution: roots acquired positive gravitropism after they evolved as distinct organs, in lycophytes (Matsunaga and Tomescu 2017).

Early Devonian strata have yielded euphyllophytes as old as 407 Ma that exhibit secondary growth (wood production) from a vascular cambium (Gerrienne and Gensel 2016) (euphyllophytes are the sister clade to lycophytes and include psilotophytes, ferns, sphenopsids, and seed plants, along with diverse related lineages). These fossils provide a minimum age for the evolution of this important structural feature. Furthermore, the fact that these early wood producers have simple sporophyte organization (undifferentiated axes) indicates that secondary growth pre-dates the evolution of complex body plans with stem-leaf-root differentiation, in euphyllophytes. The small size of these wood-producing sporophytes suggests that secondary growth evolved primarily in response to selective pressures related to maximizing hydraulic conductance and not mechanical stiffness (Gerrienne and Gensel 2016; see sections "Developmental Regulation" and "Secondary Growth").

Developmental Regulation

Plant fossils exhibit combinations of characters unknown in modern plants and preserve anatomical and morphological fingerprints for developmental processes and physiological mechanisms. Aside from implications for sequences of character evolution (see section "Sequence of Character Evolution"), when considered in their stratigraphic (temporal) and taxonomic context, these types of data provide glimpses into the systems biology of developmental regulation and its evolution. In many instances, the resulting perspectives inform understanding of the modularity of developmental regulatory networks, hierarchy of regulatory modules, synchronization in developmental processes, or relationships between physiology and development.

In isoetalean lycophytes, "rootlets" borne on the corm base (in *Isoetes*) branch apically and dichotomously and produce root cap-like structures at their tips (Fig. 7a), as well as root hairs (Fig. 7b). Fossil members of the clade provide developmental and structural evidence pointing to shoot homology of the corm base and leaf homology of the "rootlets" (Rothwell and Erwin 1985; see section "Lepidodendralean Rooting Structures"). Together, these indicate that development of these leaf homologs also involves expression of a shoot- or root-specific developmental program (for apical dichotomous branching) and of two developmental programs that are widely believed to be root-specific among living plants (for root cap and root hair production). Two implications of the expression in leaf homologs of developmental programs not usually associated with leaves are that (1) these programs include conserved gene regulatory networks that are modular, and (2) expression of these regulatory modules is independent of organ identity. In the case of root hairs, this is to be expected, given evidence available on shared developmental regulators between bryophyte gametophyte rhizoids and angiosperm sporophyte root hairs; this implies that such rooting structures are fundamentally homologous (deep homology) across embryophytes and independent of life cycle phase, let alone organ identity.

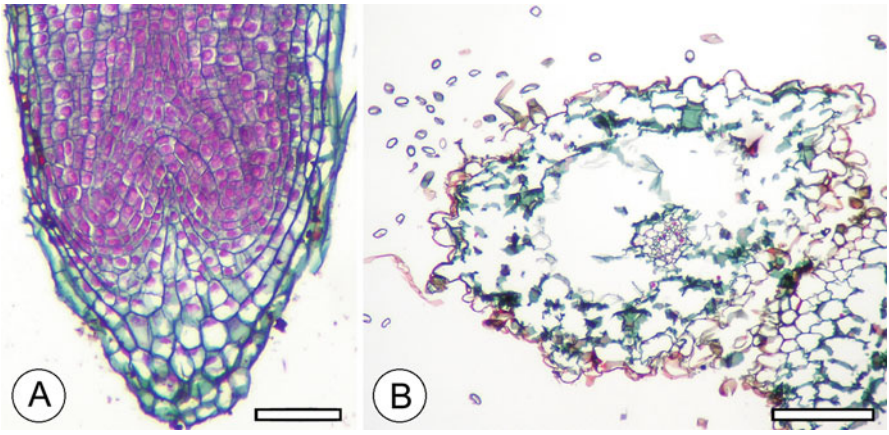


Fig. 7 Appendages ("rootlets") of *Isoetes* corm base (rhizomorph) bear structures typical of roots – a protective cap on the apical meristem (**a**) and absorptive hairs (**b**) – even though they are leaf homologs; note incipient isotomous branching of the "rootlet" apical meristem. Scales = 75 μm

In light of the homologies of the body plan of *Isoetes*, as resolved by data from the fossil record, the presence of root cap-like structures in the "rootlets" of this plant provides interesting phylogenetic perspectives. These root cap-like structures, present on organs that are not root homologues, could imply that the root cap pre-dates roots and evolved on less specialized axes with rooting function, *if* rhizomorphic lycophytes evolved directly from ancestors devoid of roots. However, because among modern tracheophytes the root cap is known exclusively in roots, another possible explanation, namely, that *Isoetes* descends from ancestors that had true roots with root caps, seems more probable. This hypothesis has implications for lycophyte phylogeny and character evolution, consistent with previous ideas that the clade of root-less lycophytes that includes *Isoetes* (rhizomorphic clade) occupies a derived position in the lycophyte clade.

In equisetacean sphenopsids, the fossil record yielded several Paleozoic fossils exhibiting character combinations that fill important gaps in terms of morphological evolution between modern *Equisetum* and ancestral forms. Considered in the developmental context provided by modern *Equisetum*, these fossils were crucial in the development of hypotheses that explain the morphology of equisetacean reproductive structures as the result of a hierarchic system of modular regulatory programs. Nested within this set of hypotheses are also implications for the developmental program of the sporangiophore, which may represent a conserved regulatory module responsible for the development of basic fertile lateral branching systems, and for timing of the evolution of this module, which may have preceded the evolution of stem-leaf-root organography (Tomescu et al. 2017; see section "The *Equisetum* Strobilus: A Case of Reciprocal Illumination").

At the scale of the sphenopsid group, the presence of intercalary meristems at the base of each internode in Equisetaceae, and in fossil Calamitaceae and Sphenophyllales, represents an anatomical fingerprint for a shared set of developmental regulators that

could imply common ancestry and, thus, inform phylogeny. Furthermore, the presence of whorled appendages in all fossil members of this group that also share the same type of apical meristematic organization as *Equisetum* suggests another shared regulatory program. This program, responsible for developmental synchronization of merophytes in a primordial ring (Tomescu et al. 2017), could then be traced back to the common ancestor of the group, somewhere in the Late Devonian – Early Carboniferous (ca. 380–325 Ma ago).

In modern tracheophytes, secondary growth from a vascular cambium is known only in seed plants and *Isoetes*. The fossil record has revealed that this mode of growth is shared by the extinct seed-free progymnosperms, which form a clade with the seed plants (the lignophyte clade), and that other groups – lepidodendralean lycophytes, sphenopsids, zygopterid fens – had also evolved secondary growth. In this context, identification of auxin swirls as anatomical fingerprints for polar auxin regulation of cambial growth points to auxin-related processes of secondary growth regulation shared across the euphyllophyte-lycophyte divide and among euphyllophytes (Rothwell et al. 2008; see section "Secondary Growth"). This has implications for the evolution of developmental regulation, because the distribution of secondary growth across tracheophyte phylogeny points to parallel evolution of this developmental pathway in different lineages. This would imply that the shared auxin-related regulatory module pre-dates the evolution of secondary growth and may have been involved in more basic developmental pathways. However, discoveries of Early Devonian basal euphyllophytes exhibiting secondary growth (Gerrienne and Gensel 2016) may indicate just the contrary, namely, that all (or most) euphyllophytes share a regulatory program for secondary growth inherited from a common ancestor but expressed only in some of its descendants. Clearly, better understanding of secondary growth regulation and continued exploration of the fossil record can bring resolution to these questions.

For another auxin-related growth response, gravitropism, fossils contribute support to a hypothesis integrating physiology, development, and homology. Plant roots exhibit typically positive gravitropic responses. Lepidodendralean lycophytes demonstrate positive gravitropism in the downward-growing rhizomorph, an organ with shoot homology (Rothwell and Erwin 1985). Interestingly, lepidodendralean rhizomorphs exhibit acropetal polar auxin transport (Rothwell et al. 2014), just like roots and like the *Selaginella* rhizophore, another organ that is not a root homolog but has rooting functions. These observations could imply that acropetal polar auxin transport is independent of organ identity (homology) and is more generally associated with positive gravitropic responses in a diverse array of organs that have absorption and anchoring roles (Matsunaga et al. 2017).

Life Cycles, Reproductive Systems

The fossil record has provided direct and indirect evidence for the timing and mode of evolution of reproductive biology and plant life cycles. For instance, fossils provide the only evidence that sphenopsids, the clade that includes homosporous *Equisetum* as

its sole living representative, evolved heterospory as early as the Early Mississippian, ca. 350 Ma ago. The fossil record also reveals that heterospory originated independently, in different variants, in several exclusively extinct plant lineages (to the extent that it has been regarded as the most iterative key innovation in the evolutionary history of plants) and provides minimum ages for those independent origins. None of these aspects could have been gleaned by the study of extant plants alone, irrespective of the methods of investigation or inference employed.

In the same vein, the fossil record contains evidence on first occurrences of plant structures associated with reproduction. Considered in a systematic context, these features can provide characters for phylogenetic analyses that inform understanding of the evolution of plant reproductive biology. Middle Pennsylvanian (ca. 310 Ma) callistophytalean seed fern ovules preserve evidence for a pollination drop mechanism and branched pollen tubes formed by the developing macrogametophyte. These features similar to those of extant gymnosperms are, consequently, known to have arisen early among basal gymnosperm groups. Late Permian (ca. 250 Ma) glossopterid seed ferns exhibit a novel combination of reproductive characters in which sperm with a helical flagellate band, similar to that of extant cycads and *Ginkgo*, is associated with pollen tubes simpler than those of other living or extinct gymnosperms.

Going back to the origins of land plants, the spore record provides minimum dates for fundamental embryophyte characters. Ordovician (Darriwilian, ca. 460 Ma) spores recovered in tetrads with characteristic configuration provide the oldest evidence for simultaneous meiosis (cytokinesis). Furthermore, the ultrastructure of these spores indicates that sporoderm development involved active secretion by a sporangial tapetum early in embryophyte evolution (Taylor et al. 2017).

Observations on Late Silurian – Early Devonian (430–410 Ma) polysporangiophyte sporophytes allowed for assessment of their nutritional status, with implications for sporophyte-gametophyte relationships early in the evolution of the group. The size and anatomy of these early sporophytes, and comparisons with extant plants taking into account physiology, demonstrate that the earliest polysporangiophytes sporophytes could not have sustained photosynthetic activity at levels high enough to ensure their nutritional independence. These sporophytes were nutritionally dependent on the gametophytes, like the sporophytes of extant bryophytes. Such observations provide a glimpse into the sequence of character evolution, demonstrating that the branched sporophyte pre-dates independence of the sporophyte from the gametophyte.

The gametophytes of the earliest embryophytes have been elusive, with only equivocal hints available to date of what their morphology may have been. However, the stable carbon isotope chemistry and internal structure of thalloid carbonaceous fossils scattered throughout the Silurian and Early Devonian, combined with experiments simulating fossilization on extant thalloid organisms, indicate that at least some of those fossils are plants. These fossils demonstrate thalloid gametophytes in early embryophytes and corroborate hypotheses that early polysporangiophyte gametophytes may have had thalloid morphology (Tomescu et al. 2014). This perspective on polysporangiophyte gametophyte morphology is at odds with predictions based on phylogenetic studies that place mosses, which have leafy gametophytes, as the sister group of polysporangiophytes.

Morphological evolution in the gametophytes of early polysporangiophytes has a story of which we have uncovered only some parts, and those thanks to the fossil record. Unlike the thalloid gametophytes of early polysporangiophytes, the next oldest known gametophytes, belonging to Early Devonian (Lochkovian–Pragian, ca. 408 Ma) protracheophytes and tracheophytes of the Rhynie chert, exhibit morphologies with no counterpart in the modern flora. Their morphology is similar to that of sporophytes, with axial organization, branched architecture, and vascular tissues. The only approximation to this morphology in living plants are the subterranean gametophytes of *Psilotum* and *Tmesipteris*, which are also axial, can branch, and can be vascularized. However, compared to these, Rhynie chert gametophytes were larger, highly branched, and developed above-ground. Currently, it is not clear how polysporangiophyte gametophytes evolved from a basic thalloid morphology to the axial forms seen in the Rhynie chert, and then back to the primarily thalloid forms seen in living seed-free vascular plants. These present questions in evo-devo whose answers will require additional data from the fossil record.

The Early Devonian Rhynie chert plants also provide the oldest direct evidence of anisospory (segregation of micro- and megaspores within the same sporangium), a reproductive system currently known only in a subset of bryophytes, in a few *Isoetes* species and, potentially, in *Equisetum* and *Ceratopteris*. Specifically, gametophytes of the Rhynie chert protracheophyte *Aglaophyton* are found forming dense populations, when preserved in situ. Because *Aglaophyton* spores were dispersed as masses representing the contents of whole sporangia, such dense gametophyte tufts probably represent the product of individual sporangia. Although spore size shows no bimodal distribution within *Aglaophyton* sporangia and gametophytes are exclusively unisexual, the gametophyte tufts always include mixtures of both sexes. These have been interpreted as indicating anisospory of *Aglaophyton* and other Rhynie chert plants (Taylor et al. 2005). Like in the case of gametophyte morphology, it is not yet clear what the anisospory of Rhynie chert plants means for the complex picture of plant life cycle evolution.

Emblematic Case Studies

Gravitropism

Tropisms play a major role in plant development and evidence is accumulating that tropic responses influenced growth of the earliest land plants. Fossils from the Early Devonian reveal that rooting organs of lycophytes appeared long before those of the other major clade of vascular plants (euphyllophytes), for which roots are not known until the Middle Devonian. Evidence for tropic responses in the aerial and rooting structures of Early Devonian lycophytes is provided by several plants, including *Drepanophycus* and *Sengelia* (Matsunaga and Tomescu 2017). These plants have leafy axes that extend in one direction (horizontally or upwards) and smaller smooth axes that bend in the opposite direction to root the plant, suggesting negative and positive gravitropism in the aerial and rooting organs, respectively (Fig. 8). Similar

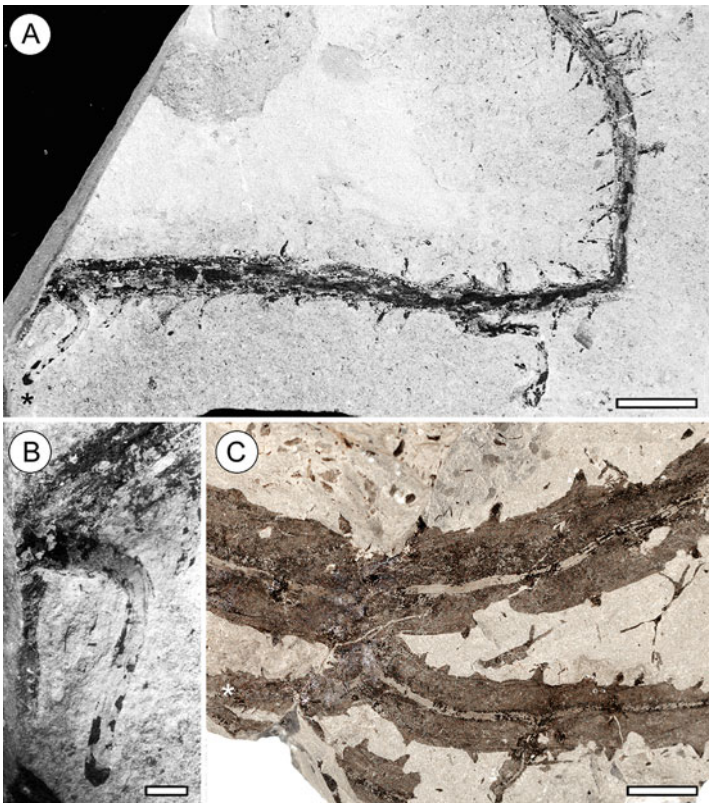


Fig. 8 Evidence for gravitropic responses in the rooting structures of Early Devonian (ca. 410 Ma) lycophytes. (**a**, **b**) *Drepanophycus spinaeformis* with smooth root-like axis (asterisk in **a**) pointing away from direction of stem growth; (**b**) is detail of (**a**). (**c**) K-branching in *Sengelia radicans*; main stem (top) produces side branch that forks very close to its base, producing a branch stem (bottom right) and a rooting axis (bottom left; asterisk), which develops in a direction opposite to growth direction of main stem and branch stem. Scales = 10 mm (**a**, **c**); 2 mm (**b**). (**a** and **b** courtesy of Patricia G. Gensel; **c** courtesy of Patrick S. Herendeen and Kelly K.S. Matsunaga)

dichotomy in growth direction, reflecting gravitropic responses, is present in the undifferentiated axes of Early Devonian zosterophylls, which produce branches with upward growth and others with downward growth; e.g., *Zosterophyllum* (Fig. 9a, b), *Bathurstia*, and *Sawdonia*.

Additional evidence for tropic responses derives from Early Devonian Rhynie Chert plants preserved in growth position, with excellent cellular detail. Plants such as *Rhynia*, *Aglaophyton*, *Horneophyton*, and *Nothia* had no stem-leaf-root differentiation, but did have aerial axes that grew both laterally and upward, suggesting negative gravitropism. These plants also have bulges on the lower surface of laterally growing axes and the base of upward growing axes, from which tufts of rhizoids diverge (Fig. 9c). Such rhizoids either extend or bend downward, demonstrating positive gravitropism similar to that of rhizoids on living plants and their green algal

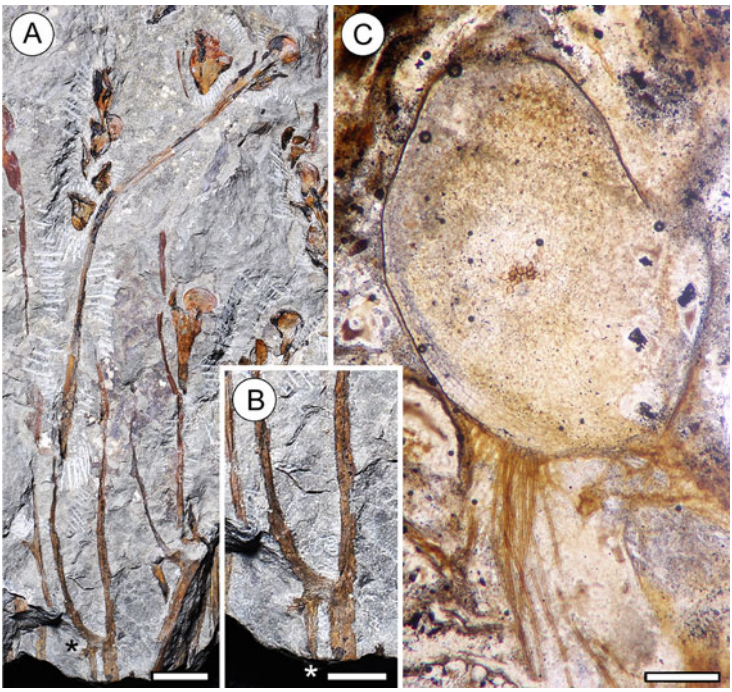


Fig. 9 Evidence for gravitropic responses in Silurian-Early Devonian (ca. 420–410 Ma) tracheophytes. (**a**) *Zosterophyllum qujingense* displaying K-branching (asterisk) similar to that of *Sengelia* (Fig. 8c) but expressed in undifferentiated naked axes. (**b**) Detail of A; one of two axes of K-branch (asterisk) diverges away from upright aerial axes. (**c**) Cross section of rhyniophyte axis bearing tuft of rhizoids on lower side that was in contact with substrate. Scales = 5 mm (**a**); 3 mm (**b**); 500 μm (**c**). (**a** and **b** courtesy of Jinzhuang Xue)

close relatives, in which the direction of rhizoid growth is controlled by a statolith mechanism. Because this tropic response is driven by the same type of statolith mechanism in both extant vascular plants and charophycean green algae, it is reasonable to infer that the molecular toolkit for the regulation of gravitropic growth was present in the earliest vascular plants, as illustrated by rhyniophytes.

It will be interesting to learn from future fossil discoveries what anatomical structures were responsible for the positive and negative gravitropic responses of the undifferentiated axes of zosterophylls or the root-bearing axes of plants such as *Sengelia*. Because statolith-based mechanisms are ubiquitous as gravity sensing systems across embryophytes and their green algal relatives, at both the cell level (rhizoids) and in multicellular organs (roots, shoots), it is safe to assume that the axes of zosterophylls and *Sengelia* had the same type of mechanism. In roots of extant angiosperms, amyloplast statoliths are located in cells of the root cap columella. We do not know whether the positively gravitropic axes of zosterophylls and *Sengelia* had root cap-like structures. Considering that zosterophylls did not possess stem-leaf-root differentiation and that the root-bearing axes of *Sengelia* are not root homologs,

it is likely that none of these axes had root cap-like structures, unless regulation of root cap development is independent of organography (as it appers to be in *Isoetes*). If a root cap was absent from these positively gravitropic axes, could such axes have housed statoliths in boundary layers (e.g., endodermis, starch sheath) like those responsible for negative gravitropism in the shoots of extant angiosperms? If so, how would the statolith-based mechanisms responsible for positive gravitropism in the below-ground axes of zosterophylls and for negative gravitropism in the above-ground axes of those plants have been different? Could they have differed only in the polarity of the response to a gravitropic stimulus sensed in a shared type of structure? And would these tropic responses have involved redistribution of polar auxin fluxes, as seen in positive and negative gravitropic responses of angiosperm roots and shoots? Answers to all of these questions and their integration into a more complete picture of the evolution of gravitropism will also require understanding of the incompletely explored structural, developmental, and physiological underpinnings of gravitropism in many lineages of extant seed-free plants (the roots and shoots of lycophytes, ferns, *Equisetum*, or the rhizophores of *Selaginella*).

Polar Auxin Transport

Auxin is among the most prominent of growth regulators, and polar auxin transport from developing leaf primordia in the apical meristem toward the base of the stem, and then toward the apical meristem(s) of the root system, regulates a wide spectrum of developmental processes. Among the most important of those processes is the patterning of primary vascular architecture and of tracheary elements in the secondary xylem.

Secondary Growth

Vascular tissue differentiation of living plants is under the control of several plant growth regulators, including gibberellins, cytokinins, and ethylene, among which auxin is the most prominent. Moreover, at least some aspects of polar auxin transport have been identified as far down the green lineage as bryophytes and charophycean algae.

Polar auxin flow within the vascular cambium of seed plants patterns the axially elongated tracheary elements of the secondary xylem, which typically follow a straight course. However, when obstacles such as buds, branches, and wounds impede polar auxin flow, auxin whirlpools form in the cambial zone, inducing the differentiation of characteristic circular patterns of tracheary elements above the obstacles (Rothwell et al. 2008). Similar circular patterns have been identified at the same positions in the wood of the Upper Devonian progymnosperm *Archaeopteris* (Fig. 10a) and serve as structural fingerprints for polar auxin regulation. This recognition revealed that polar auxin flow and auxin regulation also affect wood patterning in species from the fossil record and established the existence of structural fingerprints for the regulatory mechanisms of secondary growth in extinct plants. Subsequent studies documented similar patterns in the wood of extinct sphenopsids and lycophytes (Fig. 10b, c) (Rothwell et al. 2008; see also section "Lepidodendralean Rooting Structures").

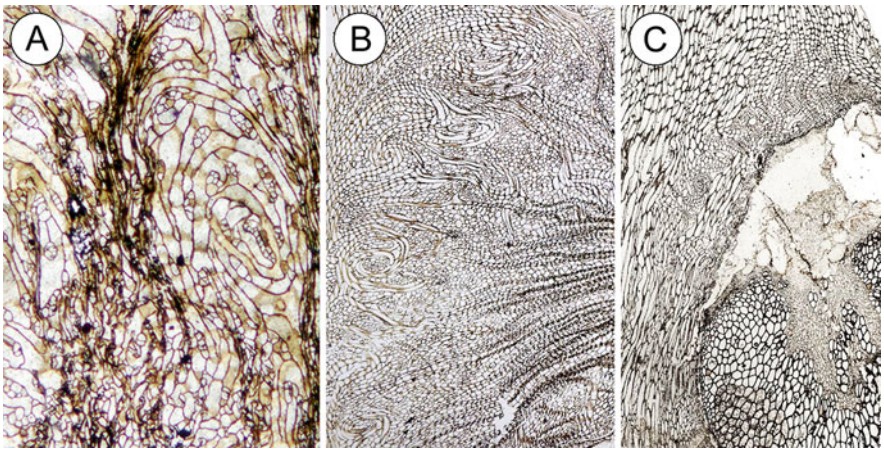


Fig. 10 Swirls formed by tracheids in wood (in tangential longitudinal view) as structural fingerprints for polarized transport of auxin (polar auxin flow) in vascular cambium. Demonstration of such auxin swirls, well documented in extant seed plants, in the wood of archaeopteridalean progymnosperms (**a**; *Callixylon*), calamitalean sphenopsids (**b**; *Arthropitys*), and lepidodendralean lycopsids (**c**; *Paralycopodites*) supports hypothesis of shared regulatory role for polar auxin in development of secondary xylem, in all three lineages

Current knowledge of early vascular plants suggests that the most recent common ancestors of lycophytes, sphenopsids, and lignophytes (i.e., seed plants + progymnosperms) were plants without secondary growth. In this context, such fingerprints demonstrating polar auxin flow regulation of cambial activity suggest that wood evolved separately (i.e., parallel evolution) in each of these groups, even though the different evolutionary pathways may have involved the same ancestral regulatory pathways associated with polar auxin transport (Rothwell et al. 2008). However, discoveries of Early Devonian plants that produced wood as early as 407 Ma ago (Gerrienne and Gensel 2016) push the origin of secondary growth very close to the base of the euphyllophyte clade, suggesting that euphyllophytes may share a common ancestor that had evolved the basic toolkit for cambial growth (see also sections "Sequence of Character Evolution" and "Developmental Regulation"). If lycophytes and euphyllophytes share the same polar auxin flow-related regulation of wood patterning, future studies of basal euphyllophytes with secondary growth from the Lower Devonian will reveal the same fingerprints for auxin regulation of wood production.

Lepidodendralean Rooting Structures

Among vascular plants, giant trees have evolved in only two major groups, modern seed plants and extinct lepidodendralean lycophytes, where that stature has been achieved by the evolution of substantial rooting systems. At the same time, we also recognize that the rooting structures of the two groups have distinctly different homologies and arose by divergent evolutionary pathways, and that those differences are understood only because the lycophytes have a rich fossil record.

In seed plants, giant trees are supported by a rooting system that arises from the radicle of a cotyledonary embryo, thus establishing bipolar growth via a system of true roots. By contrast, lycophytes do not have cotyledonary embryos and the only living descendent of the giant lepidodendraleans is the tiny quillwort, *Isoetes*. Interestingly, *Isoetes* appears to have distinctly unusual structure and growth, unless interpreted with reference to extinct relatives (Rothwell and Erwin 1985). Among lycophytes, the fossil record reveals that giant lepidodendralean trees are rooted by a shoot that is modified for rooting (known as a rhizomorph), rather than by a system of true roots. As recently emphasized by Hetherington et al. (2016b), homologies of the lepidodendralean rooting system are with the shoot system, as originally hypothesized more than a century ago.

Interest in similarities between the developmental morphology and anatomy of *Isoetes* and the lepidodendralean rooting system (e.g., the fossil genera *Stigmaria* and *Protostigmaria*) was rekindled by Stewart (1947), who emphasized that *Stigmaria* axes have anatomical features that agree more closely with stems than roots. Stewart also detailed that the leaf-like anatomy and arrangement of stigmarian lateral appendages, referred to as stigmarian rootlets, compare closely to both the leaves and rootlets of *Isoetes*. Likewise, the elongated branched rooting systems of *Stigmaria* and the cormose lobed rooting systems of *Protostigmaria* and *Isoetes* are now recognized as growth variations of a common organography (Rothwell and Erwin 1985). However, such paleontological evidence was not fully understood or appreciated until much later, despite several, additional paleontological discoveries. Frankenberg and Eggert (1969) reconstructed the overall morphology and anatomy of stigmarian rooting systems, reemphasizing both anatomical and developmental similarities of stigmarian axes to the lepidodendralean stems. The authors further demonstrated that stigmarian rootlets abscised as if they were leaves, and provided additional support for anatomical and developmental similarities between stigmarian appendages and *Isoetes* leaves. Concurrently, Jennings (1975) recognized that some lepidodendralean trees were rooted by a cormose *Isoetes*-like rooting system, thus strengthening the homologies between living and extinct rhizomorphic lycophytes. Subsequent characterizations of both embryogeny and apical development for related lycophytes clarified meristematic activity and embryogeny in the clade, and laid the groundwork for a comprehensive summary of homologies among lycophyte shoots and stigmarian rooting systems (Rothwell and Erwin 1985).

Most recently, the developmental significance of the overwhelming morphological, anatomical, developmental, and embryological evidence that stigmarian rooting systems of lepidodendralean lycophytes (and *Isoetes*) are homologous to the aboveground shoot systems (Rothwell et al. 2014) has been explained by the discovery of fingerprints for polar auxin patterning of xylem in such plants. Polar auxin swirls are now known to occur in the wood of both the stems and stigmarian rooting axes of lepidodendraleans, but polar auxin flows in opposite directions in these above- and below ground systems. In stems of the lepidodendralean *Paralycopodites*, such swirls occur above branches, demonstrating basipetal auxin flow from shoot apices. By contrast, such swirls are located, in *Stigmaria* rhizomorphs, on the basiscopic side of stigmarian rootlet traces, indicating acropetal polar auxin flow, toward the

apices of rhizomorphs (Fig. 11). These data reveal that basipetal auxin transport was lost during evolution of the stigmarian and protostigmarian rooting systems of tree-sized lycophytes, and confirm that both gravitropic response and polar auxin flow are independent of the homologies of organs in vascular plants.

Euphyllophyte Leaf Evolution

Traditionally, vascular plants have been interpreted as having either microphylls, derived from enations (i.e., lycophytes), or megaphylls derived from modified branching systems (i.e., euphyllophytes). Whereas currently there is general agreement that euphyllophyte and lycophyte leaves originated independently, the evolution of leaves within euphyllophytes has been the subject of discussion and debate for several years.

On one hand, there is the idea that all euphyllophyte leaves have a unique origin. This idea stems from phylogenetic analyses of only living species (Pryer et al. 2001), which recovered a set of relationships whereby all living seed-free euphyllophytes form a clade (referred to as Moniliformopses) that is sister to the seed plants. Because all living euphyllophytes (except for psilotophytes) have leaves, some have called on these relationships to propose that the common ancestor of all living euphyllophytes also had leaves and, therefore, euphyllophyte leaves have a single, common evolutionary origin. On the other hand, the relationships proposed by Pryer et al. (2001) are at odds with the results of analyses that include living and fossil taxa (Rothwell and Nixon 2006), which (1) do not recover ferns, sphenopsids, and psilotophytes as a clade, and (2) show leafless extinct lineages at the base of living ferns, sphenopsids, and seed plants; thus, supporting independent origins of leaves in several euphyllophyte lineages (Tomescu 2009).

Even if the relationships among living euphyllophytes proposed by Pryer et al. (2001) were supported, review of the fossil record shows that (1) the origin, deep phylogeny, and relationships of ferns, sphenopsids, and seed plants are not well understood; and (2) part of the reason for this situation is that the basal, Devonian representatives of these groups are leafless. The original Moniliformopses – as defined by Kenrick and Crane (1997) – consist of three Devonian taxa: *Ibyka*, *Pseudosporochnus*, and *Rhacophyton*. The relationships of these fossil taxa to living ferns and sphenopsids are unresolved, so equivalence between the formally defined Moniliformopses (Kenrick and Crane 1997) and living "Moniliformopses" (Pryer et al. 2001) is uncertain. Furthermore, *Ibyka* and *Pseudosporochnus* are leafless; therefore, the common ancestor of Moniliformopses did not possess leaves, which must have evolved independently more than once in the descendants of the group. Concurrently, there is little doubt today that seed plants are nested within the lignophytes, a clade that includes a paraphyletic grade of extinct leafless progymnosperms at the base and, thus, has a leafless common ancestor. In summary, from a phylogenetic perspective, the fossil record unequivocally supports a minimum of three independent origins of leaves among euphyllophytes – in ferns, sphenopsids, and seed plants.

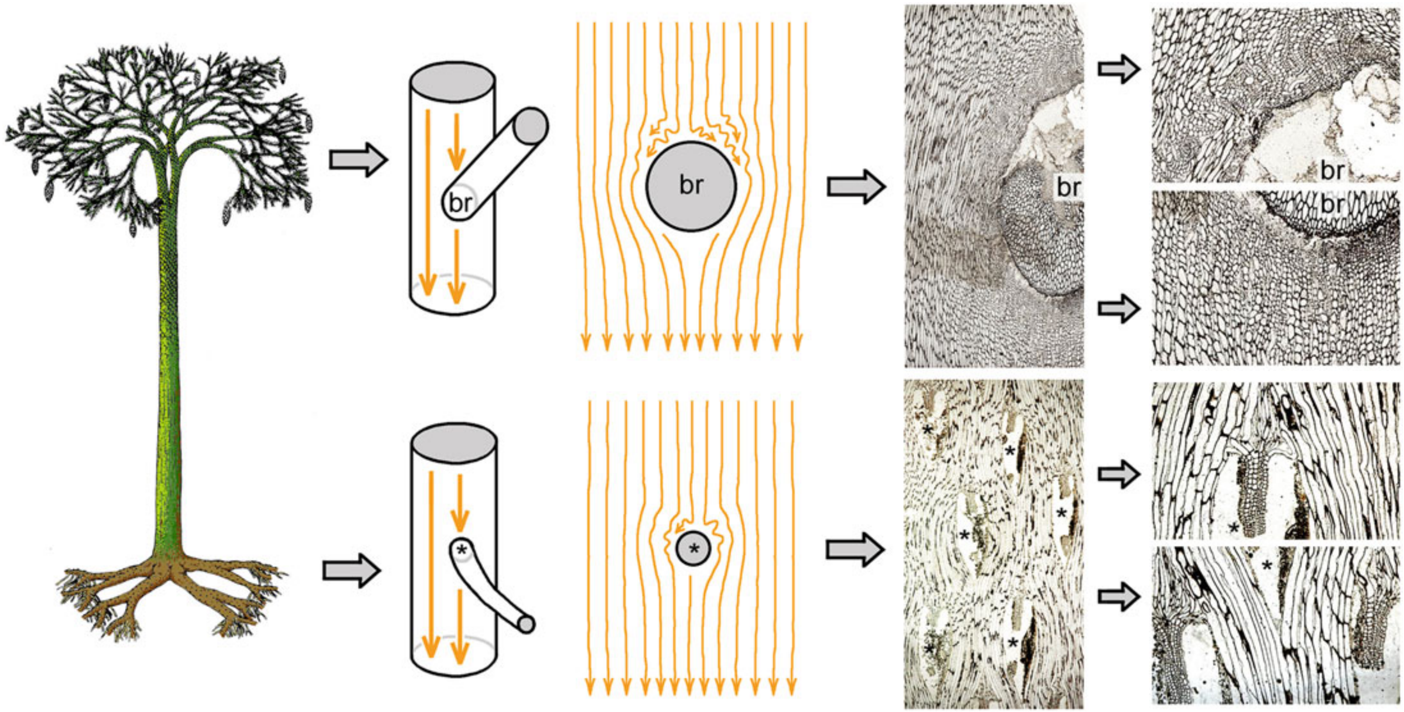


Fig. 11 Polar auxin transport in vascular cambium of arborescent lepidodendralean lycopsid (left). In stems (top panel), auxin flows basipetally, i.e., away from shoot tip. An obstacle in the cambium, represented by base of diverging branch (br), disrupts flow of auxin leading to formation of auxin swirls in wood acroscopically with respect to branch trace (above trace), but not in a basiscopic position (below trace). In positively gravitropic rooting structures (rhizomorphs; bottom panel), subtle auxin swirls are formed in wood basiscopically with respect to "rootlet" traces (asterisks), i.e., above traces, but not in acroscopic position (below traces). This demonstrates that polar auxin flow is directed acropetally in rhizomorph, i.e., toward tip. Wood anatomy illustrated by *Paralycopodites* (stem) and *Stigmaria* (rhizomorph)

The fossil record also contributes evidence for multiple origins of euphyllophyte leaves in an evo-devo perspective. One line of evidence is the demonstration that leaf evolution followed different trajectories, in terms of sequence of character evolution, in ferns and seed plants (Sanders et al. 2009; see also section "Sequence of Character Evolution"). Another line of evidence is provided by fossils demonstrating that the evolution of leaf venation followed similar trajectories, from simpler to more complex architectures, in different euphyllophyte lineages (Boyce and Knoll 2002). This corroborates the evidence for leaf evolution from leafless ancestors in each of those lineages, indicating parallel evolution of leaf venation in distinct euphyllophyte lineages, as opposed to inheritance from a common ancestor that had leaves with complex venation.

Partial homology has been proposed for the leaves of different euphyllophyte lineages at the level of their precursor structures, i.e., the lateral branching systems of their leafless Devonian ancestors (Kenrick and Crane 1997). However, because the branching architectures of Devonian tracheophytes cover a continuous range of morphologies from lateral subordinate (overtopped) branching systems all the way to the branched sporophyte axes of the ancestral polysporangiophyte, statements of homology are difficult to formulate, let alone demonstrate, along this morphological continuum. In a similar vein, Boyce and Knoll (2002) hypothesized that the independent origins of euphyllophyte leaves could have been based on modifications of a common underlying developmental system. Tomescu (2009) reviewed the genetic regulation of leaf development and concluded that interactions between shared regulatory genes are too diverse among (and sometimes within) major lineages to support a common underlying regulatory system.

Vasco et al. (2016) proposed another form of deep homology. They demonstrated expression of Class III HD-Zip transcription factors (HD-Zip III) in the sporangia of *Selaginella* (lycophyte), *Psilotum* (psilotophyte), and *Ophioglossum* (fern). Because HD-Zip III genes have also been shown to be expressed in the sporangia of *Physcomitrella* (bryophyte) and *Arabidopsis* (angiosperm), Vasco et al. hypothesized deep homology of leaves across all tracheophyte lineages, resulting from independent co-option of an ancestral sporangium developmental program that involved III HD-Zip III transcription factors. However, it is also possible that the shared expression of HD-Zip III genes in plant sporangia is not directly relevant to leaf homologies. HD-Zip III genes also have a role in vascular tissue development in all tracheophytes (Floyd and Bowman 2010), possibly evolved after duplication of the ancestral HD-Zip III, which regulated sporangium development. Therefore, it is likely that HD-Zip III expression patterns in the leaves of lycophytes and euphyllophytes have more to do with vascular tissue identity and the regulation of radial (and adaxial-abaxial) polarity in vascular tissues, than with leaf identity and homology (Floyd and Bowman 2010).

In summary, in the debate of euphyllophyte leaf evolution, the fossil record adds phylogenetic resolution by revealing leafless taxa at the base of major euphyllophyte lineages, as well as morpho-anatomical resolution, by showing plants with combinations of characters (determinacy, adaxial-abaxial polarity, venation) that could not be predicted from studies of extant plant diversity alone. When considered alongside

the living plants, these reveal patterns of phylogeny and character evolution that support multiple independent origins of leaves among euphyllophytes.

The *Equisetum* Strobilus: A Case of Reciprocal Illumination

The strobilus of *Equisetum*, a highly condensed structure, and the sporangiophores it comprises have presented a puzzle in terms of evolution and homology for many years. *Equisetum* is the only living representative of the sphenophytes, a diverse clade with a rich fossil record, and as such provides an excellent example of a long phylogenetic branch on which homology issues can only be resolved by querying the fossil record. At the same time, information from fossils is only relevant in the context of development and developmental regulation, as understood based on studies of extant plants (including *Equisetum*), in an example of reciprocal illumination between data on fossils and living plants (Fig. 12).

Transformational series assembled during the mid-twentieth century based on the sphenophyte fossil record suggest that both the leaves and the sporangiophores of *Equisetum* evolved from lateral branching systems. This implies equivalence between leaves and sporangiophores and, consequently, equivalence of their locations on shoots, which were regarded as *nodes* for both types of organs. However, a

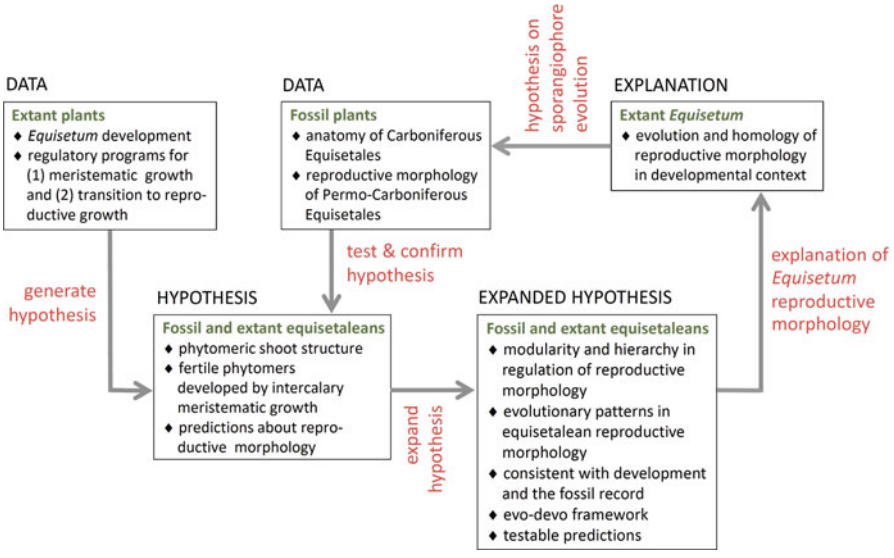


Fig. 12 Reciprocal illumination in elucidation of the origin and evolution of equisetacean reproductive morphology (Tomescu et al. 2017). Hypothesis generated by data from living plants was tested and confirmed by data from fossil record. This provided framework for subsequent hypotheses that included additional data from living *Equisetum* and fossil plants to offer novel explanation for origin of *Equisetum* strobilus and fossil plant reproductive morphologies. This new understanding of *Equisetum* strobilus was then used to formulate further hypotheses about evolution and the deep fossil record, namely, explaining origin and evolution of equisetalean sporangiophore

subset of sphenophyte fossils demonstrate quite the contrary by possessing whorls of sporangiophores attached along *internodes*. These seemingly irreconcilable interpretations, based on two distinct datasets, led to a deadlock in homology interpretations that was not resolved until the realization that a node-internode view may not be the appropriate paradigm within which to interpret homologies of *Equisetum* strobilus structure (Tomescu et al. 2017), cleared the way for a solution to the conundrum.

Studies of vegetative development in extant *Equisetum* provide a framework for hypothesis generation (Fig. 12) by showing that shoot development in this genus owes to the combined activity of the apical meristem (which generates phytomers) and intercalary meristems (responsible for growth of individual phytomers by internode elongation). Our growing understanding of the molecular regulatory mechanisms responsible for meristematic growth suggests that plant meristems of all types are equivalent in their fundamental capacities (Tomescu et al. 2017). These include the capacity to transition to reproductive growth, and molecular programs regulating this transition in meristems are shared broadly among tracheophytes. Together, these developmental capabilities of living plants suggest the hypothesis that the switch to a reproductive developmental program in the intercalary meristems could lead to production of sporangiophore whorls along internodes, as has been observed in fossil equisetaleans. Predictions based on this hypothesis for the development internodal sporangiophore whorls were tested against the anatomy and morphology of fossils. These tests confirm that extinct sphenophytes had the same mode of shoot development involving apical and intercalary meristems, and support the hypothesis that internodal sporangiophore whorls are the product of intercalary meristematic growth.

Confirmation of this *intercalary reproductive growth hypothesis* provides a new framework for formulating additional hypotheses to explain the origin of the *Equisetum* strobilus, as well as the array of different reproductive morphologies documented in extinct equisetaleans. These hypotheses integrate information on development of the *Equisetum* strobilus and observations on fossil equisetalean morphology and propose that independent regulatory modules (gene regulatory networks) are expressed in a hierarchic sequence, leading to determinate apical growth in reproductive mode, and to repression of node-internode differentiation and of intercalary meristematic activity within fertile regions.

Considered within an evo-devo framework, this set of hypotheses on developmental regulation of the *Equisetum* strobilus, in turn, offers solutions for the homology and evolution of the equisetalean sporangiophore, relevant to the deep fossil record of sphenophytes (Fig. 12). Specifically, because sporangiophore development seems to be independent of nodal/internodal identity, it is proposed that the sporangiophore followed an independent evolutionary trajectory that bypassed the evolution of shoots with node-internode structure (including leaves) and whose beginnings may have pre-dated these structures (Tomescu et al. 2017). Accordingly, the sporangiophore could represent the direct expression of a conserved regulatory module originally responsible for development of fertile lateral branching systems, a module that underwent its own evolution, which included heterotopic change, from

expression along undifferentiated axes, to expression on specialized shoot segments, the internodes.

Summarizing this case of reciprocal illumination: a hypothesis generated by data from living plants was tested and confirmed by data from the fossil record. This provided a framework for subsequent hypotheses that included additional data from living *Equisetum* and fossil plants, to offer a novel explanation of the origin of the *Equisetum* strobilus and fossil reproductive morphologies. This new understanding of the *Equisetum* strobilus was then used to formulate further hypotheses about evolution and the deep fossil record, explaining the origin and evolution of the equisetalean sporangiophore.

Conclusions and Future Outlook

Fossils are quintessential witnesses of evolution. Study of the fossil record has contributed tremendously toward resolving plant evolution, systematics, and phylogeny, and gaining a fuller understanding of the role and workings of development in evolution. Living biodiversity represents only a small fraction of the diversity of life that spans Earth's history; therefore, most of the history of plant life is revealed exclusively by the fossil record. The fossil record provides access to an extensive diversity of plant structure that allows for higher resolution in the understanding of evolutionary processes and events in deep time.

Understanding the indispensable role of fossils in addressing questions of plant evolution and phylogeny also provides a powerful argument for a much wider systematic spectrum of genomic sequencing (i.e., a species of Lycopodiaceae, *Psilotum*, *Equisetum*, a species of Ophioglossales, a species of Marattiales). Only with such data available to test hypotheses of phylogeny will we be able to resolve currently recalcitrant relationships among seed plants, euphyllophytes, ferns, and in several regions of the angiosperm clade.

Plant fossils are invaluable in documenting the pattern of evolution (for tissues, organs, modes of growth, life cycles, etc.), which illuminates structural and developmental homologies and provides a test for hypotheses that have been generated from other disciplines. Focused queries of data from the physiology, developmental molecular biology, and comparative developmental anatomy of extant plants will identify additional fingerprints like the ones discussed here. These fingerprints provide as many additional bridges over the gaps that separate living plants, in which development, physiology, genetics, and molecular biology can be studied directly, from the fossils, in which only morphology and anatomy can be observed.

Conversely, the fossil record provides data for the formulation of hypotheses that can be tested with genetic and developmental regulatory experiments. Pressing questions that are currently apparently insoluble and could be addressed by these methodologies regard patterns of evolution for plant vegetative organs (e.g., stele types, axillary branching, intercalary growth), fertile organs, and life cycles (e.g., the seed, the flower, the fruit, heterospory, angiospermous fertilization). It would be interesting to test, for example, if enhanced polar auxin transport in *Isoetes* would lead to elongation and

branching in the rhizomorph and stem, and to more active secondary growth, lepidodendralean-style. Or if discovery and silencing of the regulatory module that represses node-internode differentiation in *Equisetum* sporangiophore phytomers would lead to reproductive morphologies like those of extinct *Peltotheca*. And if further induction of indeterminacy in the fertile shoots of such plants would produce *Cruciaetheca*-like morphologies. We could also test whether abaxialized leaves of loss-of-function HD-Zip III mutants would grow and branch like the undifferentiated axes of early polysporangiophytes, if they were induced into indeterminacy.

The examples highlighted here encompass only a small number of insightful studies where hypotheses generated either from the fossil record or from regulatory developmental genetics serve as reciprocal hypothesis tests. Nevertheless, they demonstrate the exciting potential for such approaches to dramatically improve our ability to address many evolutionary questions that have thus far eluded resolution through the application of either paleontological, systematic, or regulatory genetic/developmental approaches alone. These examples also highlight the value of developmental fingerprints for employing data from the fossil record to enhance our understanding of the role of regulatory genetics in the evolution of plant structure and the origin of major clades. Because these techniques have thus far been applied to such a small number of studies distributed across a narrow sample of potentially fruitful approaches, we are optimistic that the rapidly expanding application of coordinated developmental genetic–paleontological studies will, for the first time, allow us to address some of the most poorly understood events and processes of evolutionary biology.

Cross-References

- ▶ A Process-Based Approach to the Study of Flower Morphological Variation
- ▶ Developmental Homology
- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Macroevolution
- ▶ Methods and Practices in Paleo-Evo-Devo
- ▶ The Evolution of Branching in Land Plants: Between Conservation and Diversity

Acknowledgments We are indebted to Jean Galtier, Patricia Gensel, Alexander Hetherington, Jinzhuang Xue, Patrick Herendeen, and Kelly Matsunaga, who kindly provided images for illustrations. Nevin Cullen, Kyla Garten, and Dennis Walker are thanked for preparing some of the sections. Space constraints precluded us from fully referencing this work, and we apologize to the many authors whose publications are not included.

References

Boyce CK, Knoll AH (2002) Evolution of developmental potential and the multiple independent origins of leaves in Paleozoic vascular plants. Paleobiology 28:70–100

Floyd SK, Bowman JL (2010) Gene expression patterns in seed plant shoot meristems and leaves: homoplasy or homology? J Plant Res 123:43–55

Frankenberg JM, Eggert DA (1969) Petrified *Stigmaria* from North America: part I. *Stigmaria ficoides*, the underground portions of Lepidodendraceae. Palaeontogr B 128:1–47
Gerrienne P, Gensel PG (2016) New data about anatomy, branching, and inferred growth patterns in the early Devonian plant *Armoricaphyton chateaupannense*, Montjean-sur-Loire, France. Rev Palaeobot Palynol 224:38–53
Harrison CJ (2016) Auxin transport in the evolution of branching forms. New Phytol. https://doi.org/10.1111/nph.14333
Hetherington AJ, Dubrovski JG, Dolan L (2016a) Unique cellular organization in the oldest root meristem. Curr Biol 26:1629–1633
Hetherington AJ, Berry CM, Dolan L (2016b) Networks of highly branched stigmarian rootlets developed on the first giant trees. Proc Natl Acad Sci U S A 113:6695–6700
Jennings JR (1975) *Protostigmaria*, a new plant organ from the lower Mississippian of Virginia. Palaeontology 18:19–24
Kenrick P, Crane PR (1997) The origin and early diversification of land plants. Smithsonian Institution Press, Washington, DC
Langdale JA (2008) Evolution of developmental mechanisms in plants. Curr Opin Genet Dev 18:368–373
Matsunaga KKS, Tomescu AMF (2017) An organismal concept for *Sengelia radicans* gen. et sp. nov. – morphology and natural history of an early Devonian lycophyte. Ann Bot 117:1097–1113
Matsunaga KKS, Cullen NP, Tomescu AMF (2017) Vascularization of the *Selaginella* rhizophore: anatomical fingerprints of polar auxin transport with implications for the deep fossil record. New Phytol. https://doi.org/10.1111/nph.14478
Niklas KJ (1997) The evolutionary biology of plants. Chicago University Press, Chicago
Pryer KM, Schneider H, Smith AR et al (2001) Horsetails and ferns are a monophyletic group and the closest living relatives to seed plants. Nature 409:618–622
Rothwell GW, Erwin DM (1985) The rhizomorph apex of *Paurodendron*: implications for homologies among the rooting organs of Lycopsida. Am J Bot 72:86–98
Rothwell GW, Nixon K (2006) How does the inclusion of fossil data change our conclusions about the phylogenetic history of euphyllophytes? Int J Plant Sci 167:737–749
Rothwell GW, Sanders H, Wyatt SE et al (2008) A fossil record for growth regulation: the role of auxin in wood evolution. Ann Mo Bot Gard 95:121–134
Rothwell GW, Wyatt SE, Tomescu AMF (2014) Plant evolution at the interface of paleontology and developmental biology: an organism-centered paradigm. Am J Bot 101:899–913
Sanders H, Rothwell GW, Wyatt SE (2009) Key morphological alterations in the evolution of leaves. Int J Plant Sci 170:860–868
Spencer ART, Mapes G, Hilton J et al (2015) Middle Jurassic evidence for the origin of Cupressaceae: a paleobotanical context for the roles of regulatory genetics and development in the evolution of conifer seed cones. Am J Bot 102:942–961
Stein WE (1993) Modeling the evolution of stelar architecture in vascular plants. Int J Plant Sci 154:229–263
Stein WE, Boyer JS (2006) Evolution of land plant architecture: beyond the telome theory. Paleobiology 32:450–482
Stewart WN (1947) A comparative study of stigmarian appendages and *Isoetes* roots. Am J Bot 34:315–324
Taylor TN, Kerp H, Hass H (2005) Life history biology of early land plants: deciphering the gametophyte phase. Proc Natl Acad Sci U S A 102:5892–5897
Taylor WA, Strother PK, Vecoli M et al (2017) Wall ultrastructure of the oldest embryophytic spores: implications for early land plant evolution. Rev Micropaleontol. https://doi.org/10.1016/j.revmic.2016.12.002
Tomescu AMF (2009) Megaphylls, microphylls and the evolution of leaf development. Trends Plant Sci 14:5–12
Tomescu AMF (2011) The sporophytes of seed-free vascular plants – major vegetative developmental features and molecular genetic pathways. In: Fernandez H, Kumar A, Revilla MA (eds) Working with ferns. Issues and applications. Springer, New York, pp 67–94

Tomescu AMF, Wyatt SE, Hasebe M et al (2014) Early evolution of the vascular plant body plan – the missing mechanisms. Curr Opin Plant Biol 17:126–136

Tomescu AMF, Escapa IH, Rothwell GW et al (2017) Developmental programmes in the evolution of *Equisetum* reproductive morphology: a hierarchical modularity hypothesis. Ann Bot 119:489–505

Vasco A, Smalls TL, Graham SW et al (2016) Challenging the paradigms of leaf evolution: Class III HD-Zips in ferns and lycophytes. New Phytol 212:745–758

A Process-Based Approach to the Study of Flower Morphological Variation

Chelsea D. Specht and Ana M. R. Almeida

Contents

Abstract

One of the most striking elements of angiosperm evolution is the diversity of floral forms represented. Beyond modifications of existing structures, flowers can evolve novel elements that are often linked with functions associated with effective or efficient pollination. Yet despite this diversity of floral forms, certain flower structures are highly conserved, as are many of the genes that underlie their

C. D. Specht (✉)
School of Integrative Plant Sciences, Section of Plant Biology and the Bailey Hortorium, Cornell University, Ithaca, NY, USA
e-mail: cdspecht@cornell.edu

A. M. R. Almeida
Department of Biology, California State University East Bay, Hayward, CA, USA
e-mail: ana.almeida@csueastbay.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_61

basic form. Here, a process-based approach is discussed as a means of investigating floral morphological diversity and studying the evolution of floral form. First, the advances in understanding the evolution of floral form derived from the traditional *morphogenetic* approach are discussed. Then, a discussion follows on the unique ways that a process-based approach can contribute to our understanding of developmental evolution leading to organ elaboration and morphological diversification, focusing on polarity as a case study. Finally, the current limitations of a process-based approach are discussed, while pointing out future venues of research in this area that might greatly improve our understanding of morphological diversification of flowers.

Keywords

Flower development · Developmental process · Morphological variation · Polarity · Petaloidy

Introduction

The field of evolutionary developmental biology, also known as evo-devo, aims to characterize and understand the evolution of morphological variation in multicellular organisms. In particular, evo-devo is concerned with how developmental patterns and processes mediate evolutionary changes in organismal form. As a discipline, evo-devo evolved from a mix of various approaches to the study of development and evolution (Scholtz 2008), thus resulting in different explanations for organismal diversity. It has recently been claimed that these explanations can be roughly divided into three distinct views – *transformational*, *morphogenetic*, and *process approach* views – based on the roles played by pattern and processes in explaining the variation and innovation of organic form (Nuño de la Rosa and Etxeberria 2011).

According to Nuño de la Rosa and Etxeberria (2011), *transformational views* are accredited to early studies in comparative embryology, in which development was conceived as a series of discrete or dynamic patterns corresponding to developmental stages, and distinct evolutionary lineages were compared based on these regularities. As such, the main goal was to describe developmental patterns, present in all evolutionary lineages, ultimately detecting and revealing the regularities of a biogenetic law.

The *morphogenetic approach*, in turn, is concerned with how patterns (or structures) come into being (develop). According to this view, evolutionary patterns warrant explanations that stem from developmental processes. Changes in the "construction rules" underlying developmental processes are the main features that explain evolutionary changes in observed morphological patterns (Nuño de la Rosa and Exteberria 2011). This view is based on a mechanistic ontology, in which developmental processes can be broken down into entities that can, through linear composition, explain observable evolutionary patterns and homology is explained via conserved mechanisms.

On the other hand, a *process approach* considers that processes themselves are biological phenomena to be described, independent of their role in explaining pattern formation. In this view, developmental processes and their morphological manifestations gain the center stage as the temporal and spatial "characters" of evolution, in an effort to account for the dynamic nature of living organisms. An important assumption subjacent to this view is that processes share common properties with patterns, such as stability, modularity, and homology, allowing them to become objects of scientific inquiry in their own right (Gilbert and Bolker 2001). Because both processes and patterns are described as characters, homology statements can include both phenomena. In this sense, a given process can give rise to distinct patterns, at the same time that the same pattern could conceivably originate from different processes (Nuño de la Rosa and Etxeberria 2011).

Regardless of whether these distinct views accurately represent the explanatory landscape of contemporary evo-devo, they provide an interesting framework from which one can anticipate the role of patterns and processes in the evolution of morphological variation. The implications of adhering to a pattern-centric or process-centric approach are various, and beyond the scope of this chapter. Here, a *process-based approach* already advanced by others (Scholtz 2008) is presented, in which patterns and processes are observable and tractable evolutionary entities, comparable and homologizable units of development. Most importantly, rather than excluding the role of patterns underlying the evolution of biological form, a process-based approach calls for more attention to be given to the developmental processes themselves and their roles in shaping morphological changes in evolution, both in populations and across clades. In the case of organismal biology, developmental processes are instantiated by the interactional dynamics of epigenetic landscapes and gene regulatory networks (Alvarez-Buylla et al. 2008). In this view, the explanatory power of processes, although realized by distinct entities, cannot be attributed to any particular such structure but to the resulting dynamics of the interactions of its formative components. As such, both processes and patterns are required to describe the evolution of form and function.

Patterns and Processes in Flower Evo-Devo

ABCs and Patterns

The great majority of floral evo-devo studies have focused on genes involved in the specification of organ identity within the framework of the well-established ABC model of floral organ development. The ABC model, proposed based upon knowledge of floral mutants that appeared to uniquely affect organ identity, describes the expression patterns of a group of genes that – in combination – give rise to the 4 canonical floral organs: sepals, petals, stamens, and carpels.

The original model based on *Arabidopsis* was specified as follows: a single A-class gene is represented by *APETALA-2* (*AP2*) and, when expressed alone, this gene specifies sepal identity in the primordia that comprise the outermost whorl of

the flower. B-class genes comprise *APETALA-3* (*AP3*) and *PISTILLATA* (*PI*), and together their expression domains determine the identity of petals when they are expressed in combination with A-class genes in the primordia forming the 2nd whorl of the developing *Arabidopsis* flower. B-class gene expression is also responsible for primordia taking on the identity of stamens when they are expressed in combination with C-class genes. *AGAMOUS* (*AG*) is the single C-class gene in *Arabidopsis thaliana* and is responsible for the identity of the sex organs: stamens are formed from primordia in which C-class genes are expressed in combination with B-class genes, and the gynoecium is formed from the innermost whorl of primordial where C-class genes are expressed alone. Based on mutant analysis and gene expression patterns, the main hypothesis derived from the canonical ABC model states that during flower development, the expression of the A-, B-, and C-class genes in concentric fields and the subsequent presence of their resulting regulatory proteins in those concentric domains results in the specification of floral organ identity (Coen and Meyerowitz 1991).

Early advances in flower evo-devo focused on the correlations between gene expression patterns and changes in flower morphology in different lineages (see Della Pina et al. 2014). The ABC model and its genetic underpinnings were, for the most part, successfully utilized as "construction rules" active during flower development. Deviations from the mechanics encoded by these construction rules could be hypothesized to explain evolutionary changes in morphology (observable variations in patterns in organ identity) in populations and macroevolutionary time. As such, by considering the ABC model as a mechanism for development and for evolution, flower evo-devo research has largely taken a *morphogenetic* approach in which much of the focus remains in the explanation of mechanistic principles underlying patterns, with less attention given to processes.

One problem that commonly arises in pattern-based approaches, however, relates to homology statements. For instance, while organ identity is pronounced in *Arabidopsis*, organ identity is less discrete and more variable across other flowering plant lineages giving rise to intermediate forms or organs with combinations of identity/function. For example, if a "petaloid" or petal-like organ forms in the third whorl, it remains positionally homologous to a stamen; however, gene expression in the organ may be hypothesized to be homologous to that of a petal. If indeed gene expression is petal-like, based on the ABC model in *Arabidopsis*, does that make the organ homologous to a petal? What if the petal itself, in the same organism, does not share a gene expression pattern with the petaloid stamen of the same flower? Is it then not identified as petal-like or as having petal homology? By focusing on organ identity – a pattern-based homology statement – rather than the process leading to the morphology of the organ, the morphogenetic approach can lead to ambiguities in how organs develop and manifest certain morphological properties and how that results in evolutionary changes underlying morphological diversification.

Discordant patterns of gene expression and floral organ morphology have long been described in the literature (e.g., Kanno et al. 2007) and might point to the fact that complex interactions between the canonical organ identity genes may need to be taken into account. Within the monocot lineage, for instance, the evolution of

differentiated sepal and petal whorls has occurred multiple times. While this differentiation was at one time considered evidence for a united group, it is now clear that the monomorphic perianth has become differentiated multiple times and forms independent synapomorphies for the Commelinales, Zingiberales, Eriocaulaceae, and Bromeliaceae as well as other lineages within the Poales and Alismatales (APG IV 2016). These independent evolutionary events of organ elaboration within monocot lineages likely result from the co-option of homologous developmental processes. These processes underlie our concept of organ identity via their influences on organ morphology and provide a mechanistic framework for investigating the developmental processes that affect the evolution of form and function.

Dynamic Processes and Organ Evolution

Even though a morphogenetic approach has dominated the flower evo-devo research field, process-based approaches have also been carried out within the scope of floral organ evolution. One basic assumption of this dynamic-systems (process-based) approach is that gene regulatory networks (GRN) and their corresponding epigenetic attractor landscapes are fundamental units of development, largely shared across distantly related organisms, and therefore homologous to a certain extent. In the case of the Floral Organ Specification Gene Regulatory Network (FOS-GRN), which includes the ABC organ identity genes, a recent study suggests that this regulatory module is largely shared among angiosperms and is robust to perturbation. This robustness leads to the canalization of expression patterns within lineages. It is proposed that this module has been subjected to strong functional constraints and that the dynamic interactions of the genes within the network could explain its constrained evolution (Davilla-Velderrain et al. 2013). However, while this robust network appears to function to define differential organ identity across flowering plants, the relative expression of these genes and the number of copies these gene families maintain is not conserved between divergent lineages, nor is it simple to define organ homology either by expression pattern or by position or function across large evolutionary distances. Thus, the utility of "organ identity" as a pattern for understanding morphological evolution is limited, unless taken within the scope of a clearly defined evolutionary lineage.

While specification of organ position and organ morphology involve various gene networks that may act in parallel (review in Alvarez-Buylla et al. 2010), the strict 1:1 correlation of position and structure/function in *Arabidopsis* gives the false impression that organ identity (1) is a feature of the organ rather than an emergent property of the underlying dynamic interaction of the FOS-GRN, (2) is a feature that can be derived from a single pattern of gene expression, and (3) is a feature that is discernable, based on the final morphological form of the organ. In fact, in many plants, organ position, structure, and function are characters that can vary independently from one another: for example, both outer (sepals) and inner (petals) perianth function as petals in tulips; stamens and carpels switch positions in the "inside-out" flower of *Lacandonia;* and infertile stamens (staminodes) function as petals in many

Zingiberales. Furthermore, organ identity can be complicated as the same organ type within a single flower can have multiple identities. Therefore, focusing on the processes underlying development, rather than patterns of organ identity, could overcome limitations imposed by viewing these homology issues in the light of discrete patterns.

In the next section, the establishment of abaxial-adaxial polarity is used as a case study to demonstrate the contributions that a process-based approach brings to our understanding of morphological evolution across angiosperm flowers.

Polarity as a Case Study

During development, plant structures arise from the differentiation of meristematic regions. As development proceeds, different polarity axes are established: distal-proximal, abaxial-adaxial, and apical-basal (Fig. 1a). In particular, the abaxial-adaxial polarity axis is established through an intricate interaction of several transcription factors, hormones, as well as regulatory microRNAs (Fig. 1b), first described in the context of leaf development.

Through the dynamic interaction of multiple components of an intricate GRN (Fig. 1b), three domains are established on a developing lateral organ primordia

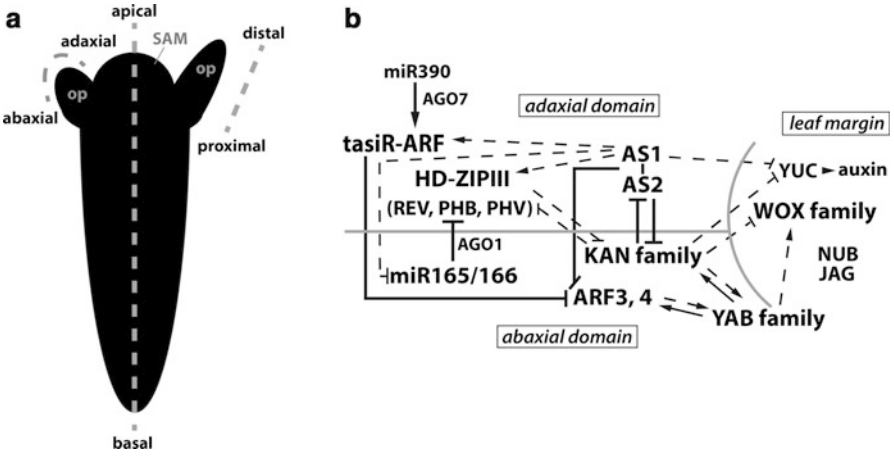


Fig. 1 Polarity axes and the abaxial-adaxial gene regulatory network. (a) Main polarity axes in a developing seedling. SAM – Shoot Apical meristem; op – lateral organ primordial; Dashed grey lines – polarity axes. (b) Main components of the abaxial-adaxial gene regulatory network. See details on text. Grey lines divide the abaxial, adaxial, and leaf margin domains. Solid black lines denote direct interaction, while dashed black lines denote either indirect or unconfirmed interactions. *YUCCA* (*YUC*); *ASYMMETRIC LEAVES* (*AS*); *REVOLUTA* (*REV*); *PHAVOLUTA* (*PHV*); *PHABULOSA* (*PHB*); *KNADI* family (*KAN*); *YABBY* family (*YAB*); *WUS* homeobox-containing family (*WOX*); *NUBBIN* (*NUB*); *JAGGED* (*JAG*); *AUXIN RESPONSE FACTOR* (*ARF*); *ARGONAUTE* (*AGO*); *trans*-acting small interfering RNAs (tasiR); micro RNA (miRNA). Interactions based on Fukushima and Hasabe (2013) and Husbands et al. (2009).

based on their relative position with respect to the shoot apical meristem (SAM). The adaxial domain is closest to the meristem and will ultimately form the dorsal surface of the mature lateral organ; the abaxial domain is furthest from the SAM and will ultimately form the ventral surface of the lateral organ. At the boundary of these two domains, the leaf margin domain forms as the result of the mutual negative regulatory interactions between players from the adaxial and abaxial domains. This leaf margin domain is where activation of downstream regulators promotes laminar outgrowth (Husbands et al. 2009, Fig. 1b). At this leaf margin, cell proliferation is induced resulting in leaf blade expansion followed by gradual cell differentiation. Most mature leaves develop into bifacial laminar (=flattened) structures, with morphologically and anatomically differentiated dorsal and ventral surfaces. Nonetheless, the ab-ad GRN has been implicated in the development of other leaf forms, reviewed in Fukushima and Hasabe (2013), as well as the shapes of other organs that appear to have evolved from ancestrally laminar structures. Thus, the ab-ad GRN can be considered to be involved in the process of establishing polarity, resulting in marginal expansion and laminar lateral organ structures when polarity is properly established, or radial lateral organ structures when polarity is disrupted.

The proper establishment of abaxial-adaxial polarity depends on the interaction of multiple factors, ranging from transcription factors to regulatory microRNAs. Abaxializing agents include *KANADI* (*KAN*), *YABBY* (*YAB*), and *AUXIN RESPONSE FACTOR* (*ARF*) transcription factors, which likely act as positive regulators of one-another while negatively regulate adaxializing agents. Adaxializing agents include members of the *HD-ZIPIII* family of transcription factors, *REVOLUTA* (*REV*), *PHABULOSA* (*PHB*), and *PHAVOLUTA* (*PHV*), as well as *ASYMMETRIC LEAF 1* and *2*. Similarly to abaxializing transcription factors, adaxializing agents negatively regulate abaxializing signals, excluding them from the adaxial domain of the lateral organ primordial.

The interaction of several of these several components (Fig. 1b) helps to establish both the abaxial and the abaxial domains. Meanwhile, *YABBY* genes likely contribute to the activation of leaf margin domain regulators at the boundary region of the abaxial-adaxial domains. Activation of *WUS* homeobox-containing genes (*WOX*), *NUBBIN* (*NUB*), *JAGGED* (*JAG*), and *YUCCA* (*YUC*) contribute to cell proliferation and leaf blade expansion at the leaf margin domain (Fukushima and Hasabe 2013).

Although the ab-ad GRN has been largely studied in leaf primordia as models of laminar growth, recent studies have suggested that this GRN was co-opted to shape the morphology of stamens across angiosperms and is involved in the evolution of novel morphologies within lineages (Almeida et al. 2014). Flower evolution in Zingiberales exhibits marked changes in stamen morphology, with stamen abortion, sterilization, and petaloidy being the most prominent features. Across the Zingiberales, stamens can be either radial (banana families) or laminar (ginger families). In some Zingiberales, stamens bearing the ancestral radially symmetric morphology (e.g. *Musa*; Fig. 2a, b), overexpression of one *YABBY* gene leads to an imbalance of ab-ab regulators, preventing blade expansion. In comparison, in species bearing laminar, petal-like stamens, such as those observed in Costaceae

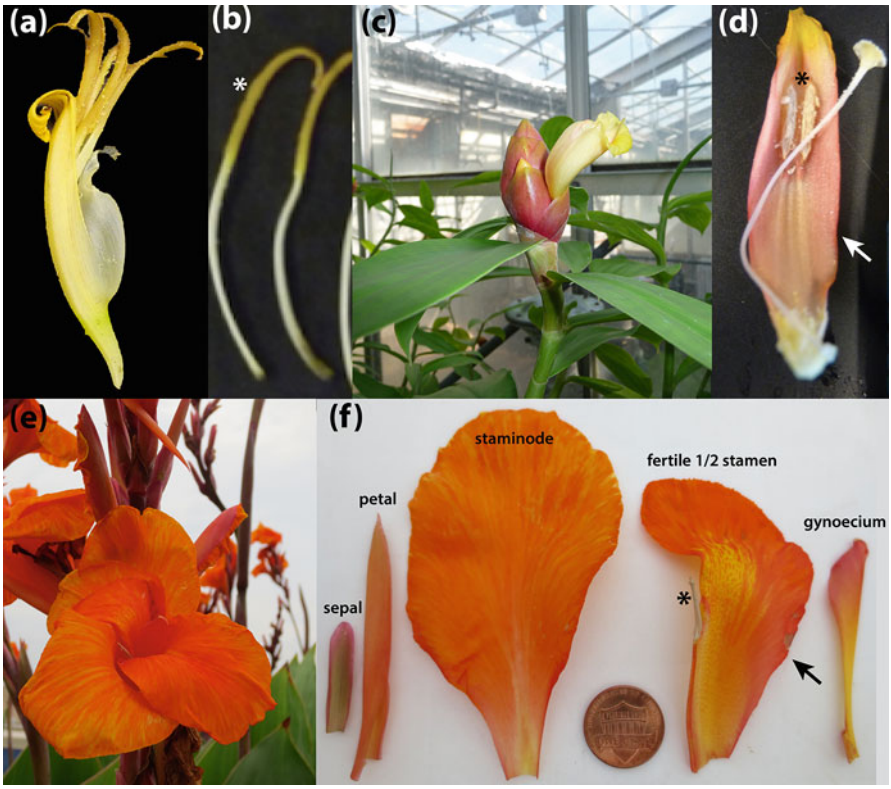


Fig. 2 Flowers and floral organs of Zingiberales species. (a) *Musa basjoo* flower; (b) *Musa basjoo* radial stamen; (c) *Costus spicatus* inflorescence and flower with showy labellum forming the floral display; (d) *Costus spicatus* fertile stamen; (e) *Canna sp.* inflorescence and flower with showy sterile male organs (staminodes); (f) *Canna sp.* dissected flower organs showing sterile laminar (staminode) and fertile laminar male organs. (*) indicates position of the anther within the fertile stamen in Musaceae (a; 5 or 6 fertile stamens), Costaceae (d; one fertile stamen) and Cannaceae (f; 1/2 fertile stamen)

(Fig. 2c, d) and Cannaceae (Fig. 2e, f), the orthologous *YABBY* gene is expressed in significantly lower levels, similar to the expression levels of other genes in the ab-ad GRN. As such, it has been hypothesized that downregulation of this *YABBY* gene in species such as *Canna* and *Costus* leads to a reconstitution of the balanced expression of abaxializing and adaxializing agents, resulting in the establishment of a "leaf" margin domain and therefore laminar expansion of the stamen filament. Similar gene expression pattern of ab-ad GRN genes is also observed in the radial stamens of *Brassica rapa*, supporting the idea that the ab-ad GRN is involved in shaping stamen morphology across angiosperms (Almeida et al. 2014).

The ab-ad GRN is a prime example of an evolutionary mechanism operating at the level of a developmental module. The co-option of an already established GRN module, linked to regulation of gene interactions within the module, leads to the

morphological diversification of lateral organs observed in angiosperms: both in leaves and in flowers. In the case of the ab-ad GRN, no singular gene expression value can explain the resulting morphological transitions. Only when taken within the dynamical context of the ab-ad GRN can one understand the resulting morphological – both developmental and evolutionary – pattern. The study of the ab-ab GRN emphasizes the importance of observing the complexities of gene interactions when focusing on organ morphologies, and only when these interactions are taken into account in a comparative context can the observed gene expression patterns underlying the development and evolution of morphologies be fully understood.

Beyond Gene Patterns to Morphological Processes

The future of flower evo-devo lies in a combination of classical functional experimentation with a more systems dynamic approach in which genes and gene regulatory networks are viewed as interacting units of larger, more complex developmental networks, ultimately responsible for the diversification of phenotypes and the evolution of morphology (see chapter ▶ "Modeling Evolution of Developmental Gene Regulatory Networks"). In this approach, developmental processes are viewed as modules that function rather independently, and are comparable units of evolutionary change. In this sense, homology of process, rather than homology of structure, becomes the focus of attention. By understanding how developmental modules, comprising several regulators and their interactions, function and evolve through comparative analysis within a well-defined phylogenetic context, one can unravel the origin of morphological variation. Thus, although it is fundamentally important to understand the role of that particular components play in the process of development, it becomes more critical to understand, at a higher level, how complex gene interactions of multiple components ultimately result in particular phenotypes.

It is important to emphasize that throughout evolution a particular GRN can be co-opted to participate in developmental processes of nonhomologous organs, which makes structural homology statements challenging. Under this paradigm, changes in regulatory modules, either through gene duplication followed by diversification or through changes in gene regulation and interaction, can lead to morphological variation through changes in gene interactions and/or function. Homology, therefore, can be assessed at the level of developmental processes or modules, but only within a phylogenetic framework of closely related species will the homology statements remain evolutionary meaningful.

Limitations of a Process-Based Approach

Process-based approaches, albeit fascinating, are not free from limitations. One such limitation comes from a theoretical standpoint. Although discussions about the ontological nature of evo-devo approaches are beyond the scope of this manuscript, one question remains to be answered: Is it possible to emphasize developmental

processes as units of development and evolution without committing to a process ontology? Some successful attempts at formalizing GRN nonlinear dynamics have claimed their mechanistic nature (Davilla-Velderrain et al. 2015), rejecting, therefore a process-driven ontology. Nonetheless, more theoretical and conceptual developments are still missing in order to fully unite a dynamic systems approach to a mechanistic ontology free of contradictions.

Potentially one of the main counter-intuitive ideas of a process-based approach is the claim that processes, as developmental modules, are stable units that can be homologized across evolutionary distances. At the same time, modifications and variation in the functioning of developmental modules are one of the main forces driving morphological diversification. The understanding of how developmental modules can and do evolve, and how this evolution contributes to morphological variation is an area of active research.

In practical terms, it is likely that the most important challenge faced by evo-devo research taking a process-based approach is the formalization of particular developmental processes. This would require not only a theoretical transition, but also the development of new tools and technologies related to formalizing and modeling developmental processes that can be applied across large evolutionary distances. With the continued refinement of computational and genomic approaches, it is likely that a process-based approach will further our understanding of developmental evolution by focusing on the role of developmental modules underlying major processes and characterizing their variation in genetic components and tissue/organ deployment through evolutionary time.

Duplications, Natural Variation, and Future Promise for Evo-Devo

Gene and/or whole genome duplications, extensively spread across the angiosperm phylogeny, present scientists with a formidable challenge, regardless of their approach to understanding the genetic mechanisms underlying developmental evolution. The functional outcomes of duplicated genes are varied and most times difficult to uncover due to subsequent gene losses or phylogenetic and/or morphological distance to model systems in which many processes have been described (e.g., Rijkema et al. 2010). For example, the ABC model of organ identity in *Arabidopsis thaliana* is characterized by the fact that most classes of genes in the ABC model comprise a single gene. However, the multitude of gene and genome duplications that have been described during the evolution of the angiosperms (Cui et al. 2006) results in complex patterns of gene regulatory interactions following sub- or neo-functionalization in distinct angiosperm lineages (see, e.g., Sharma and Kramer 2013). The study of these complex interactions would be greatly improved within a process-based approach capable of modeling these gene interactions while predicting system's stable states.

In light of the wealth of research already taking place in *Arabidopsis* development based on a morphogenetic approach, below are potential routes for the advancement of the plant evo-devo research field. It is also important to keep in mind that in a

genomics era, current approaches are being applied to a wide range of organisms, enabling the study of morphological diversity beyond model species (Tickle and Urrutia 2017). In current plant evo-devo research, natural variation can greatly inform our understanding of flower morphological evolution. Although mutant analysis has been the gold-standard for studies of gene function in individual organisms, natural phenotypic variation provides the evo-devo community with a powerful research tool for studying the mechanisms of variation across populations and among clades. Above all, naturally occurring phenotypes have the advantage of representing real biological entities that have persisted under the influence of evolutionary dynamics including selection, gene flow, drift, and mutation (Bolker 1994). Therefore, the study of natural phenotypic variation within a clear comparative phylogenetic context provides flower evo-devo researchers a powerful tool to investigate how changes in development might influence the phenotypic variation observed and which changes are under differential selection regimes, allowing researchers to tease apart correlation from causation. Natural variation also provides information about which phenotypes can co-occur within a developmental framework, providing information about interactions among gene regulatory networks that can be used to estimate developmental fitness within a dynamic evolutionary landscape.

In order to fully embrace a process-based approach, taking into account the dynamics of gene interactions and integrating the wealth of publicly available experimental data, evo-devo researchers could greatly benefit from modeling approaches. This degree of formalization, however, would require a solid mathematical account of the developmental process and, in many cases, would require the establishment of collaborations or the development of new technologies (Mabee 2006). On the biological end, however, important initial steps are within reach of any evo-devo researcher.

Extensive Literature Review can Provide Powerful Information on Gene Interactions and Their Relation to Phenotypic Variation

It is clear that a great number of genes are involved in flower development and evolution, and numerous flower mutants have already been described in *Arabidopsis* as well as in other species. Although mutant analysis has commonly led to the identification of a gene as an isolated causal agent, such screens also provide an incredible amount of information on the role of various genes in shaping a particular floral phenotype. By searching the literature and evaluating mutant experiments and resulting phenotypes, one can develop insights for the way in which various genes might interact during the development of a particular floral organ and which complex developmental processes that gene might influence. In this sense, changes in gene expression patterns and in gene interactions become potential hypothesis to be explored.

The focus on gene interactions broadens our explanatory power, as we are not limited to the way particular genes are being expressed, but how changes in interactions among genes might lead to the changes in gene expression patterns.

These changes can be of regulatory nature or can involve changes in binding affinity or protein-protein interactions, in addition to changes in copy number and regulation. Considering the array of mutant morphologies observed in *Arabidopsis* lines, new genes might end up being critical in conferring some of the morphological novelties in organ morphology, merism, flower size, symmetry patterns, and fusion that characterize the flowers of lineages that do not share the whorled and 4-organ arrangement of wild type *Arabidopsis* plants.

As a caveat, the knowledge accumulated in *Arabidopsis* or any other angiosperm lineage should be taken in perspective, as any current angiosperm lineage has its own evolutionary history and thus cannot be viewed as ancestral or as an archetype that has been modified through time. The *Arabidopsis* lineage is no exception. The trick is then to differentiate the aspects of any molecular developmental mechanisms that are largely shared across angiosperms from those that are lineage-specific. There is no easy way out of this problem, but a broader focus on a comparative process-based approach across lineages, illuminated by the model-organism's data, can provide some of these answers.

Genome-Level Information on Nonmodel Taxa Provides a Useful Tool for Studying the Evolution of Developmental Processes at a Large Scale

A considerable number of whole genome sequences are already available across various angiosperms families. These sequences provide a tremendous amount of valuable information on developmental-related genes and their evolution. As next-generation sequencing technologies advance, transcriptome research on flowers and individual floral organs becomes a feasible reality. Organ- or tissue-specific transcriptomes can be generated for almost any tissue and can provide an almost infinite wealth of information on specific gene expression patterns that can be compared with organs or tissues having a different set of features in a comparative framework. Having as a foundation the set of gene interactions gathered from the literature (experimentally derived GRN) that might underlie a particular developmental process, and postulating dynamic GRNs already established for model systems, one can make logical predictions on novel and candidate genes as well as candidate pathways by comparing observed and expected expression levels and numbers of copies with morphological patterns.

Next-generation sequencing data are capable of quickly providing large amounts of expression data that can be used to understand the dynamical interactions of genes in nonmodel organisms (Tickle and Urrutia 2017). These data can be used not only as a whole-transcriptome level comparison analysis, but also as initial screening of genes of interest to a certain developmental process under study. Based on the GRNs developed in model species, we can then infer how these candidate gene interactions behave in our nonmodel systems and lineages of interest, and acquire lists of candidate gene interactions for further studies. This targeted approach to next-generation sequencing data can become especially interesting to evo-devo if clear

gene interaction hypothesis are tested experimentally. Once candidate genes and network interactions are identified, the functions of different genes can be tested using targeted genome editing (CRISPR/Cas9) as these methods are being rapidly and effectively developed across all plant lineages. In addition, morphological variations resulting from genome editing approaches can be tested in experimental settings to test fitness and selection parameters that may influence developmental evolution.

Dynamic Models Provide a Powerful Tool for Flower Evo-Devo Research

Dynamic models can be formalized, based on experimentally informed GRN. Publically available data on functional experiments can greatly help the proposal of a solid GRN. Although the ability to produce dynamic models to represent specific developmental processes is not currently a widely used tool in flower evo-devo, it has started to prove an extremely powerful approach to developmental studies. Research on the evolution of plant development can be guided by a focus on how patterns of gene expression emerge during development as a result of complex and highly nonlinear interactions of genetic and nongenetic components, for example as GRN dynamics feedback with physical and chemical fields (see Barrio et al. 2013) and why certain patterns of gene expression are fairly robust across angiosperm evolution (Alvarez-Buylla et al. 2010). Such approaches have hypothesized that the observed combinations of gene expression characteristic of different floral organ primordia correspond to steady states or attractors of complex GRNs. The challenge becomes proposing a set of dynamic gene interactions that are sufficient to robustly recover such expression combinations and result in organ modifications. Such studies will complement the evo-devo research program in flower morphology by contributing a mechanistic explanation of how developmental processes emerged and evolved, why certain aspects of floral morphology remain widely conserved among flowering plants while others are highly variable, and how changes in dynamic GRNs yield altered patterns of floral morphology and provide the genetic raw material for the evolution of flower development (e.g., Alvarez-Buylla et al. 2008; Barrio et al. 2013). Such an approach ultimately provides a means to understand how gene interactions themselves can function to restrict the potential combinations of genes that are attainable at particular spatial positions or developmental stages and thus can be used to understand developmental constraints and predict how they might function in developmental evolution (see chapter “Developmental constraints”).

The current challenge for floral evolutionary development is to uncover other regulatory modules involved in all aspects of flower development (i.e., organ symmetry, polarity, fusion, etc.) and propose even more integrative models by linking different modules via intermodule interactions. Also, given that the models are built based on derived model species such as *Arabidopsis*, another great challenge is to understand to what extent are these modules conserved across angiosperm

lineages and how to correlate changes in these modules with morphological diversification. Such studies necessarily require collaborations with computer scientists and mathematicians to model the developmental processes in silico (Mabee 2006). But the advantage of a process-based approach to evo-devo in particular is that these methods can effectively harness the power of natural variation that occurs in a particular lineage as a source of comparative data. Some recent results of such types of collaboration, not only on flower development, but also in *Arabidopsis* root development are starting to appear (e.g., Barrio et al. 2013).

Concluding Remarks

Some (Brigandt and Love 2010) claim that the study of morphological variation, in particular the origin of morphological novelty, can benefit from different disciplinary accounts based on a problem-centered approach, instead of following under the constraints of a single theoretical framework. In this case, the diversity of plant evo-devo approaches aiming at understanding the evolution of floral diversification may, in fact, benefit from a plurality of views. In these lines, we believe that a process-based approach to plant evo-devo rather than compete with current views sheds new light on different ways of looking at the same problem: the evolution of morphological variation in angiosperm flowers.

The challenges of formalization of GRN dynamics are, indeed, daunting. Furthermore, it would require, in most cases, a solid collaboration between biologists and mathematicians, as well as computational biologists. In a large-data prone era, experiencing a wealth of publicly available data, however, will require, more and more, an approximation of biology to math-based sciences. The large amounts of available data, on the other hand, can greatly contribute to the development of GRN models that are experimentally based and will certainly contribute to a more process-based approach to the study of morphological evolution in angiosperms.

Cross-References

► A Macroevolutionary Perspective on Developmental Constraints in Animals
► Developmental Innovation and Phenotypic Novelty
► Modeling Evolution of Developmental Gene Regulatory Networks

Acknowledgments AMRA thanks CAPES (BJT069/2013) and INCT (IN-TREE, 465767/2014-1) for support. CDS acknowledges support from NSF DEB, IOS and AVAToL and animated discussions with K. Sears, P. Mabee, M. Wake, D. Howarth, D. Baum, the Specht Lab (esp. J. Martinez-Gomez, R. Bruenn, C. Tribble, J. Chery) and participants of the NSF funded NESCent workshop (grant number DBI-1249112 to C. Extavour and A. Rodrigo) on the future of evo-devo.

References

Almeida AMR, Yockteng R, Schnable JC, Alvarez-Buylla ER, Freeling M, Specht CD (2014) Co-option of the polarity network shapes filament morphology in the angiosperms. Nature Scientific Reports 4. https://doi.org/10.1038/srep06194

Alvarez-Buylla E, Chaos A, Aldana M, Benítez M, Cortes-Poza Y et al (2008) Floral morphogenesis: stochastic explorations of a gene network epigenetic landscape. PLoS One 3:e3626

Alvarez-Buylla ER, Benítez M, Corvera-Poiré A, Chaos AC, de Folter S, de Buen AG, Garray-Arroyo A, García-Ponce B, Jaimes-Miranda F, Pérez-Ruiz RV, Piñeyro_Nelson A, Sánchez-Corrales YE (2010) Flower development. In: American Society of Plant Biologists (ed) The Arabidopsis book. https://doi.org/10.1199/tab.0127

APG IV, Angiosperm Phylogeny Group (2016) An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG IV. Bot J Linn Soc 181:1–20

Barrio RA, Romero-Arias JR, Noguez MA, Azpeitia E, Ortiz-Gutiérrez E, Hernández-Hernández V, Cortez-Posa Y, Alvarez-Buylla ER (2013) Cell patterns emerge from coupled chemical and physical fields with cell proliferation dynamics: the *Arabidopsis thaliana* root as a study system. PLoS Comput Biol 9:e1003026. https://doi.org/10.1371/journal.pcbi.1003026

Bolker JA (1994) Model systems in developmental biology. BioEssays 17:451–455

Brigandt I, Love AC (2010) Evolutionary novelty and the evo-devo synthesis: field notes. Evol Biol 37:93–99

Coen E, Meyerowitz EM (1991) The war of the whorls: genetic interactions controlling flower development. Nature 353:31–37

Cui L, Wall PK, Leebens-Mack JH et al (2006) Widespread genome duplications through the history of flowering plants. Genome Res 16:738–749

Davilla-Velderrain J, Martínez-García JC, Alvarez-Buylla ER (2015) Modeling the epigenetic attractors landscape: towards a post-genomic mechanistic understanding of development. Front Genet 6:160. https://doi.org/10.3389/fgene.2015.00160.

Davilla-Velderrain J, Servin-Marquez A, Alvarez-Buylla ER (2013) Molecular evolution constraints in the floral organ specification regulatory network module across 18 angiosperm genomes. Mol Biol Evol 31. https://doi.org/10.1093/molbev/mst223

Della Pina S, Souer E, Koes R (2014) Arguments in the evo-devo debate: say it with flowers! J Exp Bot 65:2231–2242

Fukushima K, Hasabe M (2013) Adaxial-abaxial polarity: the developmental basis of leaf shape diversity. Genesis 52:1–18

Gilbert SF, Bolker JA (2001) Homologies of process and modular elements of embryonic construction. J Exp Zool 291:1–12

Husbands AY, Chitwood DH, Plavskin Y, Timmermans MCP (2009) Signals and prepatterns: new insights into organ polarity in plants. Genes Dev 23:1986–1997

Kanno A, Nakada M, Akita Y, Hirai M (2007) Class B gene expression and the modified ABC model in nongrass monocots. Sci World J 7:268–279

Mabee PM (2006) Integrating evolution and development: the need for dioinformatics in evo-devo. Bioscience 56:301–309

Rijkema A, Vandenbussche M, Koes R, Heijmans K, Gerats T (2010) Variations on a theme: changes in the floral ABCs in angiosperms. Semin Cell Dev Biol 21:100–107

Nuño de la Rosa L, Etxeberria A (2011) Pattern and process in evo-devo: descriptions and explanations. In: de Regt H, Hartmann S, Okasha S (eds) . Springer, Amsterdam, pp 263–274

Scholtz G (2008) On comparisons and causes in evolutionary developmental biology. In: Minelli G, Fusco G (eds) Evolving pathways: key themes in evolutionary developmental biology. Cambridge University Press, Cambridge, pp 144–159

Sharma B, Kramer E (2013) Sub- and neo-functionalization of APETALA3 paralogs have contributed to the evolution of novel organ identity in aquilegia (columbine, Rnnunculales). New Phytol 197:949–957

Tickle C, Urrutia AO (2017) Perspectives on the history of evo-devo and the contemporary research landscape in the genomics era. Philosophical Transactions R Sco B 372:20150473

The Impact of Atmospheric Composition on the Evolutionary Development of Stomatal Control and Biochemistry of Photosynthesis over the Past 450 Ma

Matthew Haworth, Giovanni Marino, and Mauro Centritto

Contents

Abstract

The conversion of carbon dioxide (CO_2) and water into glucose and oxygen (O_2) by photosynthesis has been a central component of the atmosphere and climate system over Earth history. The diffusive uptake of CO_2 and its biochemical assimilation have in turn been strongly affected by atmospheric composition. Here, we illustrate how declining [CO_2] and rising [O_2] have exerted selective pressures to reduce the uptake of O_2 (photorespiration) in favor of CO_2 (photosynthesis) by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco). In the last 10 Myr when [CO_2] fell to less than 300 ppm, C3 photosynthesis became less efficient and mechanisms concentrating CO_2 at the rubisco active site were favored leading to the expansion of C4 photosynthesis. The need to optimize carbon gain relative to water-loss has acted as a key selective pressure in the evolutionary development of stomatal function and epidermal patterning, to not only maximize diffusion of CO_2 into the leaf but also regulate excessive transpirative water-loss. This stomatal control of photosynthesis generally allows angiosperms to sustain greater levels of stomatal conductance and CO_2-uptake than species with more ancient evolutionary origins. This is particularly evident in the grasses, where dumb-bell stomata and the allocation of a higher percentage of the epidermis to gas exchange permit greater rates of stomatal conductance and

M. Haworth (✉) · G. Marino · M. Centritto
Tree and Timber Institute, National Research Council (CNR – IVALSA), Florence, Italy
e-mail: haworth@ivalsa.cnr.it; marino@ivalsa.cnr.it; mauro.centritto@cnr.it

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_171

photosynthesis than species with kidney-shaped stomata. The diffusive and biochemical components of photosynthesis have been strongly influenced by declining CO_2:O_2 over the past 100 Myr. However, current rising [CO_2] may affect these selective pressures, having implications for future plant growth.

Keywords

Rubisco · Evolution of stomata · Photorespiration · Diffusive limitations · Atmospheric carbon dioxide · Atmospheric oxygen

Introduction

Photosynthesis utilizes light energy to convert carbon dioxide (CO_2) and water into glucose and oxygen (O_2). This process has shaped the land, atmosphere, and oceans over Earth history. In turn, the evolutionary development of photosynthesis has also been influenced by environmental changes through the selective pressures exerted by the respective rates of carboxylation and oxygenation, alongside the need to take-up, transport, and conserve water. These selective pressures can be encapsulated by the measure "water use efficiency" (WUE) that indicates the amount of CO_2 taken-up relative to water-loss (Fig. 1). Ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) is the central enzyme involved in photosynthesis, determining rates of carboxylation (photosynthesis) and oxygenation (photorespiration) of ribulose-1,5-bisphosphate. Photosynthetic rubisco likely evolved from similar proteins involved in methane formation and sulphur metabolism in anoxic environments 4.0 to 3.0 Ga. The expansion of photosynthetic microbes led to the oxygenation of the Earth's atmosphere 2.4 Ga. The increased abundance of atmospheric oxygen made the environment less favorable to the previously dominant anaerobic microbial organisms, and over time led to the development of the ozone layer, shielding the Earth from high energy UV radiation. The drawdown of atmospheric CO_2 associated with the oxygenation of the atmosphere may also have induced a global glaciation (early Proterozoic Snowball Earth 2.4 Ga); possibly marking the first occurrence whereby photosynthesis significantly influenced the Earth's climate through its effects on atmospheric composition (Nisbet and Nisbet 2008). However, such alterations in atmospheric composition and temperature would also influence photosynthesis via the lower availability of CO_2, greater abundance of oxygen, and effects on enzyme activity. The action of photosynthetic organisms over Earth history has been proposed to have led to the development of comparative stability in the levels of atmospheric [CO_2] and [O_2] that mirror the kinetics of rubisco (Tolbert et al. 1995; Tcherkez et al. 2006). It is the selective pressures exerted by this interaction between photosynthetic processes and the environment that we hope to briefly outline in this chapter on the evolutionary development of the diffusive characteristics and biochemistry of photosynthesis. We will focus on the evolutionary development of photosynthesis and its accompanying metabolic and regulatory processes in vascular plants over the past 450 million years, as experiments with

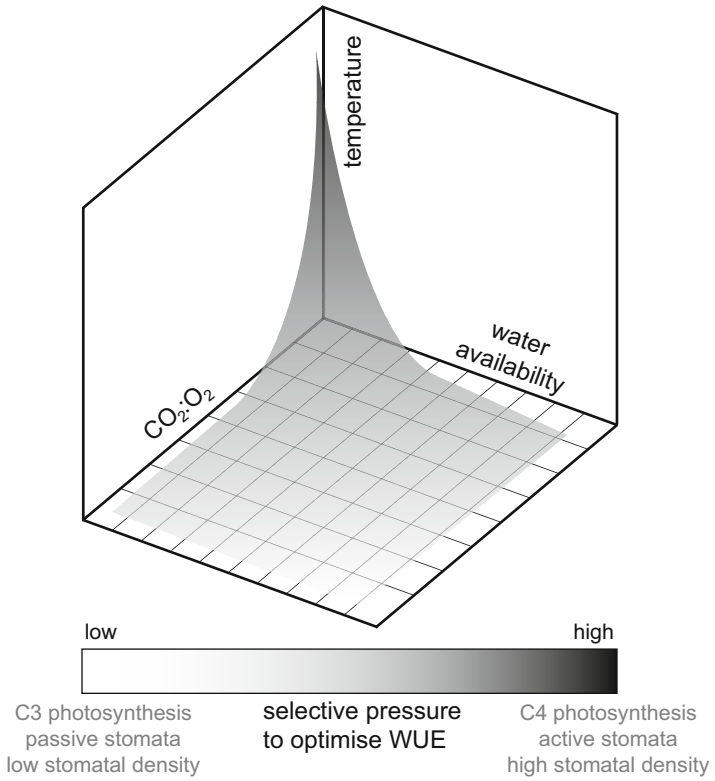


Fig. 1 An illustration of the impact of mean temperature during the growing season, water availability, and the atmospheric CO_2:O_2 ratio on selective pressures to optimize water use efficiency (*WUE*). High temperatures reduce the specificity of rubisco, decrease the activity of rubico activase, and increase the solubility of O_2 in comparison to CO_2. Low water availability restricts potential for stomatal conductance and CO_2 uptake. Low atmospheric CO_2:O_2 ratios favor photorespiration over photosynthesis

living plants with ancient evolutionary histories have allowed an understanding of the selective pressures that have shaped present day vegetation. Moreover, it is these same selective pressures that will determine the response of terrestrial vegetation to current climatic and atmospheric changes.

Rubisco Specificity and Levels of Atmospheric CO_2 and O_2

Photosynthetic rubisco does not have identical affinities for both CO_2 and O_2. Rubisco will preferentially select CO_2 over O_2; this is known as the specificity of rubisco. However, there is vastly more O_2 in the atmosphere than CO_2, with O_2 measured as a percentage while CO_2 is determined on a parts-per-million (ppm: i.e., μmol $mol^{-}1$) basis. This disparity in the concentrations of [CO_2] and [O_2] in

the atmosphere has acted as a selective pressure on the evolution of plants throughout the past 450 Ma exerted via the competing processes of photosynthesis and photorespiration. As the CO_2:O_2 ratio rises, the proportion of photosynthesis to photorespiration increases in C3 plants (Fig. 2). Analysis of rubisco specificity in phylogenetically diverse plants suggests that the atmospheric CO_2:O_2 ratio over Earth history (Fig. 3a) has affected the characteristics of rubisco. Those species originating during periods of comparatively lower CO_2:O_2 tend toward higher specificity for CO_2, but lower maximum rates of carboxylation. This lower carboxylation capacity is generally compensated by possession of higher concentrations of rubisco (Galmes et al. 2014). However, rubisco is a comparatively expensive compound for plants as it requires the investment of nitrogen (the availability of nitrogen frequently limits plant growth in many terrestrial habitats), and rubisco often accounts for more than 25% of leaf nitrogen. At [CO_2] levels below 200 ppm, characteristic of atmospheric [CO_2] during glacial episodes, the rates of photorespiration begin to approximate those of photosynthesis (Tolbert et al. 1995). The increase in photorespiration and the decline in photosynthesis at low [CO_2] make C3 photosynthesis less economic (Taylor et al. 2014). This exerted selective pressures favoring adaptations to reduce photorespiration that led to the origination in the Eocene (or possibly earlier) and expansion 5–7 Ma, of C4 photosynthesis (Sage et al. 2012). C4 photosynthesis reduces photorespiration by effectively concentrating CO_2 within the bundle sheath at the active site of rubisco (Fig. 2). This mechanism is also favored at higher temperatures where the specificity of rubisco for CO_2 declines and

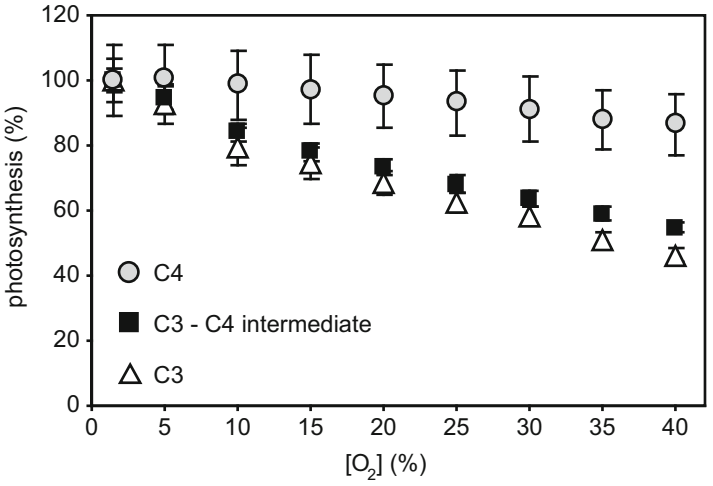


Fig. 2 The effect of increasing atmospheric concentration of oxygen on the proportion of photosynthesis relative to photorespiration of plant species with C3 photosynthesis (*Rosoideae rosa* hybrid tea rose; *triangle white fill*), C3–C4 intermediate photosynthesis (*Moricandia arvensis*; *square black fill*) and C4 photosynthesis (*Zea mays*; *circle grey fill*). Photosynthesis is expressed as a percentage of its value at 1.5% [O_2] where levels of photorespiration are assumed to be negligible. Error bars indicate one standard error either side of the mean. The concentration of [CO_2] remained constant at 400 ppm at all [O_2] levels

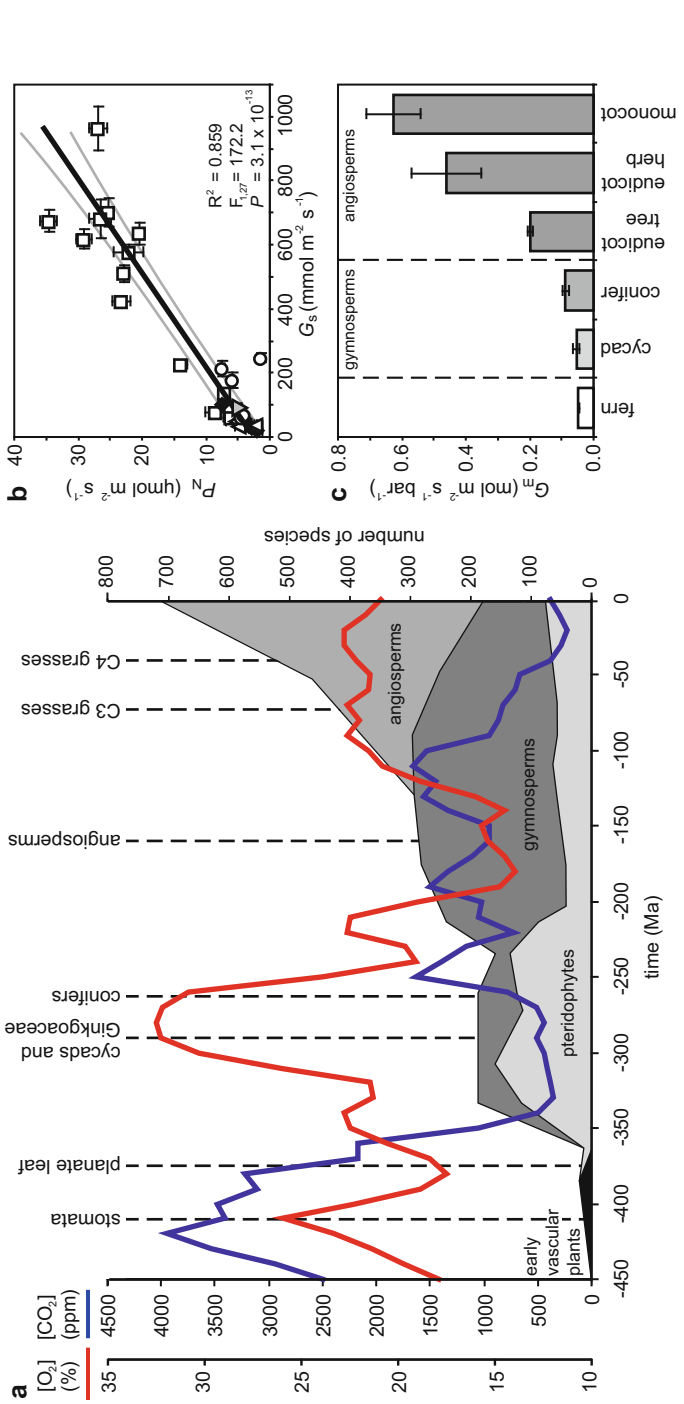


Fig. 3 (**a**) Levels of atmospheric $[CO_2]$ (*blue line*) and $[O_2]$ (*red line*) (from Berner 2009) and the diversification of major plant groups (Niklas et al. 1983) over the past 450 Ma. The origination of plant groups and/or occurrence of morphological/physiological adaptations are marked by *dashed vertical lines*. (**b**) The relationship between photosynthesis (P_N) and stomatal conductance (G_s) of vascular plants under present atmospheric conditions (ferns = *white circle*; cycads = *white triangle*; *Ginkgo biloba* = *inverted grey triangle*; conifers = *black diamonds*; angiosperms = *white squares*). The *black line* indicates best fit and the two *grey lines* either side indicate 95% confidence intervals of the mean. (**c**) Mesophyll conductance to CO_2 (G_m) of a fern (*Cyrtomium fortunei*), cycad (*Lepidozamia perroffskyana*), conifer (*Agathis australis*), eudicot tree (*Olea europea*), eudicot herb (*Gossypium hirsutum*), and monocot (*Avena sativa*). All error bars indicate standard error

the solubility of O_2 relative to CO_2 increases (Fig. 1) (Crafts-Brandner and Salvucci 2002). The transition from C3 to C4 is complex, involving a large number of genetic mutations to produce the morphological (Kranz anatomy) and physiological (principally the enzyme phosphoenolpyruvate carboxylase as a CO_2 acceptor and nicotinamide adenine dinucleotide phosphate-malic enzyme) adaptations responsible for C4 photosynthesis (Sage et al. 2012). However, this transition likely occurred in stages of mutations that offered incremental selective advantages. This has resulted in species with photosynthesis bearing characteristics of both C3 and C4 physiologies, the so-called C3–C4 intermediates (also known as "C2" plants). These C3–C4 intermediates do not minimize photorespiration to an extent comparable to C4 plants, but rather recapture for photosynthesis a higher percentage of the CO_2 released via photorespiration (the development of a so-called photorespiratory bypass) (Fig. 2). The transition from C3 to C4 has occurred more than 60 times in different lineages of plants (Sage et al. 2012; Osborne and Beerling 2006), suggesting that the selective pressures of low $[CO_2]$/high $[O_2]$ are highly influential, and that many C3 plants possess the genetic capability to "switch on" C4 attributes – this has formed the basis for numerous projects to improve the WUE of C3 staple crops by modifying their physiologies toward C4 photosynthesis (Gowik and Westhoff 2011). Plants still require a degree of photorespiration, as it is an important component of carbon metabolism and plays a protective role against abiotic stresses such as excess light energy. Mutant plants without the capacity for photorespiration are unable to tolerate abiotic stress and have low levels of survival. Nonetheless, the reduction of photorespiratory losses of carbon would imply that C4 plants should experience a selective advantage under the present atmospheric conditions of comparatively low $[CO_2]$/high $[O_2]$ in the context of much of the preceding 450 Myr. However, C4 photosynthesis is energetically more expensive than C3, requiring two thirds more adenosine triphosphate to fix each molecule of CO_2. Indeed, the majority of C4 plants occur in warm to hot regions where water is limited and temperatures unfavorable to C3 photosynthesis (some C4 plants also occur in warm humid nutrient poor environments where C4 photosynthesis ameliorates the impact of low nutrient availability on CO_2-uptake), illustrating the costs and benefits associated with both photosynthetic physiologies (Fig. 1).

Diffusive Resistances to CO_2-Uptake and Stomatal Control of Photosynthesis

As the availability of CO_2 for photosynthesis declines, a frequent response of plants is to promote diffusion of CO_2 into the leaf by opening stomata and increasing stomatal conductance (G_s). Stomata are the pores covering the leaf that contribute to the maintenance of leaf homeostasis by regulating the uptake of CO_2 for photosynthesis against the loss of water via transpiration. A stomatal complex consists of two guard cells surrounding a stomatal pore through which CO_2 is taken-up for photosynthesis and water lost via transpiration. Turgor changes in the guard cell regulate the size of the stomatal aperture. The guard cells may be surrounded by

specialized epidermal cells known as subsidiary cells. The actions of stomata are closely coordinated with the availability of CO_2 and rates of photosynthesis in the mesophyll driven by the carboxylation activity of rubisco outlined above (Engineer et al. 2016). Stomata first occur in the fossil record more than 400 Ma and played a central role in the colonization of the land by early plants. These early stomata are largely identical to "kidney-shaped" stomata found in most dicots, suggesting that their structure and function (particularly in preventing desiccation) have remained largely unchanged (Edwards et al. 1998). However, the earliest stomata-like structures were probably not involved in gas exchange, but the distribution of spores by allowing spore-bearing tissues to dry (Duckett et al. 2009). The genes encoding proteins responsible for the development of stomata in spore-bearing tissues of the moss Physcomitrella patens are orthologs of the genes responsible for stomatal development in the model angiosperm *Arabidopsis thaliana* (Chater et al. 2016). The evolutionary exaptation of stomata for gas exchange allowed early terrestrial plants to take-up CO_2 for photosynthesis at a faster rate. As [CO_2] fell during the late Devonian, the number of stomata on the leaf surfaces of Devonian plants increased. The higher rates of transpirative cooling associated with this increased G_s are proposed to have facilitated the evolutionary development of the planate leaf by reducing thermal stress; larger leaves intercept more light energy resulting in increased temperature, and also lose less heat energy via transpiration due to the presence of a more developed "boundary layer" of still air over the leaf surface. This increase in leaf area served as a selective advantage, enabling plants to capture greater amounts of light for photosynthetic carbon gain (Beerling et al. 2001).

The origination of many groups of plants or major physiological/morphological adaptations has coincided with intervals of low or falling [CO_2] and/or high [O_2] (Fig. 3a). This may have favored those species with greater stomatal control that are able to maximize G_s and potential diffusive uptake of CO_2, but also capable of restricting water-loss under unfavorable growth conditions. When measured under current ambient [CO_2] of 400 ppm, the rates of photosynthesis are closely linked to G_s in phylogenetically diverse plants (Fig. 3b). Generally, more recently derived angiosperms exhibit higher photosynthesis and G_s than more ancient groups (ferns, cycads, Ginkgoales, conifers) that originated in atmospheres of higher CO_2:O_2. Moreover, analysis of the conductance of CO_2 across the mesophyll layer from the substomatal air-space to the chloroplast (termed "mesophyll conductance": G_m) would suggest such an evolutionary trajectory is replicated, with angiosperms possessing greater capacity for the uptake of CO_2 (Fig. 3c). However, more extensive screening of G_m in different plant phylogenies is necessary before any firm conclusions may be drawn regarding evolutionary development of CO_2 transport across the mesophyll. Nonetheless, the greater potential for gas exchange observed in the angiosperms would only constitute a selective advantage if accompanied by more effective stomatal control to regulate water-loss when photosynthesis is limited and prevent desiccation during drought or high evapotranspirative demand (Haworth et al. 2011). Stomatal control is the speed of stomatal aperture adjustment to a change in conditions (e.g., light, [CO_2], water availability) and the tightness of stomatal closure when photosynthesis is not taking place (i.e., at night or under severe

drought). There are two main modes of stomatal behavior: passive and active. The guard cells of passive stomata follow changes in leaf water potential. As such, passive stomatal behavior is generally slower when responding to an external stimulus. In contrast, the active pumping of osmolytes across the plasma membrane of guard cells allows species with active stomatal behavior to regulate G_s more rapidly. This has led to a hypothesis proposing an evolutionary trajectory from passive to active stomatal behavior, with the stomata of angiosperms exhibiting increased sensitivity to light, super-ambient [CO_2], leaf-to-air vapor pressure deficit (a measure of evapotranspirative demand placed on the leaf), and the stress hormone abscisic acid (McAdam and Brodribb 2012). However, genetic analysis of mosses and lycophytes (Chater et al. 2013) and gas exchange analyzes of G_s in phylogenetically diverse plants (Hasper et al. 2017) indicate that active stomatal behavior originated in early plant lineages. Nevertheless, the higher potential rates of G_s and photosynthesis observed in angiosperms would indeed constitute a selective advantage in a "low [CO_2]" world.

Evolutionary developments in epidermal patterning may account for the higher rates of G_s observed in angiosperms via an increase in the proportion of the cuticle allocated as stomatal complexes and therefore potentially available for gas exchange (de Boer et al. 2016). Larger numbers of smaller stomata are thought to be more efficient in achieving high G_s but also offering greater stomatal control, as smaller stomata are considered to close more rapidly (Raven 2014). This may account for observations of generally low densities of large stomata in groups such as the ferns and cycads in comparison to higher densities of small stomata in angiosperms (Franks and Beerling 2009). This epidermal patterning in angiosperms may also allow for more complex vein architecture within the leaf to enable more efficient transport of water (Roth-Nebelsick et al. 2001). While a eudicot (angiosperm) with a high density of small kidney-shaped stomata sustains higher G_s than a cycad, when the rates of stomatal closure are normalized they are broadly consistent. This may indicate that the individual kidney-shaped stomata of an evolutionarily ancient cycad are behaving in a manner similar to those of the more recently derived angiosperm (Fig. 4). However, while the majority of species possess kidney-shaped stomata, the stomatal complexes of grasses are morphologically different, termed "dumb-bell" stomata. Dumb-bell stomata are mechanically different to kidney-shaped stomata in their opening/closing mechanism. As dumb-bell stomata are larger, to fully open they rely not only on an increase in the turgor of guard cells, but also a greater loss in turgor pressure in the adjacent subsidiary cells to accommodate full stomatal opening. This permits larger and more rapid stomatal movements in grasses (Fig. 4) (Franks and Farquhar 2007) and may have contributed to the expansion of grasses over the past 20 Myr as atmospheric [CO_2] has declined (Robinson 1994).

These stomatal modifications are important not only in terms of optimizing carbon gain relative to water-loss, but also in the protection of water transport systems. Under conditions of low water availability and high evapotranspirative demand plants may experience xylem embolism, where air enters the xylem causing a loss of water movement. Such xylem embolisms may be fatal to plants in a low [CO_2] world (particularly at high altitude where the partial pressure of CO_2 is further

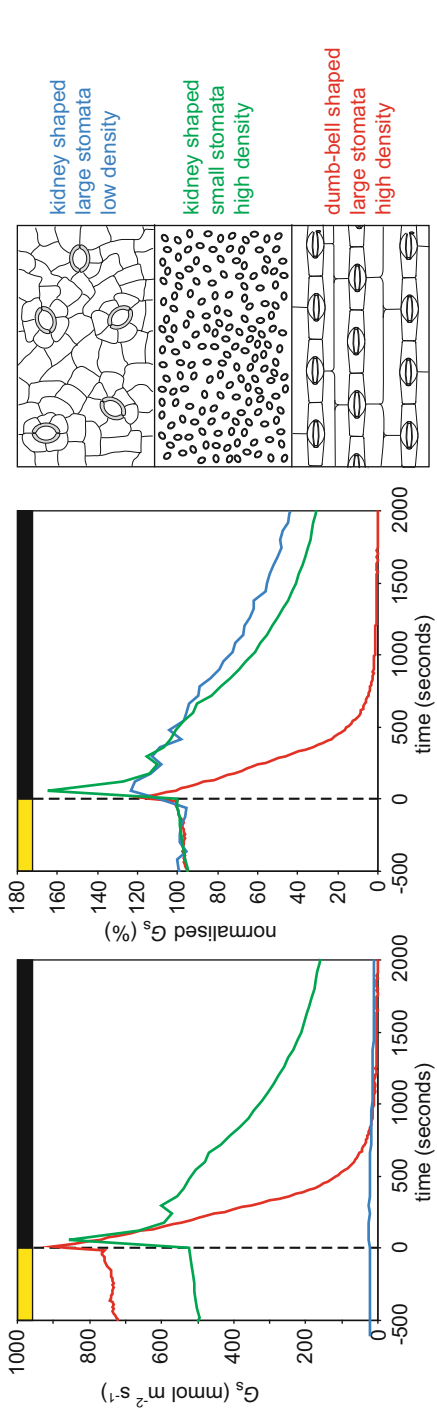


Fig. 4 Absolute and normalized rates of stomatal closure to darkness (indicated by a *vertical dashed line* and change from *yellow* to *black* in the *upper horizontal bar*) of a cycad (*Cycas siamensis* – *blue line*) and angiosperm (*Chenopodium quinoa* – *green line*) with kidney-shaped stomata and an angiosperm monocot (*Arundo donax* – *red line*) with dumb-bell stomatal complexes. Panels on the right provide an illustration of stomatal morphology and stomatal patterning

reduced) where high G_s is required for sufficient uptake of CO_2 for photosynthesis. Indeed, the shift from xylem tracheids to vessels with higher conductivity and/or resistance to embolism may be related to increased stomatal control allowing prevention of high levels of transpiration and development of excessive negative pressures within the xylem (Meinzer et al. 2009; Sperry et al. 2006).

Conclusion

Over the past 100 Ma, atmospheric composition has been characterized by declining [CO_2] toward the point during quaternary glaciations where the carboxylation and oxygenation rates of rubisco were broadly similar. The selective pressures resulting from this low [CO_2] have induced the evolutionary development of rubisco with increased specificity for CO_2 in C3 plants, C4 photosynthesis to concentrate CO_2 at the active site of rubisco, more efficient water transport systems, enhanced stomatal control, and modified epidermal patterning to allocate a greater proportion of the leaf surface to gas exchange. These changes have likely contributed to the decline of more ancient plant groups and the expansion and diversification of the angiosperms (Fig. 3a). However, atmospheric [CO_2] has risen at a greater rate over the past 250 years than at any time in the past 65 Myr (with the possible exception of the Paleocene-Eocene Thermal Maximum 55.8 Ma). This has the potential to alter the strength of the selective pressures induced by atmospheric composition that have driven modifications in the biochemistry of photosynthesis, diffusive limitations to CO_2 uptake, and stomatal control of photosynthesis over the past 65 Myr. A greater understanding of the genetic regulation of photosynthesis, and its integration with supporting gas exchange and water transport systems, will advance our understanding of not only the evolutionary development of photosynthesis in major plant groups, but the likely effects of future rising [CO_2] on agriculture and natural vegetation.

Acknowledgments We are grateful to funding from the EU FP7 project 3 to 4 (289582).

References

Beerling DJ, Osborne CP, Chaloner WG (2001) Evolution of leaf-form in land plants linked to atmospheric CO_2 decline in the late Palaeozoic era. Nature 410(6826):352–354

Berner RA (2009) Phanerozoic atmospheric oxygen: new results using the Geocarbsulf model. Am J Sci 309(7):603–606. https://doi.org/10.2475/07.2009.03

de Boer HJ, Price CA, Wagner-Cremer F, Dekker SC, Franks PJ, Veneklaas EJ (2016) Optimal allocation of leaf epidermal area for gas exchange. New Phytol 210(4):1219–1228. https://doi.org/10.1111/nph.13929

Chater C, Gray JE, Beerling DJ (2013) Early evolutionary acquisition of stomatal control and development gene signalling networks. Curr Opin Plant Biol 16(5):638–646. https://doi.org/10.1016/j.pbi.2013.06.013

Chater CC, Caine RS, Tomek M, Wallace S, Kamisugi Y, Cuming AC, Lang D, MacAlister CA, Casson S, Bergmann DC (2016) Origin and function of stomata in the moss *Physcomitrella patens*. Nat Plant 2:16179

Crafts-Brandner SJ, Salvucci ME (2002) Sensitivity of photosynthesis in a C4 plant, maize, to heat stress. Plant Physiol 129(4):1773–1780. https://doi.org/10.1104/pp.002170

Duckett JG, Pressel S, P'ng KM, Renzaglia KS (2009) Exploding a myth: the capsule dehiscence mechanism and the function of pseudostomata in sphagnum. New Phytol 183(4):1053–1063. https://doi.org/10.1111/j.1469-8137.2009.02905.x

Edwards D, Kerp H, Hass H (1998) Stomata in early land plants: an anatomical and ecophysiological approach. J Exp Bot 49:255–278

Engineer CB, Hashimoto-Sugimoto M, Negi J, Israelsson-Nordström M, Azoulay-Shemer T, Rappel W-J, Iba K, Schroeder JI (2016) CO_2 sensing and CO_2 regulation of stomatal conductance: advances and open questions. Trends Plant Sci 21(1):16–30. https://doi.org/10.1016/j.tplants.2015.08.014

Franks PJ, Beerling DJ (2009) Maximum leaf conductance driven by CO_2 effects on stomatal size and density over geologic time. Proc Natl Acad Sci U S A 106(25):10343–10347. https://doi.org/10.1073/pnas.0904209106

Franks PJ, Farquhar GD (2007) The mechanical diversity of stomata and its significance in gas-exchange control. Plant Physiol 143(1):78–87. https://doi.org/10.1104/pp.106.089367

Galmes J, Kapralov MV, Andralojc P, Conesa MÀ, Keys AJ, Parry MA, Flexas J (2014) Expanding knowledge of the Rubisco kinetics variability in plant species: environmental and evolutionary trends. Plant Cell Environ 37(9):1989–2001

Gowik U, Westhoff P (2011) The path from C3 to C4 photosynthesis. Plant Physiol 155(1):56–63

Hasper TB, Dusenge ME, Breuer F, Uwizeye FK, Wallin G, Uddling J (2017) Stomatal CO_2 responsiveness and photosynthetic capacity of tropical woody species in relation to taxonomy and functional traits. Oecologia 184(1):43–57

Haworth M, Elliott-Kingston C, McElwain JC (2011) Stomatal control as a driver of plant evolution. J Exp Bot 62(8):2419–2423. https://doi.org/10.1093/jxb/err086

McAdam SAM, Brodribb TJ (2012) Stomatal innovation and the rise of seed plants. Ecol Lett 15(1):1–8. https://doi.org/10.1111/j.1461-0248.2011.01700.x

Meinzer FC, Johnson DM, Lachenbruch B, McCulloh KA, Woodruff DR (2009) Xylem hydraulic safety margins in woody plants: coordination of stomatal control of xylem tension with hydraulic capacitance. Funct Ecol 23(5):922–930. https://doi.org/10.1111/j.1365-2435.2009.01577.x

Niklas KJ, Tiffney BH, Knoll AH (1983) Patterns in vascular land plant diversification. Nature 303(5918):614–616

Nisbet EG, Nisbet RER (2008) Methane, oxygen, photosynthesis, rubisco and the regulation of the air through time. Philos Trans R Soc B 363(1504):2745–2754

Osborne CP, Beerling DJ (2006) Nature's green revolution: the remarkable evolutionary rise of C4 plants. Philos Trans R Soc B 361(1465):173–194

Raven JA (2014) Speedy small stomata? J Exp Bot 65(6):1415–1424. https://doi.org/10.1093/jxb/eru032

Robinson JM (1994) Speculations on carbon dioxide starvation, late tertiary evolution of stomatal regulation and floristic modernization. Plant Cell Environ 17(4):345–354

Roth-Nebelsick A, Uhl D, Mosbrugger V, Kerp H (2001) Evolution and function of leaf venation architecture: a review. Ann Bot 87(5):553–566

Sage RF, Sage TL, Kocacinar F (2012) Photorespiration and the evolution of C4 photosynthesis. Annu Rev Plant Biol 63:19–47

Sperry JS, Hacke UG, Pittermann J (2006) Size and function in conifer tracheids and angiosperm vessels. Am J Bot 93(10):1490–1500. https://doi.org/10.3732/ajb.93.10.1490

Taylor SH, Ripley BS, Martin T, De-Wet LA, Woodward FI, Osborne CP (2014) Physiological advantages of C4 grasses in the field: a comparative experiment demonstrating the importance of drought. Glob Chang Biol 20(6):1992–2003

Tcherkez GG, Farquhar GD, Andrews TJ (2006) Despite slow catalysis and confused substrate specificity, all ribulose bisphosphate carboxylases may be nearly perfectly optimized. Proc Natl Acad Sci 103(19):7246–7251

Tolbert NE, Benker C, Beck E (1995) The oxygen and carbon dioxide compensation points of C3 plants: possible role in regulating atmospheric oxygen. Proc Natl Acad Sci USA 92:11230–11233

Alternation of Generations in Plants and Algae

Simon Bourdareau, Laure Mignerot, Svenja Heesch, Akira F. Peters, Susana M. Coelho, and J. Mark Cock

Contents

Abstract

Photosynthetic organisms are found in most of the branches of the eukaryotic tree of life, and these organisms have diverse life cycles. There has been a tendency toward dominance of the diploid phase of the life cycle in the land plant lineage, and recent analyses suggest a similar trend in the brown algae. A number of hypotheses have been proposed to explain the evolutionary stability of different types of life cycle, and in some cases these hypotheses are supported by empirical studies. Molecular analyses are elucidating the regulatory molecules that control life cycle progression and are providing insights into the developmental pathways associated with the construction of each generation of the life cycle.

S. Bourdareau · L. Mignerot · S. Heesch · S. M. Coelho · J. M. Cock (✉)
UMR 8227 Integrative Biology of Marine Models, Algal Genetics Group, Station Biologique de Roscoff, CS 90074, Sorbonne Universités, UPMC University Paris 06, CNRS, Roscoff, France
e-mail: simon.bourdareau@sb-roscoff.fr; laure.mignerot@sb-roscoff.fr; svenja.heesch@sb-roscoff.fr; coelho@sb-roscoff.fr; cock@sb-roscoff.fr

A. F. Peters
Bezhin Rosko, Santec, France
e-mail: akirapeters@gmail.com

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_58

Keywords

Diploid · Epigenetic · Gametophyte · Haploid · Sporophyte

Introduction

The term "algae" groups together photosynthetic organisms from a broad range of lineages, with representatives in almost all the supergroups of the eukaryotic tree of life (Fig. 1, see the glossary for definitions of the terms used). From a strict, taxonomic point of view, "plants" correspond to the kingdom Plantae (equivalent to the modern group Archaeplastida; Fig. 1), but this term is often used loosely to include any macroscopic photosynthetic organism, particularly those in terrestrial habitats. In any event, plants are therefore a subset of the algae.

While it is preferable to use more taxonomically precise names when discussing phylogeny, the terms plants and algae are nonetheless extremely useful because they

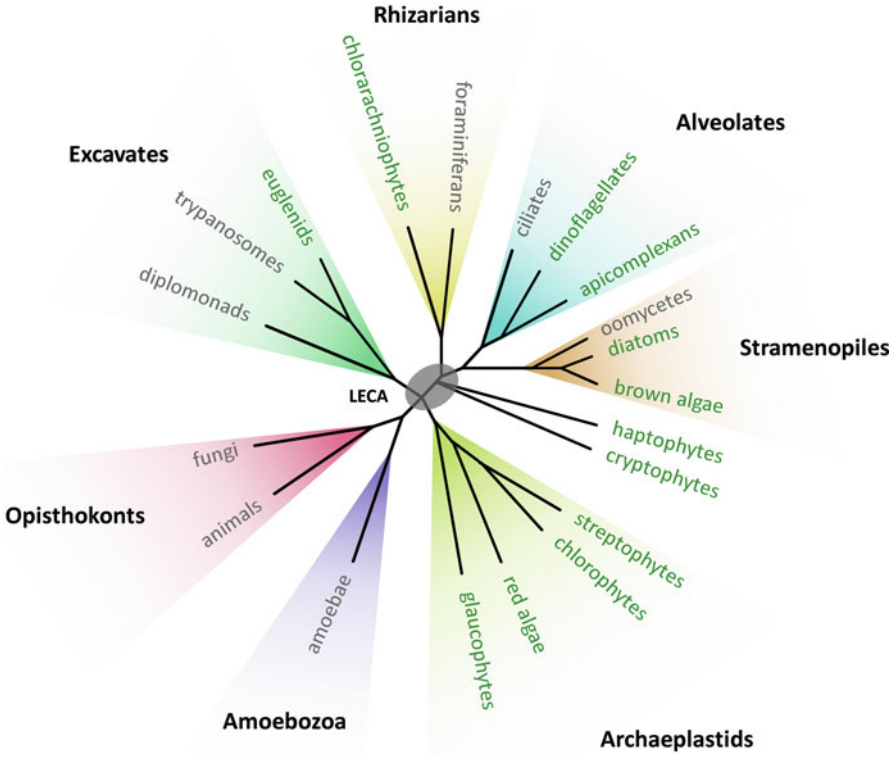


Fig. 1 Schematic tree of the eukaryotes showing the positions of algal groups. Green lettering indicates groups that include photosynthetic organisms (algae). *LECA* last eukaryotic common ancestor

group together organisms that share many common biological features that stem from their autotrophic lifestyles based on photosynthesis. The broad taxonomic distribution of these organisms can be traced back to the various mechanisms whereby they have acquired the ability to carry out photosynthesis. For algae in the archaeplastid group (which includes green algae, red algae and glaucophytes; Fig. 1), photosynthetic capacity arose due to a primary endosymbiotic event, which involved the engulfment of a cyanobacterium by a common ancestral eukaryotic cell. The enslaved cyanobacterium became the plastid.

The other eukaryotic lineages acquired photosynthesis by more complex secondary (and perhaps even tertiary) endosymbiotic events, in which a photosynthetic eukaryote (usually a red or green alga) was enslaved by another eukaryotic cell. It is this process of secondary endosymbiosis that has led to the occurrence of photosynthetic organisms in such a diverse array of eukaryotic supergroups (stramenopiles, alveolates, rhizarians, haptophytes, cryptophytes, and excavates). Given the complicated evolutionary history of plants and algae, it is not surprising that they exhibit a high level of diversity with regard to many characters, including their life cycles, the feature that will be discussed in this chapter.

The basic eukaryote sexual life cycle involves an alternation between two key processes: meiosis, which allows the chromosome number to be reduced by half, and syngamy or gamete fusion, which restores the level of ploidy by bringing together the chromosomes of the fusing gametes in a single nucleus within the zygote (John 1994). Before meiosis the cells are diploid, after meiosis they are haploid, and syngamy restores the diploid state. Variations on this basic life cycle can be defined based on the relative importance of these two phases, i.e., whether the organism grows (undergoes mitotic cell divisions) during the haploid or the diploid phase, or both (Fig. 2). When growth occurs during the diploid phase, the life cycle is called a diploid life cycle (the human life cycle is one example). When growth occurs during the haploid phase, the life cycle is called a haploid life cycle (e.g., that of the green microalga *Chlamydomonas*). Finally, in some organisms, growth occurs during both the haploid and diploid phases. These organisms are said to have haploid-diploid life cycles. Examples include angiosperms, where the macroscopic plant is the diploid phase and microscopic pollen grains and embryo sacs constitute the haploid phase.

The above paragraph applies to both unicellular and multicellular organisms. For the latter, mitosis serves not only to increase cell number (asexual reproduction) but is also the process that underlies construction of the multicellular body plan (development). Multicellular organisms with haploid-diploid life cycles have two multicellular generations. For plants and algae, these two generations are called the sporophyte and the gametophyte, i.e., the spore-producing "plant" (where meiosis occurs to produce spores) and the gamete-producing "plant" (i.e., which generates the gametes), respectively. For these organisms, the alteration of generations referred to in the title of this chapter is the repeated cycle of sporophyte and gametophyte generations produced as a haploid-diploid life cycle progresses.

This chapter will summarize current knowledge about the evolutionary origins and evolutionary trajectories of plant and algal life cycles. We will provide an overview of the different types of life cycle in the major algal groups, look at

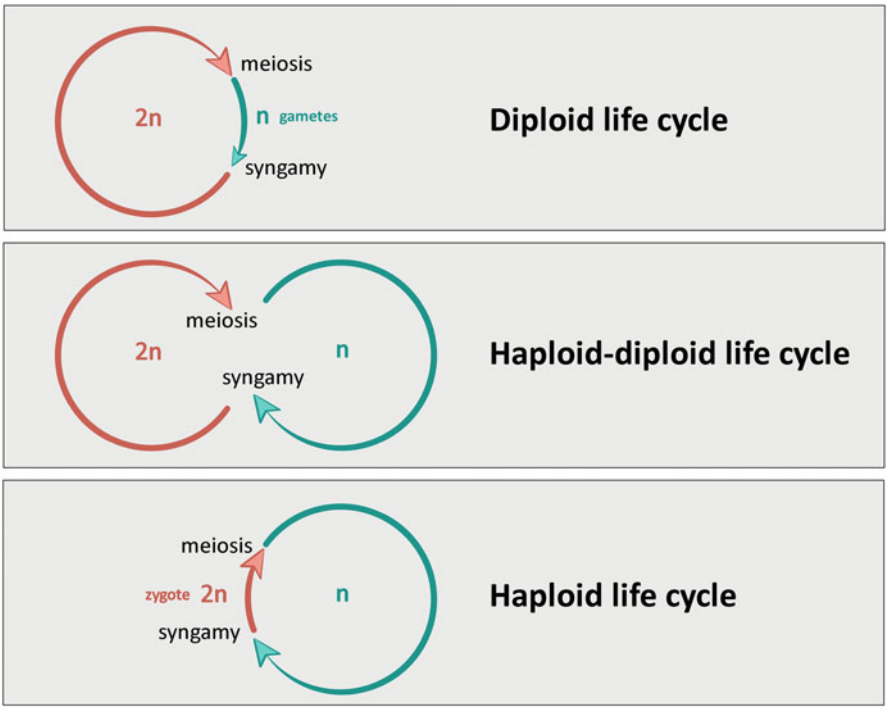


Fig. 2 Main types of sexual life cycle found in eukaryotes. Differences between eukaryotic sexual life cycles depend principally on two key events, meiosis, and gamete fusion (syngamy). The relative positioning of these events determine if the organism spends the majority of its time in the diploid phase (i.e., has a diploid life cycle) or in the haploid phase (i.e., has a haploid life cycle). Many organisms have intermediate life cycles with two generations, one that is haploid and the other diploid (haploid-diploid life cycles)

evolutionary trends within each group and will attempt to relate these trends to theoretical predictions. We will also describe recent advances in understanding how life cycles are controlled at the genetic and epigenetic levels.

The Diversity of Plant and Algal Life Cycles

The phylogenetic group that includes the green algae and terrestrial plants, the Viridiplantae, consists of two main taxa, the chlorophytes and the streptophytes (Leliaert et al. 2012; Fig. 1). Most chlorophytes have haploid life cycles (e.g., the unicellular alga *Chlamydomonas*), but some taxa with multicellular members exhibit haploid-diploid life cycles. The haploid-diploid life cycles of multicellular chlorophyte algae can either involve an alternation between morphologically similar generations (i.e., isomorphic life cycles, e.g., *Ulva*) or the two generations can be morphologically dissimilar (i.e., heteromorphic life cycles). Clear evolutionary

trends are difficult to discern within this group because individual sub-taxa can exhibit highly diverse morphologies and because there is still some uncertainty about the phylogenetic relationships between groups within the chlorophytes. In contrast, within the streptophytes, there has been a clear general trend toward increased multicellular complexity and dominance of the diploid phase of the life cycle. The common ancestor of the streptophytes was probably a unicellular organism with a haploid life cycle. The diversification of the charophytes saw an increase in the complexity of the haploid phase with the emergence of a complex, multicellular haploid generation. The shift from haploid-dominated to diploid-dominated life cycles began when the embryophytes emerged from within the charophytes, with the acquisition of a multicellular diploid generation that tended to increase in complexity as new taxa emerged through evolutionary time.

The red algae include both unicellular and multicellular species, but sexual cycles have not been described for the unicellular species. With the exception of some filamentous species, most multicellular red algae belong to one of the two most recently evolved classes within the red algae, the Bangiophyceae or the Florideophyceae. All of the species in these two classes have haploid-diploid life cycles, although the detailed structure of the life cycle can be quite complicated. The edible seaweed *Pyropia yezoensis* (formerly *Porphyra yezoensis*), a member of the Bangiophyceae, alternates between a leaflike gametophyte and a microscopic, filamentous sporophyte generation. The majority of florideophytes have complex "triphasic" life cycles, with what can be considered to be two sporophyte generations. Gamete fusion occurs on the female gametophyte, and the zygote grows to form the first sporophyte generation (the cystocarp), a small "organism" that grows parasitically on the gametophyte. The cystocarp releases spores that develop into the second, free-living sporophyte generation (the tetrasporophyte), on which meiosis occurs when mature. Such triphasic life cycles can be considered to be variants on the standard haploid-diploid life cycle; the two sporophyte stages serve to multiply this generation of the life cycle.

The brown algae (Phaeophyceae) have diverse life cycles ranging from haploid-diploid life cycles (with various levels of dominance of the haploid and diploid phases) to simple diploid life cycles (Bell 1997; Cock et al. 2013). Basal brown algal lineages all have haploid-diploid life cycles, suggesting that the last common ancestor of the brown algae also had a life cycle of this type (Silberfeld et al. 2010). The most developmentally complex brown algae are found in recently evolved orders such as the Laminariales (kelps) and the Fucales, and there is marked tendency within these orders for the diploid phase to be the dominant phase of the life cycle. For example, the large thalli of kelps, which can attain up to 50 meters in length in some species, correspond to the sporophyte generation while kelp gametophytes are microscopic, filamentous organisms. On the other hand, the Ectocarpales, which are the sister order to the kelps, tend to be less developmentally complex and exhibit diverse haploid-diploid life cycles that include both haploid- and diploid-dominant cycles (i.e., cycles with two generations but with one generation larger than the other). The Fucales, which originated about 52–80 Mya (Kawai et al. 2015) and have relatively large, complex thalli, have diploid life cycles. Hence,

although perhaps not as strongly marked as in the archaeplastid lineage, there appears to be a tendency within the brown algae for diploid-dominant life cycles to have been associated with the emergence of developmental complexity.

With the recent availability of well-supported phylogenies for the brown algae (Silberfeld et al. 2010; Kawai et al. 2015), it has become clear that there has been considerable switching between life cycle types over the course of the emergence of this lineage (Cock et al. 2013). The brown algae therefore potentially represent an interesting group in which to correlate life cycle structure with other parameters such as environmental and ecological context.

Algae in other eukaryotic supergroups also exhibit various types of life cycle, with, for example, most dinoflagellates having haploid life cycles and the occurrence of haploid-diploid life cycles in the chlorarachniophytes, but the sexual life cycles of many of these algae are unknown.

A Relationship Between Life Cycle Type and Degree of Multicellular Complexity

If we focus on the two most developmentally complex eukaryotic lineages, the land plants and the animals, there appears to be a strong correlation between dominance of the diploid phase of the life cycle and the emergence of developmental complexity. As mentioned above, the emergence of land plants corresponded to a gradual reduction in the importance of the gametophyte generation and an increase in the relative importance of the sporophyte. In animals, dominance of the diploid phase was established very early, with the vast majority of these organisms having diploid life cycles. There is also some evidence for a similar correlation in brown algae (the third most complex group of multicellular organisms), with recently evolved, developmentally complex taxa showing a tendency toward diploid-dominant haploid-diploid life cycles or diploid life cycles. No clear trend is observed in other multicellular groups such as the red algae, but this may be because these organisms exhibit lower levels of developmental complexity.

In order to understand the relationship between life cycle structure and the evolution of multicellular complexity, it is important to take into account the possible theoretical advantages and disadvantages of different types of life cycle. These aspects are discussed in the following section (see Otto and Gerstein 2008 and Coelho et al. 2007 and references therein for further details).

Theoretical Advantages and Disadvantages of Different Types of Life Cycle

It has been proposed that diploid genomes may be advantageous in a number of respects. The presence of two copies of each chromosome can result in masking of recessive deleterious mutations, reducing the negative effects of mutations. Also, more genes are present, increasing the probability of advantageous mutations

arising. Diploidy may also be important for long-lived multicellular organisms that have to deal with rapidly evolving parasites in that a larger battery of alleles is available to provide resistance. Also, because cell size is often correlated with ploidy, it may be advantageous to be diploid if large cells are required (or, conversely, haploid if small cells are advantageous). On a more mechanistic level, the presence of homologous chromosomes in diploids provides a template for the repair of double-stranded DNA breaks. Some of these proposed advantages, such as increased cell size or a possible increased capacity to resist parasites, may be relevant to the emergence of complex multicellularity.

As far as haploid genomes are concerned, while masking of deleterious mutations in diploid genomes may be an advantage in the short term, the more effective elimination of deleterious mutations from haploid genomes due to the absence of masking may be advantageous in the long term. Similarly, although advantageous mutations may have a lower probability of arising in a haploid (because there are fewer gene copies), recessive advantageous mutations will be immediately beneficial. Haploid genomes could also have an energetic advantage, as less resources are required to replicate a smaller genome.

While these different advantages and disadvantages may help explain the dominance of either diploid or haploid life cycles, they do not provide any explanation for the emergence (or evolutionary stability) of haploid-diploid life cycles. A possible advantage of haploid-diploid life cycles is that they reduce the cost of sex (because sexual reproduction occurs over a period of two generations rather than one). However, the cost of sex can also be reduced by increasing the amount of asexual reproduction. Most attempts to explain the prevalence and stability of haploid-diploid life cycles have concentrated on ecological considerations. Such a life cycle may be advantageous, for example, if the two phases are able to exploit different ecological niches, particularly if environmental conditions are variable. Here "environmental conditions" can be understood in a broad sense, not only in terms of the physical environment but also in terms of interactions with other organisms within the ecosystem. For example, if the two phases of the life cycle have different levels of susceptibility to a particular pathogen, life cycle alternation could allow the organism to "escape" from an infection (the so-called Cheshire cat strategy; Frada et al. 2008). Note that, while these hypotheses may explain the existence of two generations, they do not explain why the two generations should have different levels of ploidy. It has been proposed that, in some instances, alternation between two generations may allow one generation to be optimized for spore production (favoring dissemination) and the other for gamete production (favoring gamete fusion) (Bell 1997), but this hypothesis is unlikely to apply to all cases, particularly for isomorphic life cycles for example. It is possible, however, that the level of ploidy of each generation is irrelevant and the main role of the life cycle in these instances is to ensure a cyclic alternation between the two different generations. In other words, in situations where it is advantageous for an organism to alternate between two different forms, the pre-existing alternation between haploid and diploid phases, inherent to all life cycles, may provide a good starting point for the evolution of the two alternating variant forms.

A number of studies have attempted to test the predictions of the various hypotheses discussed above (Otto and Gerstein 2008). For example, there is evidence for unicellular organisms that masking of deleterious mutations in diploids can make them better adapted to a mutagenic environment (but see below for multicellular organisms). Similarly, haploid life cycles appear to be advantageous if population sizes are large because more mutations tend to arise in the population, but selection is limiting. As far as haploid-diploid life cycles are concerned, the ecological roles of the two generations have also been studied for a number of taxa across the different algal groups. For heteromorphic cycles, where the sporophyte and gametophyte are morphologically different, the differences between the ecological roles of each generation can be quite evident. However, even for isomorphic haploid-diploid life cycles, where the sporophyte and gametophyte are morphologically similar, there are often subtle differences between the two generations that result in them being better adapted to different niches.

Genetic Regulation of Life Cycle Transitions

In multicellular organisms it is crucial that the initiation and progress of multicellular development be coordinated with the life cycle. Indeed, initiation of developmental processes at the wrong stage of the life cycle could have catastrophic consequences. The regulatory link between life cycle and development is still poorly understood, but there have been some important advances over the last decade. When considering such systems, one obvious starting hypothesis is that the regulation of development during the life cycle involves some sort of system that senses the level of ploidy (DNA content) of the cell. However, there is currently no evidence to support such a mechanism. For example, it has been shown for several different organisms that experimental modifications of ploidy, such as the creation of tetraploids, do not necessarily disrupt coupling between life cycle progression and development. These observations indicated that the coupling of life cycle and development is more likely under genetic control. Moreover, given that the different stages of a life cycle are all produced from the same genome, the genetic components are expected to be influenced by, and integrated with, epigenetic regulatory processes.

Genetic analyses of several organisms have identified key regulators associated with syngamy (the step of the life cycle where gametes fuse to create a zygote leading to a doubling of the chromosome number) (Goodenough and Heitman 2014; Bowman et al. 2016). The green alga *Chlamydomonas reinhardtii*, for example, produces gametes of two different mating types, called plus and minus gametes (Fig. 3a). Two different three-amino acid length extension (TALE) homeodomain transcription factors (TALE HD TFs) called gamete-specific plus 1 (GSP1) and gamete-specific minus 1 (GSM1) are expressed specifically in the plus and minus gametes, respectively. When a plus and a minus gamete fuse, during syngamy, these two transcription factors are brought together in the same cell, the zygote. In the zygote, GSP1 and GSM1 form a heterodimer, which orchestrates the expression of processes associated with the diploid phase of the life cycle (Lee et al. 2008).

Therefore, in *C. reinhardtii*, a simple genetic system allows the cell to detect when there has been a transition from the haploid to the diploid state.

A similar system has been identified in the moss *Physcomitrella patens* (Sakakibara et al. 2013; Horst et al. 2016). The *C. reinhardtii* proteins GSP1 and GSM1 are members of the BELL and KNOX2 classes of TALE HD TFs, respectively. Analysis of a *P. patens* strain carrying mutations in two KNOX2 TALE HD TF genes, *PpMKN1* and *PpMKN6*, showed that it produced a diploid gametophyte instead of the sporophyte stage of the life cycle (Sakakibara et al. 2013). Similarly, overexpression of the BELL TALE HD TF gene *PpBELL1* resulted in apogamous sporophytes (i.e., the production of haploid sporophytes without syngamy) (Horst et al. 2016).

Interestingly, similar molecular systems have been described in another eukaryotic supergroup, the fungi. In *Cryptococcus neoformans*, for example, gametes of the *α* and *a* mating types express two different homeodomain transcription factors, sex-inducer 1α and sex-inducer 2a, respectively. These transcription factors form a heterodimer in the zygote and trigger sexual development, including basidium and meiospore formation (Hull et al. 2005). There is therefore a recurring theme of association of homeodomain transcription factors with the regulation of key life cycle transitions across diverse eukaryotic supergroups. It is not clear at present whether these similarities represent convergent evolution or if the different homeodomain-based regulatory systems are derived from a common, ancestral system that would therefore date back to the last eukaryotic common ancestor (LECA; Fig. 1).

There is also direct genetic evidence for the involvement of epigenetic processes in life cycle control. In *P. patens*, for example, knockout experiments indicate that *curly leaf* (*PpCLF*) and *fertilization-independent endosperm* (*PpFIE*), which are components of the chromatin-regulating polycomb repressive complex 2 (PRC2), downregulate the expression of *PpBELL1* during the gametophyte stage by trimethylating lysine 27 of histone H3 (H3K27me3) in nucleosomes at the *PpBELL1* locus (Pereman et al. 2016). PRC2 proteins are not expressed in the zygote after syngamy, and upregulation of *PpBELL1* leads to the development of the sporophyte generation, presumably through an interaction with *PpMKN1* and *PpMKN6* (Okano et al. 2009; Horst et al. 2016) (Fig. 3b).

The Origins of Sporophyte and Gametophyte Developmental Programs

To understand the emergence of multicellular complexity, it is often very important to take into consideration the context of the life cycle. In the land plants, for example, the increase in developmental complexity over evolutionary time was associated with a transition from dominance of the haploid phase to dominance of the diploid phase (Pires and Dolan 2012). There has been considerable debate as to whether the emergence of the sporophyte generation in this lineage involved de novo evolution of developmental pathways (the so-called “antithetic” hypothesis), or whether the

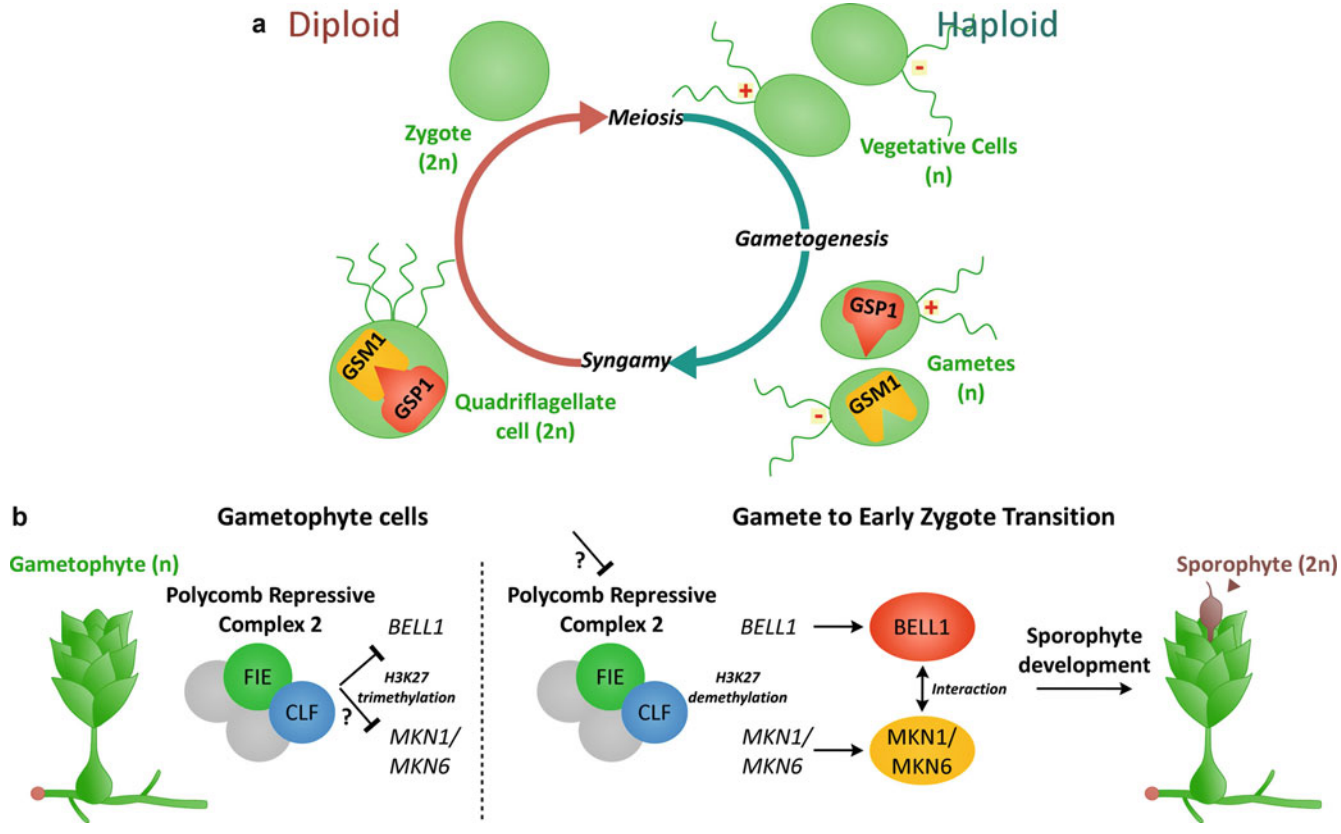


Fig. 3 Molecular regulators of life cycle progression. (**a**) The life cycle of the unicellular green alga *Chlamydomonas reinhardtii* showing the expression of the TALE homeodomain transcription factors GSP1 and GSM1 in plus and minus gametes, respectively, and the formation of a GSP1/GSM1 heterodimer after

developmental plan was an adapted version of the gametophyte program (the "homologous" hypothesis). Genomic approaches are starting to resolve this question, and the emerging picture is that recruitment of regulatory networks from the gametophyte generation played a very important role in this process, although there have also been sporophyte-specific innovations such as the employment of TALE homeodomain transcription factors of the KNOX2 family as developmental regulators.

Consequences of Life Cycle Type on Genome Evolution

The life cycle of an organism is expected to have consequences for the evolution of its genome. For example, in organisms with haploid-diploid life cycles, selection should act more efficiently on genes expressed during the haploid phase because recessive alleles of genes that are expressed during the diploid phase can be masked by dominant alleles that are also present in the diploid genome (Otto and Gerstein 2008). There is evidence that this phenomenon of masking occurs in unicellular organisms, but, surprisingly, it may not play an important role in multicellular organisms. A recent analysis of two land plant species with haploid-diploid life cycles, the angiosperm *Arabidopsis thaliana* and the moss *Funaria hygrometrica*, did not find any evidence that diploid phase-specific genes evolved more rapidly than haploid-phase-specific genes (Szovenyi et al. 2013). In fact, the evolution of life cycle-regulated genes was found to be influenced more strongly by another factor: breadth of expression. The strength of selection on a gene sequence is related to its pattern of expression because a gene that is expressed in multiple tissues and at multiple stages of development is exposed to selection more sustainedly than a gene with a very restricted pattern of expression. In land plants at least, this phenomenon appears to influence the evolution of life cycle-regulated genes more strongly than the masking effect.

Conclusion

In this chapter, we have seen that plant and algal life cycles are highly varied and often very complex. As far as the emergence of multicellularity is concerned, there appears to be a correlation between the dominance of the diploid phase and

Fig. 3 (continued) gamete fusion. (**b**) Regulators of the gametophyte-to-sporophyte transition in the moss *Physcomitrella patens*. *Left* panel: The polycomb repressive complex (PRC2) represses expression of the TALE homeodomain transcription factor BELL1 (and MKN1/MKN6?) during the gametophyte generation by laying down a repressive chromatin mark. *Right* panel: BELL1 and MKN1/MKN6 are required for initiation of the sporophyte program, and this process probably involves the formation of transcription factor heterodimers. Proteins are indicated by *colored shapes*. Genes are indicated by *italics*

multicellular complexity, at least in the most developmentally complex groups such as animals, land plants, and brown algae. The diversity of algae provides a rich source of variation to test theoretical predictions about the relative advantages of different types of life cycle. Algal systems are also providing exciting new insights into the molecular mechanisms regulating life cycle progression and the evolutionary processes that have led to the emergence of the sporophyte and gametophyte generations of the life cycle. These various themes illustrate the importance of life cycles as key processes underlying important evolutionary transitions, including adaptations to new environments and the evolution of multicellular complexity.

Glossary

Alga	Photosynthetic eukaryotes, other than land plants
Diploid	Phase of the life cycle with two sets of chromosomes
Epigenetic	A change in gene expression that is not due to modification of the DNA sequence of the genome
Gametophyte	The gamete-producing generation of a plant or algal life cycle
Generation	The organism produced at each stage of a life cycle. We use generation here to distinguish morphological/functional stages of the life cycle such as the sporophyte and the gametophyte from the ploidy phases (haploid and diploid phases)
Haploid	Phase of the life cycle with a single set of chromosomes
Haplodiploidy	Sometimes used as a synonym for haploid-diploid life cycles, but this term can lead to confusion because it is also used to describe Hymenoptera life cycles that involve development of haploid males from unfertilized eggs and diploid females from fertilized eggs (also called arrhenotoky)
Meiosis	Cell division process that results in daughter cells that contain half as many chromosomes as the parent cells. Recombination between chromosomes during meiosis generates new combinations of alleles in the chromosomes of the daughter cells
Phase	Stage of a life cycle with a specific level of ploidy, e.g., the diploid or the haploid phase
Plant	Macroscopic photosynthetic eukaryote. When used in a taxonomic sense, this term refers to a member of the kingdom Plantae, equivalent to the modern taxonomic group the Archaeplastida (Fig. 1)
Primary endosymbiosis	Capture of a cyanobacterium by a eukaryotic cell and enslavement to form a plastid

Secondary endosymbiosis	Capture and enslavement of a photosynthetic eukaryote by another eukaryotic cell leading to the production of a secondary plastid
Sporophyte	The spore-producing generation of a plant or algal life cycle
Syngamy	Fusion of gametes leading to doubling of the chromosome number in the resulting zygote

References

Bell G (1997) The evolution of the life cycle of brown seaweeds. Biol J Linn Soc 60:21–38

Bowman JL, Sakakibara K, Furumizu C, Dierschke T (2016) Evolution in the cycles of life. Annu Rev Genet 50:133–154

Cock JM, Godfroy O, Macaisne N et al (2013) Evolution and regulation of complex life cycles: a brown algal perspective. Curr Opin Plant Biol 17:1–6

Coelho S, Peters AF, Charrier B et al (2007) Complex life cycles of multicellular eukaryotes: new approaches based on the use of model organisms. Gene 406:152–170

Frada M, Probert I, Allen MJ et al (2008) The "Cheshire cat" escape strategy of the coccolithophore *Emiliania huxleyi* in response to viral infection. Proc Natl Acad Sci USA 105:15944–15949

Goodenough U, Heitman J (2014) Origins of eukaryotic sexual reproduction. Cold Spring Harb Perspect Biol 6:a016154

Horst NA, Katz A, Pereman I et al (2016) A single homeobox gene triggers phase transition, embryogenesis and asexual reproduction. Nat Plants 2:15209

Hull CM, Boily M-J, Heitman J (2005) Sex-specific homeodomain proteins Sxi1alpha and Sxi2a coordinately regulate sexual development in *Cryptococcus neoformans*. Eukaryot Cell 4:526–535

John DM (1994) Alternation of generations in algae: its complexity, maintenance and evolution. Biol Rev 69:275–291

Kawai H, Hanyuda T, Draisma SGA et al (2015) Molecular phylogeny of two unusual brown algae, *Phaeostrophion irregulare* and *Platysiphon glacialis*, proposal of the Stschapoviales ord. nov. and Platysiphonaceae fam. nov., and a re-examination of divergence times for brown algal orders. J Phycol 51:918–928

Lee JH, Lin H, Joo S, Goodenough U (2008) Early sexual origins of homeoprotein heterodimerization and evolution of the plant KNOX/BELL family. Cell 133:829–840

Leliaert F, Smith DR, Moreau H et al (2012) Phylogeny and molecular evolution of the green algae. Crit Rev Plant Sci 31:1–46

Okano Y, Aono N, Hiwatashi Y et al (2009) A polycomb repressive complex 2 gene regulates apogamy and gives evolutionary insights into early land plant evolution. Proc Natl Acad Sci USA 106:16321–16326

Otto SP, Gerstein AC (2008) The evolution of haploidy and diploidy. Curr Biol 18: R1121–R1124

Pereman I, Mosquna A, Katz A et al (2016) The Polycomb group protein CLF emerges as a specific tri-methylase of H3K27 regulating gene expression and development in *Physcomitrella patens*. Biochim Biophys Acta 1859:860–870

Pires ND, Dolan L (2012) Morphological evolution in land plants: new designs with old genes. Philos Trans R Soc Lond Ser B Biol Sci 367:508–518

Sakakibara K, Ando S, Yip HK et al (2013) KNOX2 genes regulate the haploid-to-diploid morphological transition in land plants. Science 339:1067–1070

Silberfeld T, Leigh JW, Verbruggen H et al (2010) A multi-locus time-calibrated phylogeny of the brown algae (Heterokonta, Ochrophyta, Phaeophyceae): investigating the evolutionary nature of the "brown algal crown radiation". Mol Phylogenet Evol 56:659–674

Szovenyi P, Ricca M, Hock Z et al (2013) Selection is no more efficient in haploid than in diploid life stages of an angiosperm and a moss. Mol Biol Evol 30:1929–1939

The Evolution of Branching in Land Plants: Between Conservation and Diversity

Yoan Coudert

Contents

Abstract

The evolution of branching was pivotal to the diversification of plant architecture, providing ways to colonize the environment and optimize resource acquisition both above and below ground. Fossil and phylogenetic evidence indicates that branching evolved independently in the two generations of the land plant life cycle. In this chapter, I focus on shoot systems and discuss two contrasting patterns, occurring at different levels: conservation and diversity. I show that two similar branching modes, terminal and lateral, are found across extant and

Y. Coudert (✉)
Laboratoire Reproduction et Développement des Plantes, Ecole Normale Supérieure de Lyon, Lyon, France
e-mail: coudertlab@gmail.com

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_63

extinct land plant lineages. Despite conservation of gross morphology, branching morphogenesis is highly disparate at the cellular level and is orchestrated in various manners between and within lineages. In contrast, the molecular mechanisms underpinning branch development could be largely shared among land plants.

Keywords

Branching · Land plants · Conservation · Diversity · Shoot

Introduction

The evolution of branching accompanied the successful colonization of terrestrial ecosystems by plants. This innovation provided a capacity to occupy space in the three dimensions both above and below ground and contributed to the optimization of photosynthetic capacity and reproductive success. There are two alternating phases in the life cycle of land plants: the haploid gametophyte and the diploid sporophyte, gamete-bearing and spore-producing generations, respectively (See chapter ▶ "Alternation of Generations in Plants and Algae"). In bryophytes, extant representatives of the earliest land plants, the gametophytic body is dominant and has indeterminate branching growth, whereas the sporophytic body is highly reduced and limited to a single unbranched stem with determinate growth (Sussex and Kerk 2001). The divergence between bryophytes and early vascular plants, i.e., plants with lignified conducting tissues, was accompanied by a progressive reduction of the gametophyte and the evolution of indeterminate and branching shoots in the sporophyte. Phylogenetic and fossil data support bryophytes as sister to other land plants (Wickett et al. 2014), which suggests that branching in the sporophyte evolved from bryophyte-like unbranched ancestors and originated independently from branching in the gametophyte (Harrison 2016). While the genetic control of branching has been extensively reviewed, mostly from flowering plant studies (Domagalska and Leyser 2011; Kebrom et al. 2012; Schmitz and Theres 2005), few if any syntheses on the cellular basis of branch formation in land plants are available. This chapter focuses on the evolution of branching in the vegetative systems of major land plant groups and aims to highlight three major trends. The first is the repeated evolution of branching forms in both generations of the land plant life cycle, indicating that similar architectural strategies have arisen multiple times independently in evolution. The second is the diversity in cell division patterns underpinning branch formation, that is, the occurrence of diverse morphogenetic pathways within and between lineages to attain similar forms, suggesting that morphology is more constrained than underlying cellular processes. Although our knowledge of the genetic control of branching in bryophytes and early vascular plants is still limited, the third trend is the partial conservation of molecular regulatory pathways underpinning branch development between distantly related plant lineages.

Why Branching Evolved: The Axial Nature of Plants

All extant land plant lineages are unified by the presence of an indeterminate vegetative body in the dominant generation of their life cycle (Fig. 1). The vegetative body is formed by a flattened leafless mat of photosynthetic tissue, has a bilateral symmetry in the gametophyte of hornworts and thalloid liverworts, and is typically characterized by leafy shoots with a radial or bilateral symmetry in the gametophyte of mosses and leafy liverworts, and in the sporophyte of vascular plants. Although plants have formed a diverse group with morphologies adapted to a wide range of environments since their emergence on land around 470 million years ago, they all develop through the activity of meristems localized at the growing tips of their vegetative bodies. Meristems comprise one to multiple stem cells that are responsible for producing all the other cells that will differentiate, i.e., acquire a fate, and form leaf, stem, or thallus tissues. Stem cells proliferate continuously, but the size of

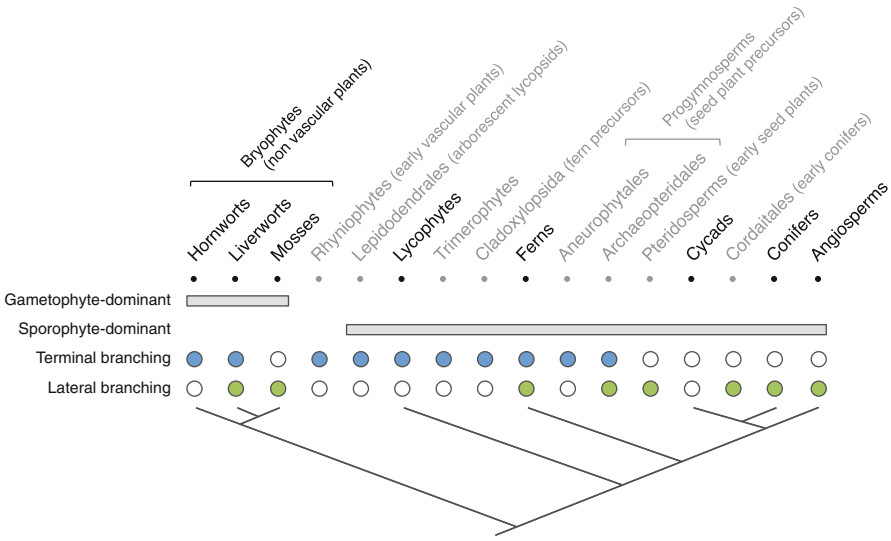


Fig. 1 Terminal and lateral branching in extinct and extant land plant lineages. Summary of the main branching modes in the dominant life cycle stage of bryophytes (gametophyte) and vascular plants (sporophyte). Rhyniophytes had isomorphic alternation of generations. Terminal and lateral branching are indicated by blue and green filled circles, respectively. Extant and extinct plant groups are shown in black and grey, respectively. Phylogenetic relationships between extant groups are based on (Wickett et al. 2014) and relative position of extinct groups on (Tomescu 2009). Branching mode in extinct lineages is documented from the following research articles: *Rhynia* (Edwards 2003), Lepidodendrales (Thomas 1978), trimerophytes (Kasper and Andrews 1972), Cladoxylopsida (Giesen and Berry 2013), ferns and Aneurophytales (Galtier 1999), Archaeopteridales (Meyer-Berthaud et al. 1999) and Cordaitales (Baxter 1959). In *Archaeopteris*, considered as the earliest known modern tree, non-permanent branches arise from the pseudomonopodial division of the main apex but another type of branch, that is associated with a leaf-type organ, develops laterally in a similar manner to the axillary branching of seed plants marking the transition between two key branching modes (Meyer-Berthaud et al. 1999)

the meristem is kept relatively constant and its position remains fixed at the apex throughout development (Fig. 2a). Molecular signaling mechanisms underpinning meristem size maintenance are well characterized in flowering plant models like *Arabidopsis thaliana* (thale cress) or *Oryza sativa* (rice). Briefly, these mechanisms involve cytokinins, a class of stem cell-promoting phytohormones, and a negative

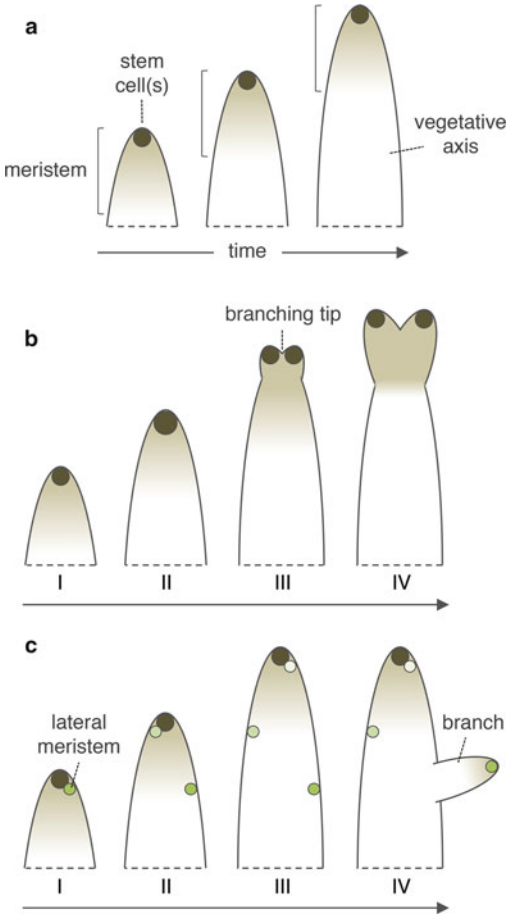


Fig. 2 Schematic representation of growing and branching vegetative axes. (**a**) Vegetative growth in a land plant axis occurs through the activity of a meristem (light brown region) that is continuously maintained in an apical position during development, as indicated by the bracket. Dark brown filled circle at the tip marks stem cell position. (**b**) Terminal branching or bifurcation. Stem cell number may increase prior to branching (*I–II*). Branching involves equal or unequal splitting of the tip into two new tips (*III*), and subsequent growth of branches is driven by newly formed meristems (*IV*). Terminal branching occurs independently from leaf position and leads to a complete reorganization of the apical meristem. (**c**) Lateral branching. Lateral meristems (green filled circles) form in the axil of leaves without disrupting apical meristem structure (*I–III*). Lateral meristems may remain dormant or grow out into branches in response to endogenous and exogenous cues (*IV*). Empty leaf axils may be found in some species. Leaves are not shown

feedback loop between a stem cell-promoting factor (*WUSCHEL, WUS,* Homeodomain transcription factor family), expressed in a central domain of the meristem flanking the stem cells, and a peptide (*CLAVATA, CLV,* secreted peptide ligand family) that is secreted by stem cells and restricts *WUS* activity (Stahl and Simon 2010). It has been proposed that cytokinins produced in the meristem epidermis diffuse into the deeper tissues and, together with *WUS/CLV* signalling, act as positional cues to scale and maintain the stem cell niche in the apical position. These data suggest that the apical-basal axis of the shoot is specified within the apical meristem. As cells exit the meristem, oriented patterns of division and highly anisotropic expansion are further needed to determine the growth direction of the stem and produce its axial shape. For instance, proper activity of the *REPLUMLESS* gene *(RPL,* Homeodomain transcription factor family) is required to orientate cell divisions in the rib zone of the shoot apex that produces inner stem tissues, and loss of function mutants in this gene show various developmental defects including reduced axial elongation. Although the structure of shoot apical meristems varies across land plants and may be unicellular (e.g., bryophytes) or multicellular (e.g., seed plants), anisotropic shoot growth driven by apical activity is a conserved property of plants. This fundamental characteristic underpins the axial nature of plant shoots, but also represents a structural constraint that restricts the range of theoretically possible architectures. An acquired capacity to branch, i.e., to establish new meristems and growth axes from preexisting shoots has therefore been essential to the diversification of plant shape (see chapter ▶ "Developmental Innovation and Phenotypic Novelty").

Similar Branching Modes Are Found Across Land Plants

Branching mechanisms evolved repeatedly in both generations of the plant life cycle, and two major branching modes are usually recognized in land plants: terminal and lateral (Fig. 1). Terminal branching occurs by equal (isotomous) or unequal (anisotomous) splitting of the shoot apex into two new apices and is a reiterative process that gives to the plant a forked appearance. This branching mode, also called apical dichotomy or bifurcation, is a direct consequence of cellular rearrangements occurring at the shoot apex and usually involves a complete re-organization of meristematic tissues. In the strict sense, dichotomy refers to an equal or near equal division of an apical meristem, and in the case of single-celled meristems to the formation of two new apical cells by division of the stem cell (Bierhorst 1977). Terminal branching was the prevalent branching mode in many extinct plant lineages including rhyniophytes (early vascular plants), Lepidodendrales (arborescent lycopsids), trimerophytes, *Cladoxylopsida* (fern precursors), Aneurophytales and Archaeopteridales (progymnosperms). It is mainly found today in plants with meristems comprising one to a few stem cells, including hornworts, liverworts, lycophytes, and ferns and is rare in plants with multicellular meristems such as gymnosperms and angiosperms. In terminal branching, branch meristem initiation and outgrowth are not distinct processes, which means that branch development proceeds directly following the specification of a new branch meristem without a growth arrest. Some dichotomously branching plants are

leafless, like thalloid liverworts or hornworts, and others including lycophytes and ferns have leaves. In these plants, the position and timing of meristem bifurcation is independent of leaf initiation patterns, which suggests that branch and leaf development are controlled by distinct mechanisms.

Lateral branching differs from terminal branching in various aspects. Most importantly, branches develop in lateral positions with regard to the main shoot, in association with leaves and without disrupting the cellular architecture of the shoot apical meristem. Two phases are typically distinguished during lateral branching: lateral meristem initiation and outgrowth. Lateral shoot meristems usually initiate in the main shoot apex during leaf initiation and in association with the leaf meristem. Lateral meristems may develop immediately into branches, in which case initiation and outgrowth is a single continuous process, or form a few leaves and enter a period of dormancy. The inhibition of branch development is controlled at a distance by the main shoot apex, a mechanism called apical dominance (see section "Are Regulatory Mechanisms of Branching Shared Between Land Plants?"). Growth arrest may last a few days to several years and may never cease. Release from dormancy is triggered in response to internal or environmental cues, such as phytohormones, light or temperature, or following the loss or termination of the main shoot apex. Lateral shoot meristems may also form de novo at some distance from the apex from differentiated tissues. Lateral meristems generally develop in leaf axils (axillary branching), but they may also form in relation to a leaf but at a distance from the axil (extra-axillary branching), or directly on a leaf (epiphyllous branching). Lateral branching is the prevalent branching mode in mosses and seed plants and is common in leafy liverworts and ferns. While branching types are generally conserved at a gross morphological level across land plants, the cellular basis of both terminal and lateral branching is highly disparate between and within lineages.

Diversity in the Cellular Mechanisms of Terminal Branching

Histological and clonal analyses of bifurcating apices have been performed to investigate how terminal branching is controlled at a cellular level. They have revealed distinct cellular mechanisms, distinguished by the number of apical cells comprising the meristem, and whether these cells disappear and are replaced by newly formed branch meristem cells during the branching process or remain functional and become incorporated into branch meristems (Gola 2014).

Hornworts

Hornworts comprise c. 250 species and might represent the most basal lineage of land plants. Although they are key to understanding the origins of plant evolution (Wickett et al. 2014), they are among the least studied plant groups. In hornworts, the gametophyte is dominant and consists of a photosynthetic thallus growing by the activity of single apical cells located in peripheral notches. The structure and

function of the apical meristem is still debated. Bifurcation has been proposed to take place by a series of divisions of the apical cell to form a row of meristematic cells, in which two daughter cells acquire an apical cell function and promote the development of new growth axes (Schuster 1984). Studies in different hornwort species have evidenced the presence of multiple identical cells lying side-by-side in meristematic notches (Fig. 3a), but the main apical cell has not yet been identified. Such cellular configuration might be transient and could correspond to an intermediate state of the branching process or to the state of the apical meristem during normal vegetative growth.

Liverworts

Liverworts comprise about 7500 species, and their phylogenetic position with regards to other bryophytes has not been fully resolved. Most phylogenetic studies place liverworts as sister to all other land plants, but recent phylotranscriptomic analyses recovered a clade comprising liverworts and mosses as sister to vascular plants, with the hornworts sister to all other land plants (Fig. 1) (Wickett et al. 2014). Besides their evolutionary relationships, liverworts are classified into three groups based on their overall gametophytic morphology: leafy liverworts, and simple and complex thalloid liverworts. Terminal dichotomous branching is predominant in the gametophyte of complex thalloid liverworts, such as the well-studied species *Marchantia polymorpha*. Thalloid liverworts are characterized by a vegetative body lacking lateral foliar appendages and growing horizontally from apical notches in which one to several meristematic cells are embedded, similarly to hornworts. Histological analyses of cell division patterns in *Marchantia emarginata* have suggested that the notch meristem comprises two initial cells (Fig. 3b). During bifurcation, the number of initials expands up to eight and this group of cells divides into two branch meristems, each one comprising a pair of initials (Burgeff 1943). A new bifurcation occurs every five divisions of the initials, suggesting that this process is endogenously controlled. Alternative cell division patterns have been described in other liverwort species. For instance, observations in *Metzgeria furcata* suggested that the meristem comprises a single apical cell and that branching involves the formation of a new initial cell next to the apical cell rather than its direct division (Goebel 1905).

Lycophytes

Lycophytes form the oldest extant group of vascular plants and are distributed between three orders, the Lycopodiales (c. 388 species), Isoëtales (c. 250 species), and Selaginellales (c. 700 species). Descriptions of the cellular organization at the surface of fixed and cleared shoot apices have been used to infer meristem structure in the sporophyte of the firmoss *Huperzia lucidula.* These studies suggested that shoot growth is driven by four initial cells, and these cells may have a transient

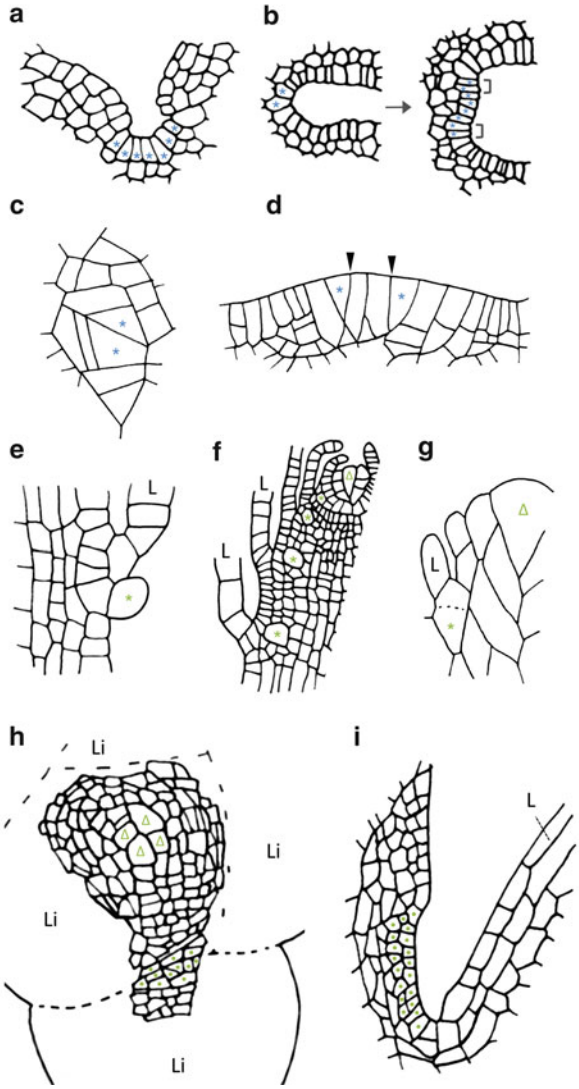


Fig. 3 Terminal and lateral branching at a cellular level. (**a–d**) Terminal branching. (**a**) Line drawing of a bifurcating apical notch in the hornwort *Anthoceros laevis*. After Schuster (1984). (**b**) Line drawings of bifurcating apical notches in the liverwort *Marchantia emarginata*. Increase in stem cell number from two (left) to eight (right) is followed by the formation of two new apical meristems (indicated by brackets). After Burgeff (1943). (**c**) Line drawing of surface cellular patterns observed immediately after dichotomy of the shoot apical cell in the fern *Dipteris conjugata*,. After Bierhorst (1977). (**d**) Line drawing of a shoot apex histological section in the fern *Dennstaedtia scabra* showing two newly formed branch apical cells. Apical cells are in contact with square cell packets (indicated by arrowheads), suggesting that the shoot apical cell stops functioning before branch apical cells are formed. After Imaichi (1984). (**e–g**) Lateral branching. (**e, f**) Line drawings of longitudinal stem sections showing formation of branch initials in leafy

character and be replaced during development (Gola 2014). Dichotomy could occur through the division of an initial cell and the subsequent displacement of one of the two resulting sister cells away from its original position to form two independent meristematic centers. This early stage of branching is associated with apex broadening, and later stages coincide with a switch in apex shape and specific cell division patterns (Gola 2014). Histological observations in various *Selaginella* species (spikemosses) have suggested that shoot meristems have a single apical cell and that dichotomy occurs in a two-step process. First, the apical cell ceases its mitotic activity and loses its meristematic features, and second, two new initials are simultaneously initiated next to the former apical cell. Whereas histological studies provide a static view of tissue architecture, clonal analysis allows elucidation of cell lineage relationships and monitoring of the fate of individual cells. This approach has been used in *Selaginella kraussiana,* in which dichotomous branching is anisotomous, and demonstrated that shoot development is underpinned by the activity of two transient apical initial cells (Harrison et al. 2007). During apex bifurcation, the number of initials increases to four or five and the direct descendants of these cells are allocated to two distinct groups of unequal size to give a minor and a major branch. Anisotomous dichotomy results in the formation of a so-called pseudo-monopodium where overtopping of major branches leads to the formation of the main shoot axis and minor branches are displaced to the side. Further observations in various species of Lycopodiales corroborate the existence of multicellular shoot meristems and support a notion of dichotomous branching whereby the entire meristem splits to generate two even or uneven branch apices (Gola 2014).

Ferns

Ferns comprise about 10,500 species and are sister to the seed plants. Apical dichotomy is recognized as the major branching mode in the sporophyte of ferns (Bierhorst 1977), and many fern species (e.g., *Davallia*, *Stenochlaena*, *Polypodium*, or *Microsorum*) have a pseudomonopodial habit with a major creeping shoot axis – called a rhizome – serving to forage the substrate for nutritive resources. Branching rhythm is usually regular in a given species and may be described in terms of the number of dichotomies per leaf initiated, although branching and leaf initiation are

Fig. 3 (continued) liverworts. After Stotler (1972). In *Radula*, branch initials form from outer cell layers (**e**), wheras in *Bazzania* they develop from ventral medullary cells (**f**). (**g**) Line drawing of a longitudinal stem section showing formation of leaf and branch initials in the moss *Fontinalis*. After Berthier (1973). (**h**) Line drawing of a detached meristem in a needle axil of *Sequoia sempervirens*. After Fink (1984). (**i**) Line drawing of surface cell patterns of an *Arabidopsis* shoot apex. An axillary meristem is visible at the boundary (dash line) between the apex dome and an incipient leaf initium (Li). After Burian et al. (2016). For (**a**–**g**), blue asterisks indicate apical stem cells, L indicates a leaf, and green triangles and asterisks indicate main and lateral (branch) meristems, respectively

not directly related in development. Genera like *Dipteris* may branch once every five to eight leaves produced, while others like *Schizaea* or *Actinostachys* may branch two to four times more than the number of leaves formed. Extensive imaging of the surface of fixed apices from dichotomizing shoots from more than 50 fern species has revealed peculiar cellular configurations, suggesting that a single shoot apical cell may directly undergo dichotomy to generate two similar shoot apical cells (Bierhorst 1977) (Fig. 3c). Histological studies of *Dennstaedtia* and *Hypolepis* shoot apices have led to a different conclusion. In these species, the shoot apical cell may disappear and be replaced by a group of non-meristematic cells; branching would subsequently occur by de novo specification of two apical cells at its flanks (Fig. 3d).

Diversity in the Cellular Mechanisms of Lateral Branching

Liverworts

Lateral branching is common in the gametophyte of leafy liverworts, and eleven types of branching have been identified and assigned to two main classes defined by the presence or absence of a basal sheath (Stotler 1972). In sheathless branching types, the branch apical cell is formed by three oblique divisions of the branch initial cell, whereas in sheathed branching types the branch initial undergoes random divisions to form a multicellular branch primordium from which the apical cell develops. Within each class, branching types can be further distinguished by the following features: (1) the cellular origin of branch initials (in merophytes (group of cells produced from the same initial cell) near the stem apex, in cortical cells from older merophytes or from the medulla), (2) the position of merophytes from which branches initiate (ventral or dorsal), (3) the type of leaves that branches are associated with (half-leaves or unmodified lateral leaves), (4) the distance separating the leaf and the point of branch origin, and (5) the ability to displace the branch to the axil of the next oldest leaf (Fig. 3e–f) (Stotler 1972).

Mosses

Mosses form the largest group among bryophytes and comprise c. 13,000 species. Their exclusively lateral gametophytic branching mode underpinned their architectural diversification (Coudert et al. 2017). The shoot apical meristem in mosses comprises a single apical cell that cleaves regularly, producing segments and regenerating itself. In most moss species, each segment divides several times consecutively and generates a leaf and a branch initial cell. The order of cell division interpreted from histological sections differs between authors, but the branch initial cell is always described in a basal position with regards to the associated leaf (Fig. 3g). As growth proceeds, high cell division rates between the leaf and the branch initial cell displace the branch meristem into the axil of the leaf

located underneath. Branches appear in axillary positions but are truly "hetero-axillary" as they originate from cellular territories that are distinct from the subtending leaf (Berthier 1973). After it is formed, a branch apical cell may immediately grow out into a branch (immediate branching), produce a few leaves and turn into a dormant bud, or directly enter dormancy without developing further (delayed branching). Release from dormancy may be triggered in response to various internal and external cues (see section "Are Regulatory Mechanisms of Branching Shared Between Land Plants?"). In the moss *Physcomitrella patens*, dormant branch apical cells have not been observed, and branch initiation and outgrowth are a single continuous process occurring at a distance from the shoot apical meristem (Coudert et al. 2015). In other species like *Sphagnum quinquefarium*, *Polytrichum formosum* or *Dendroligotrichum dendroides*, most leaf axils do not have a branch apical cell and the proportion of empty axils may vary depending on environmental conditions.

Ferns

Apical dichotomy is a major branching mode in the sporophyte of ferns, but lateral branching is also frequent and has been well studied in the Hymenophyllaceae family (filmy ferns). Histological descriptions in *Trichomanes* and *Hymenophyllum* species showed that merophytes cut from the single shoot apical cell divide periclinally (parallel to the tissue surface) and produce an inner procambium cell and an outer prismatic cell. The latter undergoes a second periclinal division, which gives rise to adjacent frond (fern leaf) and lateral bud initials. Both initial cells then divide anticlinally (perpendicular to the tissue surface) several times to produce the frond and bud apical cells, respectively. The bud apical cell cuts off a few merophytes and ceases growth to enter dormancy, whereas the development of the frond continues. Similar observations have been made in other fern species (Bierhorst 1977), which suggests that early patterns of cell division are generally conserved within the fern lineage. However, the orientation of lateral buds with regards to the position of fronds shows a high degree of variation between species (Hébant-Mauri 1984), suggesting that subsequent cell division and growth patterns are poorly conserved (Hébant-Mauri 1993). In *Trichomanes* species, buds may form in an axillary position with respect to the nearest frond or at a distance from the frond axil. In *Histiopteris* and *Hypolepis*, buds are extra-axillary and form laterally at the frond base. In *Adiantum*, buds develop on the abaxial side of the frond, i.e., on the lower surface of the leaf. In *Stromatopteris*, branching occurs in bare portions of the stem, with no apparent connection to fronds. Epiphyllous branching, in which new shoots develop on the frond in non-axillary positions, represents an extreme case of lateral branching and may serve as a means for the clonal propagation of plantlets identical to the mother plant. Interspecific variation in relative bud-to-frond position might reflect differences in shoot symmetry, phyllotaxis, and growth habit (Hébant-Mauri 1993). Importantly, lateral and terminal branching are often combined in ferns. For example, *Onoclea sensibilis* spreads by means of dichotomizing creeping rhizomes

on which fronds develop, and small lateral buds may be observed in association with the fronds in specific positions.

Gymnosperms and Angiosperms

Cycads (c. 310 species), *Ginkgo biloba* (1 species), gnetophytes (c. 90 species), and conifers (c. 615 species) form the gymnosperms. Together with the angiosperms (c. 300,000 species), the gymnosperms represent the seed plants (Christenhusz and Byng 2016). Lateral branching is a conserved feature of seed plants but cycads that lack lateral buds and have an unbranched growth habit (Fig. 1).

Several theories, including the apical cell, histogen, and tunica/corpus theories, have provided comprehensive frameworks for describing the organization of multicellular shoot apical meristems and the cellular origins of lateral meristems in angiosperms. In the tunica-corpus concept, the "*tunica*" corresponds to the more superficial layers that divide only anticlinally, and the "*corpus*" is formed by the remaining layers located underneath in which cell division patterns are more variable. This theory has been extended to all seed plants, although it may not be widely applicable and the cellular architecture of gymnosperm shoot apices may be better captured in terms of cytohistological zones. Two main zones are typically described in gymnosperms: the apical initial group, a layer of slow dividing initial cells at the meristem surface from which all other cells are derived, and the central mother cell zone, a group of cells produced by periclinal divisions of the apical initials. By comparison, five cytohistological zones have been identified in angiosperm shoot apices, suggesting increased meristem complexity in this group.

In both angiosperms and gymnosperms, lateral meristems form on the adaxial (upper) side of the leaf in a zone called the leaf axil, and lateral meristems are therefore referred to as axillary. Genetic evidence supports the relationship between the axillary meristem and the adaxial side of the leaf. For instance, in the angiosperm *Arabidopsis thaliana*, the *PHABULOSA* gene (*PHB*, Homeodomain-Leucine Zipper Class III transcription factor family) is required for the specification of abaxial (lower side) leaf fate. In mutant plants lacking *PHB* activity, the abaxial side of leaves acquires adaxial fate, resulting in the ectopic development of meristems all round the leaves. There are two main hypotheses regarding the ontogenetic origin of axillary meristems in seed plants. The "detached meristem" hypothesis proposes that lateral meristems derive directly from a persistent population of shoot apical meristem cells associated with the leaf primordium during its initiation. The alternative view proposes that there is no continuum with the shoot apical meristem and lateral meristems initiate "de novo" from differentiated tissues. While both hypotheses are supported by the expression patterns of molecular markers associated with meristematic activity such as the *SHOOT MERISTEMLESS* gene (*STM*, Homeodomain transcription factor family) (Long and Barton 2000), clonal analyses based on time-lapse imaging of tomato and *Arabidopsis* apices have evidenced that axillary meristems are specified early within the shoot apical meristem (Burian et al. 2016) (Fig. 3h). Most cell divisions occur between the center and lower boundary of the

apical meristem, and mitotic activity is almost null when the axillary meristem leaves the apex. In the *Arabidopsis* shoot apical meristem, the tunica is formed by the epidermal L1 and subepidermal L2 cell layers, and the corpus is formed by the L3 cell layer and subtending tissues. Clonal analyses performed using cell autonomous genetic markers have suggested that axillary meristems are derived from tunica cells, including a minimum of two L2 cells located in a central position of the "*axillant*" leaf, but cells from the L3 do not contribute to lateral meristem formation. However, these observations do not hold true for all angiosperms. Histological analyses in the grass *Elymus repens* have shown that axillary meristems initiate by periclinal divisions in corpus derivatives. These data suggest that distinct patterns of cell divisions underpin lateral meristem formation among angiosperms. In conifers, leaves show limited degrees of lateral flattening and lateral meristems develop in their axils. Most species are assumed to develop few lateral buds and have empty leaf axils (Fink 1984), but detailed histological observations in *Taxus baccata* and other species have revealed that most leaf axils contain a dormant meristem (Fig. 3i). As the shoot expands and its tissues differentiate, the superficial cell layers in the axils of developing leaves remain undifferentiated and do not display any obvious global organization. After several rounds of division, the outer cell layers become suberized and form a rigid protection for underlying meristematic cells. These axillary meristems may stay dormant for several years, and following the release from dormancy bud outgrowth may be very slow and occur over years.

Together, these observations suggest that the patterns of cell division during axillary meristem formation differ between angiosperms and gymnosperms, and lateral meristem organization is anatomically simpler in gymnosperms than angiosperms.

Are Regulatory Mechanisms of Branching Shared Between Land Plants?

As reported in the previous sections, terminal and lateral branching mechanisms evolved multiple times in the sporophyte and gametophyte generations of land plants and may be orchestrated in several manners at a cellular level, leading to similar morphologies by convergence. This raises important questions about underlying regulatory mechanisms, and in particular whether similar or distinct molecular pathways control the development of convergent branching forms.

Branching is a major determinant of yield in plants of agronomic importance, and its genetic control has mainly been studied in flowering plants (Domagalska and Leyser 2011; Schmitz and Theres 2005). Forward genetic studies have identified hypo- and hyper-branching mutant plants impaired at various stages of the lateral branching process, revealing the key role of transcription factors in the establishment of leaf axil identity, the maintenance of competence for meristem formation in leaf axils, the patterning of lateral meristems, and the formation and outgrowth of lateral buds (Kebrom et al. 2012; Schmitz and Theres 2005). Corresponding gene families have also been found in the genome of bryophytes, such as the moss *Physcomitrella*

patens, and non-flowering vascular plants, such as the lycophyte *Selaginella moellendorffii*, but functional studies are still limited to bryophytes and only one gene encoding a transcription factor has a reported function in branching control in these plants to date. *TEOSINTE BRANCHED1* gene (*TB1,* class II *TCP* domain transcription factor family) represses lateral branching in maize and homologues in other flowering plants (e.g., *BRANCHED1, BRC1*) have conserved functions in the sporophyte. *TCP* genes predate the origin of land plants and suppressing *TCP5* function in the moss *Physcomitrella patens* promotes sporophytic branching, although bryophyte sporophytes are normally unbranched.

Phytohormones provide another level of control for branch development. In seed plants, the growth of lateral buds is usually inhibited at a distance by the main shoot apex, a phenomenon called "apical dominance." Seminal experiments in *Vicia faba* have shown that the removal of a dominant apex (decapitation) is sufficient to allow lateral meristems to grow out (Thimann and Skoog 1933), and investigations in *Pisum sativum* have proven that the inhibitory effect is mainly due to developing leaves at the shoot apical meristem. Auxin is a phytohormone with pleiotropic effects on plant development and is mainly synthesized in young leaves in the shoot. The exogenous application of auxin to the stump of decapitated plants inhibits lateral bud outgrowth, which suggests that auxin mediates apical dominance (Cline 1996). This effect seems to be broadly conserved in herbaceous angiosperms, although weaker inhibition has been observed in some species (Cline 1996). Similar auxin replacement experiments performed in temperate woody species, such as the flowering plants *Quercus*, *Fraxinus* and *Acer* and the conifer *Araucaria*, have shown that apical dominance is conserved in shoot systems with distinct phenologies. The role of auxin in apical dominance is not direct and is relayed by two other phytohormone classes with antagonistic functions, cytokinins, and strigolactones (Domagalska and Leyser 2011). The direct application of cytokinins to buds and the endogenous increase of cytokinin levels in transgenic lines promote bud outgrowth, and auxin downregulates cytokinin biosynthesis in the stem. Conversely, the direct application of strigolactone analogues to buds inhibits their development, and the disruption of strigolactone biosynthesis and signaling pathways in mutants promotes axillary branching. Such mutants are resistant to the inhibitory effects of apical auxin, and auxin signaling genes activate strigolactone biosynthesis. Buds are also auxin sources, and the outgrowth of buds depends on their capacity to export auxin toward the stem. Auxin movement is mediated by the polar auxin transport protein PIN1 (PIN-FORMED1, PIN auxin efflux carrier family), and strigolactones regulate competition between lateral buds by reducing the accumulation of PIN1 proteins at the plasma membrane and dampening auxin transport capacity in the stem. Auxin, cytokinin, and strigolactone thus form a systemic regulatory network that is coordinated globally, via the PIN1-dependent polar auxin transport stream, and integrated locally by specific factors, such as the *BRC1* gene whose expression is activated by strigolactones and repressed by cytokinins in buds (Domagalska and Leyser 2011).

The extent to which these hormonal regulatory pathways are conserved across land plants is largely unknown. Decapitation experiments performed in bryophytes (Coudert et al. 2015), lycophytes, and ferns have shown that apical dominance is

conserved in all plant lineages and could provide a universal mechanism for branching control at a distance from a dominant apex. Although auxin can replace the function of apices that have been artificially removed in the liverwort, moss, and lycophyte species that have been tested, it is unable to do so in some ferns, suggesting variation in molecular mechanisms between lineages. A recent study in pea demonstrated that sugar demand, rather than auxin, is the initial regulator of apical dominance, and resource-based mechanisms might explain the lack of visible effect in auxin replacement experiments in some non-flowering plants. Genetic evidence supports an inhibitory role for auxin and strigolactones, and a promoting role for cytokinins, in gametophytic shoot branching in the moss *Physcomitrella patens*, which suggests the function of phytohormones was co-opted from the gametophyte to the sporophyte generation during land plant evolution. Although *PIN* genes are conserved in mosses and regulate shoot development in *Physcomitrella*, the disruption of *PIN* function or polar auxin transport promotes branching in the sporophyte, but has weak effects in the gametophyte (Bennett et al. 2014; Coudert et al. 2015). These data suggest that PIN-mediated polar auxin transport is conserved in the sporophyte generation of land plants, but other mechanisms are involved in the gametophyte (Harrison 2016). An alternative diffusive auxin transport mechanism, regulated by callose-dependent plasmodesmal gating, could instead regulate branching in the moss gametophyte (Coudert et al. 2015). This mechanism might be shared with liverworts and hornworts that display non-polar or weakly polar auxin transport in both generations.

Perspective

Although our knowledge is strongly biased towards flowering plant species, the data presented here suggest that molecular pathways regulating branching are at least partially similar between major plant groups and across life cycle stages. These molecular interaction networks could have been inherited from land plant ancestors and re-used repeatedly in evolution to regulate branching, irrespective of the cellular processes underlying branch formation. Recent progress in functional genomics has enabled the establishment of new model hornwort, liverwort, moss, and fern species, and the role of key genes identified in flowering plant models may now be investigated in more basal lineages through reverse genetics. Forward genetic screens in early diverging land plant models will also be important in discovering non-conserved regulators of branching. Combining these approaches will allow us to determine the extent of deep homology (Shubin et al. 2009) in the development of branching forms in land plants.

Cross-References

► Alternation of Generations in Plants and Algae
► Developmental Innovation and Phenotypic Novelty

Acknowledgments I am grateful to Jill Harrison, Florian Jabbour and Jeremy Solly for helpful suggestions and proofreading the manuscript. I thank the CNRS (ATIP-Avenir programme) and the Natural History Museum Paris (ATM programme) for funding research in my laboratory.

References

Baxter R (1959) A new Cordaitean stem with paired axillary branches. Am J Bot 46:163–169
Bennett TA, Liu MM, Aoyama T, Bierfreund NM, Braun M, Coudert Y, Dennis RJ, O'Connor D, Wang XY, White CD et al (2014) Plasma membrane-targeted PIN proteins drive shoot development in a moss. Curr Biol 24:2776–2785
Berthier J (1973) Recherches sur la structure et le développement de l'apex du gamétophyte feuillé des mousses. Revue bryologique et lichénologique 38:421–551
Bierhorst DW (1977) On the stem apex, leaf initiation and early leaf ontogeny in filicalean ferns. Am J Bot 64:125–152
Burgeff H (1943) Genetische Studien an Marchantia. Verlag von Gustav Fischer, Jena, pp 1–12
Burian A, de Reuille PB, Kuhlemeier C (2016) Patterns of stem cell divisions contribute to plant longevity. Curr Biol 26:1385–1394
Christenhusz M, Byng JW (2016) The number of known plants species in the world and its annual increase. Phytotaxa 261:201–217
Cline MG (1996) Exogenous auxin effects on lateral bud outgrowth in decapitated shoots. Ann Bot 78:1–12
Coudert Y, Palubicki W, Ljung K, Novák O, Leyser O, Harrison CJ (2015) Three ancient hormonal cues co-ordinate shoot branching in a moss. eLife Sci 4:2061
Coudert Y, Bell NE, Edelin C, Harrison CJ (2017) Multiple innovations underpinned branching form diversification in mosses. New Phytol 22:810
Domagalska MA, Leyser O (2011) Signal integration in the control of shoot branching. Nat Rev Mol Cell Biol 12:211–221
Edwards D (2003) Embryophytic sporophytes in the Rhynie and Windyfield cherts. Trans R Soc Edinb Earth Sci 94:397–410
Fink S (1984) Some cases of delayed or induced development of axillary buds from persisting detached meristems in conifers. Am J Bot 71:44–51
Galtier J (1999) Contrasting diversity of branching patterns in early ferns and early seed plants. In: Kurmann MH, Hemsley AR (eds) The evolution of plant architecture. Royal Botanic Gardens, Kew, pp 51–64
Giesen P, Berry CM (2013) Reconstruction and growth of the early tree calamophyton (pseudosporochnales, cladoxylopsida) based on exceptionally complete specimens from Lindlar, Germany (mid- Devonian): organic connection of calamophyton branches and duisbergia trunks. Int J Plant Sci 174:1–23
Goebel K (1905). Organography of plants especially of the archegoniatae and spermophyta. Part II. Special Organography, Oxford: Clarendon Press. pp 1–10
Gola EM (2014) Dichotomous branching: the plant form and integrity upon the apical meristem bifurcation. Front Plant Sci 5:263
Harrison CJ (2016) Auxin transport in the evolution of branching forms. New Phytol 215:545–551
Harrison CJ, Rezvani M, Langdale JA (2007) Growth from two transient apical initials in the meristem of Selaginella Kraussiana. Development 134:881–889
Hébant-Mauri R (1984) Branching patterns in Trichomanes and Cardiomanes (hymenophyllaceous ferns). Can J Bot 62:1336–1343
Hébant-Mauri R (1993) Cauline meristems in leptosporangiate ferns: structure, lateral appendages, and branching. Can J Bot 71:1612–1624
Imaichi R (1984) Developmental anatomy of the shoot apex of leptosporangiate ferns. I Leaf ontogeny and shoot branching of Dennstaedtia scabra. J Jap Bot 59:367–379

Kasper AE Jr, Andrews HN Jr (1972) Pertica, a new genus of Devonian plants from northern Maine. Am J Bot 59:897

Kebrom T, Spielmeyer W, Finnegan E (2012) Grasses provide new insights into regulation of shoot branching. Trends Plant Sci 18:41–48

Long J, Barton MK (2000) Initiation of axillary and floral meristems in Arabidopsis. Dev Biol 218:341–353

Meyer-Berthaud B, Scheckler SE, Wendt J (1999) Archaeopteris is the earliest known modern tree. Nature 398:700

Schmitz G, Theres K (2005) Shoot and inflorescence branching. Curr Opin Plant Biol 8:506–511

Schuster RM (1984) Morphology, phylogeny and classification of the Anthocerotae. *New Man Bryol* 2:1071–1092. (RM Schuster, ed)

Shubin N, Tabin C, Carroll S (2009) Deep homology and the origins of evolutionary novelty. Nature 457:818–823

Stahl Y, Simon R (2010) Plant primary meristems: shared functions and regulatory mechanisms. Curr Opin Plant Biol 13:53–58

Stotler B (1972) Morphogenetic patterns of branch formation in the leavy Hepaticae: a resume. Bryologist 75:381–403

Sussex IM, Kerk NM (2001) The evolution of plant architecture. Curr Opin Plant Biol 4:33–37

Thimann K, Skoog F (1933) Studies on the growth hormone of plants: III. The inhibiting action of the growth substance on bud development. Proc Natl Acad Sci U S A 19:714

Thomas BA (1978) Carboniferous lepidodendraceae and lepidocarpaceae. Bot Rev 44:321–364

Tomescu AMF (2009) Megaphylls, microphylls and the evolution of leaf development. Trends Plant Sci 14:5–12

Wickett NJ, Mirarab S, Nguyen N, Warnow T, Carpenter E, Matasci N, Ayyampalayam S, Barker MS, Burleigh JG, Gitzendanner MA et al (2014) Phylotranscriptomic analysis of the origin and early diversification of land plants. Proc Natl Acad Sci 111:E4859–E4868

The Origin of Angiosperms

Charles P. Scutt

Contents

Abstract

The origin of the angiosperms, or flowering plants, is a major question of evolutionary biology, famously described by Charles Darwin as an *abominable mystery*, This group arose from a yet-to-be-identified ancestral lineage and diversified to form over 350,000 species alive today. Recent advances in molecular phylogeny and genetics have combined to provide much information on the origin of the angiosperms and their synapomorphic features, such as the carpel, outer ovule integument, bisexual reproductive axis, and double fertilization. This chapter covers the likely character states of the first angiosperms, their date and place of origin, and possible contenders for close relatives of the angiosperm stem lineage. Later sections show how molecular analyses of living groups are

C. P. Scutt (✉)
Laboratoire Reproduction et Développement des Plantes, Univ Lyon, ENS de Lyon, UCB Lyon 1, CNRS, INRA, Lyon, France
e-mail: charlie.scutt@ens-lyon.fr

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_60

providing clues on the origin of angiosperm-specific developmental programs and suggest how this information might be integrated with paleobotanical data to refine hypotheses on the origin of the angiosperms.

Keywords

Angiosperms · Flowering plants · Flower · Carpel · Outer integument · Evo-devo

Introduction: What Are Angiosperms?

The angiosperms are a monophyletic group of seed plants in which the ovules form inside a specialized female reproductive organ termed the carpel. Indeed, the term *angiosperm* derives from the Greek for seeds within a vessel and contrasts with *gymnosperm*, the name given to the remaining seed plants (conifers, etc.), which refers to naked seeds. The angiosperm carpel functions to protect the ovules within it, receive pollens grains, and guide pollen-tube growth towards the ovules. The carpel can also act as a selective barrier to fertilization, thus preventing close inbreeding and/or interspecific crosses. After fertilization, the ovary tissues of the carpel become the fruit, which protects the developing seeds and may finally contribute to their dissemination through a wide variety of mechanisms. The carpel, fruit, and other specific features of the angiosperms are thought to have contributed to the great evolutionary success of this group, which arose abruptly in the early Cretaceous and rapidly expanded to dominate most terrestrial habitats, today numbering over 350,000 species. In parallel, the gymnosperms have declined to only around 1000 living species.

Carpels almost invariably arise at the center of the angiosperm reproductive axis, or flower. While the flower is also considered unique to angiosperms, it is difficult to provide a simple and rigorous definition of this structure, other than the presence with it of the carpel (and even that definition fails to cover unisexual male flowers). Rather, it is easier to list the typical features of flowers, noting both exceptions to these and cases of evolutionary convergence with the angiosperms' sister group, the living gymnosperms.

Flowers, like gymnosperm cones, generally form as compact reproductive axes from which lateral organs arise in close juxtaposition. Flowers in some angiosperms display, like gymnosperm cones, a spiral phyllotaxy leading to somewhat variable number of organs, though the majority of angiosperms show a whorled arrangement, resulting in relatively fixed numbers and positions of floral organs. A typical flower contains, from inside to outside: a gynoecium of carpels, an androecium of pollen-producing stamens, and a perianth of sterile, bract-like organs. The perianth is most frequently divided into an outer whorl of sepals, which typically function to protect the floral bud, and an inner whorl of petals, which may facilitate pollination by interacting with animal pollinators. However, the perianth may alternatively be undifferentiated, in which case its organs can be termed *tepals*. The perianth may also contain specialized organs such as the lodicules of Poaceae (grasses) or the

nectar spurs sporadically present in several families including Orchidaceae, Ranunculaceae, and Asteraceae.

Angiosperm ovules are generally surrounded by two integuments, except in specific groups that show a secondary reduction to one integument, such as the asterids or certain *Prunus* spp. Gymnosperm ovules, by contrast, possess a single integument, though are surrounded by additional tissue layers in Gnetales. Arils, which take on a fruit-like appearance, also surround the ovules and seeds in some gymnosperm groups including *Juniperus,* Taxaceae, and Podocarpaceae. However, true fruits, derived from the ovary tissues of the carpel, are specific to angiosperms. Though gymnosperms contain pollen-producing microsporangia, which may be considered as homologous to the anthers of angiosperm stamens, the latter have a unique 4-loculate structure, not seen in gymnosperms. Angiosperms possess double fertilization (though a distinct form of this is also present in Gnetales), leading to the production of both an embryo and a biparental reserve tissue termed the endosperm.

Angiosperms also show a number of synapomorphies in their vegetative anatomy and ecophysiological traits (Feild and Arens 2005). These include the presence of xylem vessels (though vessels are absent in some basally diverging angiosperms), and net-veined leaves. Interesting, xylem vessels, though with bordered pits resembling those of conifer tracheids, are also present in Gnetales, while net-veined leaves are present in the Gnetales genus *Gnetum.* Novel ecophysiological adaptations to cope particularly with falling atmospheric carbon dioxide concentrations, may have facilitated the rapid expansion of the angiosperms in the Cretaceous (see chapter ▶ "The Impact of Atmospheric Composition on the Evolutionary Development of Stomatal Control and Biochemistry of Photosynthesis over the Past 450 Ma"). Another factor which almost certainly contributed to the success of the angiosperms was extensive coevolution with insect pollinators, while the relatively short life cycle and rapid growth of early angiosperms may also have helped to improve their fitness by reducing herbivory by the low-browsing herbivorous dinosaurs which came to prominence at that time, as discussed by Willis and McElwain (2013).

When, Where and from What Did the Angiosperms Arise?

Most molecular phylogenetic analyses indicate that the living gymnosperms and angiosperms form two sister clades whose lineages separated some 300 million years ago (MYA). However, the radiation of the extant angiosperms dates from much more recently: most molecular clock estimates place this divergence between 180 and 140 MYA (Bell et al. 2005), while the earliest unequivocal fossilized angiosperm pollen, found in Israel, Morocco and southern England, dates from around 135 MYA (Willis and McElwain 2013). Molecular and fossil data therefore combine to suggest a paleotropical origin for the flowering plants in the late Jurassic/early Cretaceous. Accordingly, we may conclude the living angiosperms to derive from a stem lineage of perhaps some 140 MY in length, from which no other living groups are available for study.

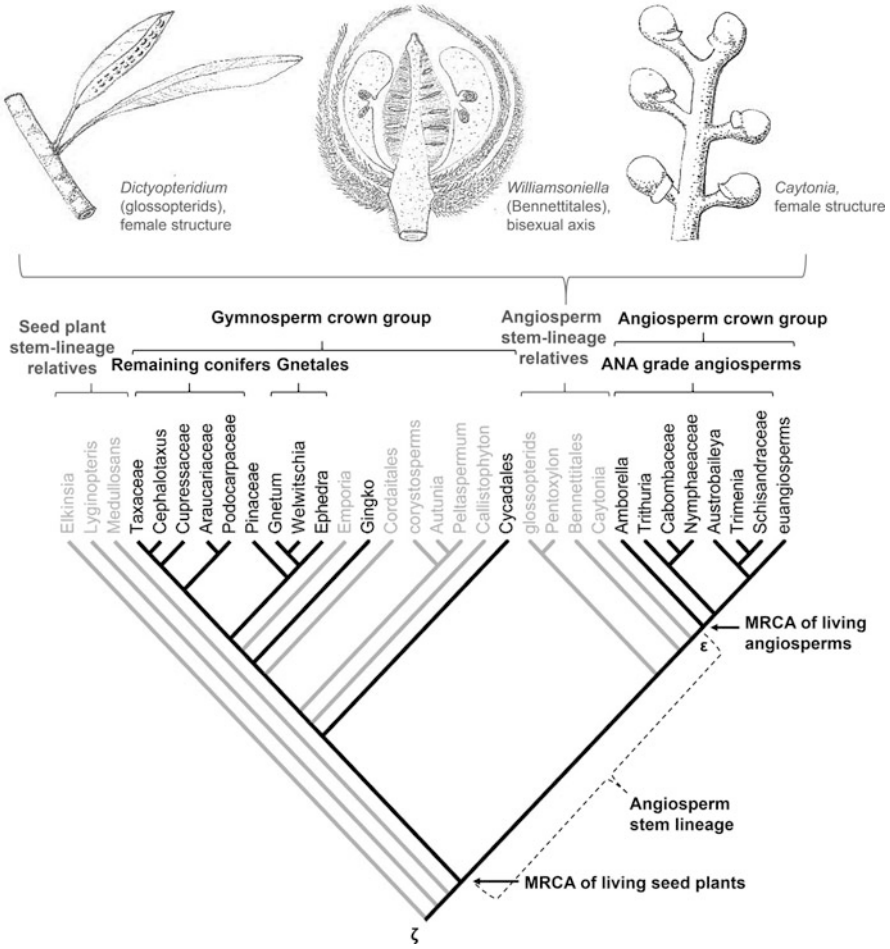


Fig. 1 A phylogeny of extant and extinct seed plants, redrawn from Doyle (2012), focusing on ANA-grade angiosperms and potential angiosperm stem-lineage relatives. Fossil taxa are shown in gray. The approximate positions of the zeta (ζ) and epsilon (ε) whole genome duplications, of probable key importance for the origin of seed plants and angiosperms, respectively, are indicated. Reconstructions of three angiosperm stem-lineage relatives are illustrated (of which *Williamsoniella* is shown in median longitudinal section), redrawn from published sources

Molecular phylogenetic studies (Fig. 1) indicate the earliest bifurcations in angiosperm phylogeny to separate the three orders, Amborellales, Nymphaeales, and Austrobaileyales, collectively termed the ANA-grade, from all other extant angiosperms, termed the euangiosperms or mesangiosperms. Amborellales are represented by the single living species *Amborella trichopoda*, which is a scrambling shrub, endemic to the understory of the tropical rainforests of New Caledonia. Most molecular phylogenetic analyses suggest Amborellales (and hence *A. trichopoda)* to be sister to all other angiosperms, though a few studies support a slightly different

topology in which Amborellales are sister to Nymphaeales (reviewed by Fogliani et al. 2017). Nymphaeales include three families of aquatic or semiaquatic angiosperms, while Austrobaileyales comprise three families of woody plants, including shrubs, trees, and lianas. By contrast to the situation in angiosperms, the internal phylogeny of living gymnosperms is less clear (Doyle 2012), though in the topology which most frequently emerges, shown in Fig. 1, cycads occupy the most basal position, while Gnetales are nested within conifers as sister to Pinaceae.

Morphological phylogenetic analyses have permitted the tentative placement of extinct gymnosperm groups on a molecular phylogenetic backbone of living seed plants (Doyle 2008, 2012; Fig. 1). Two of the most likely relatives of the angiosperm stem lineage are Caytoniales and Bennettitales. The first member of Caytoniales to be discovered was the female reproductive structure *Caytonia* (Fig. 1), which is probably of the same species as the pollen-bearing *Caytonanthus* and leaf-bearing *Sagenopteris* fossils. These plants, like many gymnosperms, possessed saccate pollen grains and a probable droplet-based pollination mechanism but had angiosperm-like net-veined leaves. *Caytonia* ovules developed within laminate cupules, whose potential homology to reproductive structures in angiosperms is discussed below. Interestingly, several further extinct gymnosperms also possessed multiovulate cupules. These include glossopterids (Fig. 1), corystosperms, and peltasperms, of which the former group is considered as a possible stem-lineage relatives of the angiosperms, while the latter two may be more closely related to extant gymnosperms (Fig. 1).

Bennettitales, which are considered as particularly strong candidates for a close relationship to angiosperms, lacked multiovulate cupules but showed the angiosperm-like features of net-veined leaves, nonsaccate pollen, and, in some species, a bisexual reproductive axis (Fig. 1). Interestingly, Bennettitales and angiosperms also share the capacity to synthesize oleananes, which are highly resistant terpenoid compounds that persist even in fossils. Potential strategies to use data from living groups and reconstructed ancestors to help choose between fossil gymnosperm candidates for the ancestor of angiosperms are discussed in the final section of this chapter.

ANA-Grade Angiosperms Provide Clues on the Morphological, Ecological, and Molecular Characteristics of the First Flowering Plants

By mapping the character-states of ANA-grade and other angiosperm species onto molecular phylogenies, it has been possible to reconstruct numerous aspects of the first angiosperms (Fig. 2). Earlier work generally used parsimony-based methods to reconstruct angiosperm features, but some recent studies have incorporated more sophisticated maximum likelihood and/or Bayesian model-based reconstructions (e.g., Willis et al. 2014; Sauquet et al. 2017). A synthesis of reconstruction studies indicates that early angiosperms were probably rapidly growing shrubs, perhaps with some liana-like tendencies, that grew in shaded and disturbed environments such as

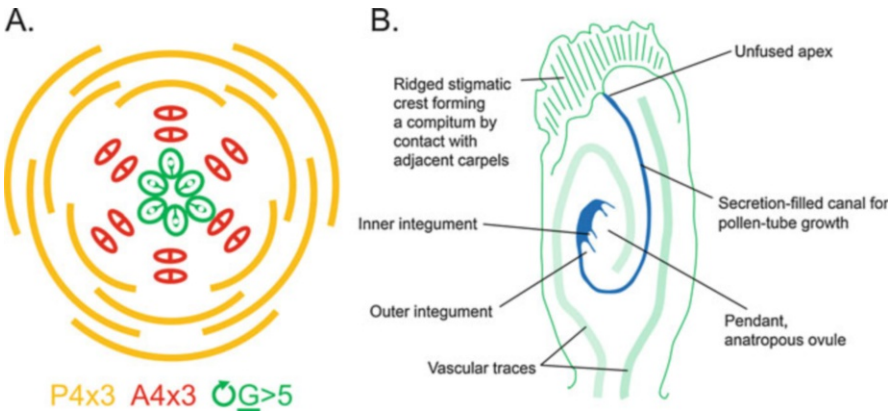


Fig. 2 (**a**) A floral diagram and floral formula representing a reconstruction of the MRCA of living angiosperms, based on Sauquet et al. (2017) and other references in the text. (**b**) Diagram of carpel anatomy in *Trimenia* (ANA grade, Austrobaileyales), redrawn from Doyle (2012), which conserves, with the possible exception of ovule-number (see Sauquet et al. 2017), the major inferred features of the carpel in the reconstructed MRCA of living angiosperms

the borders of rapidly flowing streams running through dense forest (Feild and Arens 2005). Their flowers were probably small (Endress 2001), bisexual (Sauquet et al. 2017), protogynous, actinomorphic and contained an undifferentiated perianth of tepals (Endress and Doyle 2015). Both the perianth and androecium may have contained several subwhorls, each composed of three organs (Sauquet et al. 2017).

The first angiosperms were probably pollinated by generalist insect pollinators and provided pollen rather than nectar as a reward (Endress 2001; Thien et al. 2009). The gynoecium in the MRCA of living angiosperms was almost certainly superior and probably contained more than five carpels (Sauquet et al. 2017). Sauquet et al. (2017) conclude these carpels to have probably been spirally arranged, in contrast to the likely whorled arrangement proposed by these same authors for the perianth and androecium in the MRCA of living angiosperms. However, Sokoloff et al. (2018) have pointed out that flowers may be constrained to be either whorled or spiral throughout, rather than composed of a mixture of spiral and whorled territories, questioning therefore the interpretation of Sauquet et al. (2017). Whatever their phyllotaxy, the carpels of the MRCA of living angiosperms were most likely separate, ascidiate (bottle-shaped), and incompletely fused at the apex, being closed instead by the secretion of substances into an aperture or canal that permitted the penetration of pollen tubes (Endress 2001). By contrast, the carpels of many of the more recently evolved angiosperm groups are plicate (folded), completely closed at the apex, and fused together into a syncarpic gynoecium.

The stigmatic surfaces in the MRCA of living angiosperms were probably covered with multicellular striations, rather than the unicellular papillae more prevalent in later diverging angiosperms. These surfaces may have been in physical contact between adjacent carpels and thus contributed to a compitum that permitted the growth of pollen tubes between carpels and thereby improved the efficiency

of fertilization (Endress and Doyle 2015). The ovary of each carpel probably contained one or a few pendant, anatropous ovules. Each ovule almost certainly possessed two integuments and a large nucellus (or maternal nutritive tissue), and probably contained a four-celled embryo sac (Friedman and Ryerson 2009). Double fertilization would have occurred to generate a diploid zygote and a diploid endosperm. Interestingly, in a few ANA-grade angiosperms, including *Trimena* (Austrobaileyales), several embryo sacs persist in the mature ovule and grow through the nucellus towards the pollen tubes to bring about fertilization. Thus, the growth of female gametophytes, as well as that of pollen tubes, may have contributed to a filter for fitness in early flowering plants (Bachelier and Friedman 2011). Seeds of the ancestral angiosperm probably possessed a form of morphophysiological dormancy, in which the embryo remained relatively small in the mature seed (Willis et al. 2014; Fogliani et al. 2017).

A comparative approach in living species can also be used to determine the likely molecular mechanisms underpinning the phenotypic characters of early angiosperms. Such studies mostly begin from functional genetic data in model angiosperms. Comparative studies in ANA-grade angiosperms can then be used to determine which of the molecular mechanisms under consideration have likely been conserved since the MRCA of extant angiosperms, and which were added later. For the moment, there are no viable functional genetic models among the ANA-grade angiosperms. The methods currently used to ascertain gene functions in these species are therefore largely indirect and are often based on the conservation of expression patterns (Fig. 3) and/or of protein functions in vitro or in heterologous in vivo systems (e.g., in transgenic model angiosperms).

Using a range of comparative molecular approaches, it has been possible to conclude that many of the mechanisms controlling flower development in eudicot models such as *Arabidopsis* have probably been conserved since the MRCA of extant angiosperms. Notably, the ABC model of flower development, initially constructed in *Arabidopsis* and *Antirrhinum*, shows considerable conservation in ANA-grade angiosperms. In this model, which is extensively described in the chapter ▶ "Evolution of Floral Organ Identity," the overlapping expression domains in the floral meristem of several classes of transcription factors, almost all of which belong to the MADS-box gene family, control the identity of sepals, petals, stamens, and carpels in the first to fourth floral whorls, respectively. The ABC model was extended from a genetic to a biochemical model by the addition of the MADS-box E-function, which is expressed in all floral whorls. E-function MADS-box proteins are capable of forming heterotetramers of different combinations with other classes of floral MADS-box proteins, and each type of complex formed is hypothesized to interact with pairs of so-called CArG motifs (of consensus sequence $CC[A/T]_6GG$) in the promoters of distinct sets of target genes, thus bringing about the development of the various different floral organ types. The expression domains of floral MADS-box genes appear generally conserved in ANA-grade angiosperms (Kim et al. 2005; Fig. 3), suggesting the ABCE model to have already functioned in early angiosperms. One of the main differences in floral MADS-box gene expression between ANA-grade angiosperms such as *Amborella* and eudicot models such as *Arabidopsis*

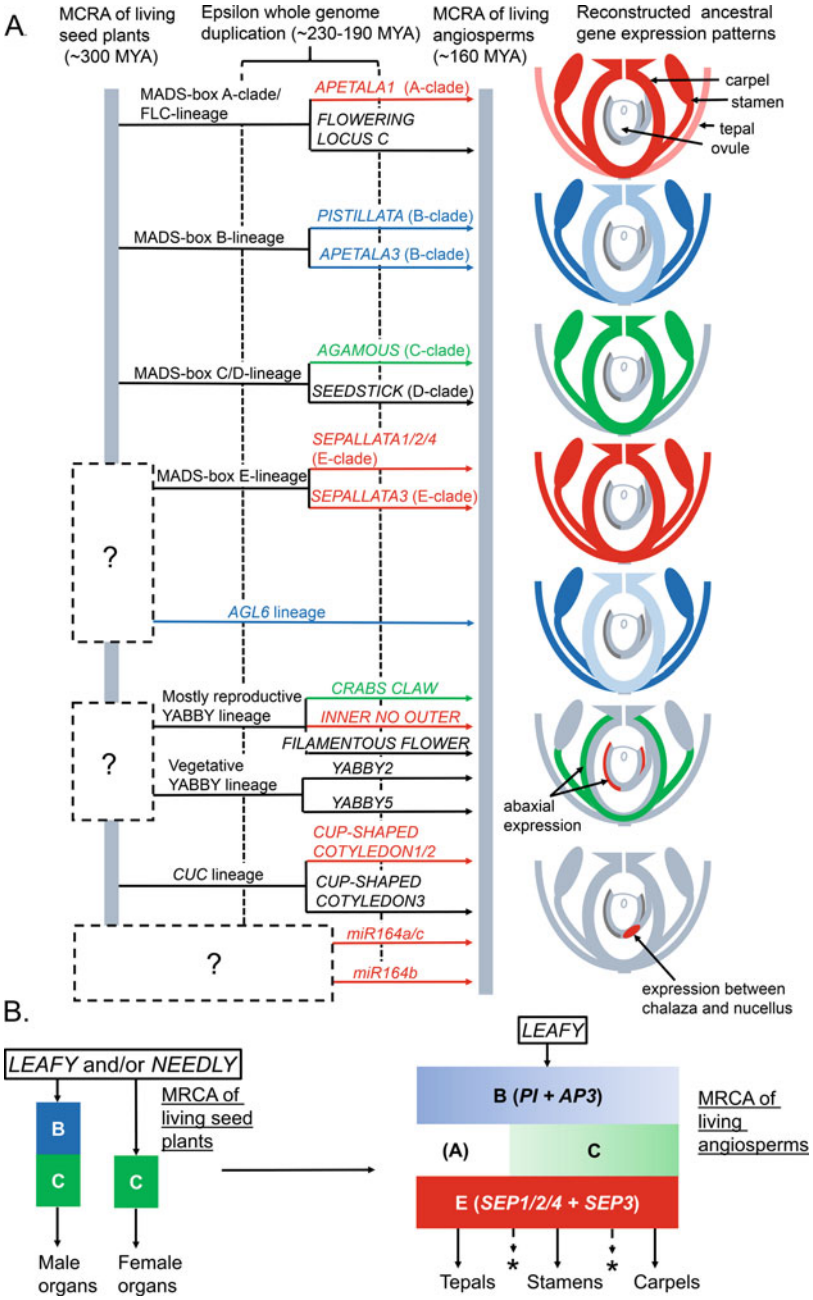


Fig. 3 (**a**) Evolution of flower-development regulators along the angiosperm stem lineage and their reconstructed expression patterns in the MRCA of living angiosperms. Gene lineages are named following their first-published descendent(s) in *Arabidopsis*. Uncertainties in the order or timing

is that expression domains appear less clearly delimited in the ANA-grade (Fig. 3b), which may explain the typically gradual transformations observed between floral organ types in the ANA grade, particularly in species such as *Amborella* in which floral organs are arranged spirally rather than in whorls.

Besides MADS-box genes, numerous other classes of regulators are known to control floral development in model angiosperms, and some of these have also been investigated in ANA-grade angiosperms to evaluate the potential conservation of their functions since the MRCA of extant angiosperms. These regulators include the YABBY transcription factors INNER NO OUTER (INO) and CRABS CLAW (CRC), which have been concluded to control abaxial-adaxial polarity and organ expansion in the outer integument and carpel, respectively, since the base of living angiosperms (Yamada et al. 2003; Fourquin et al. 2005). The developmental module involving CUP-SHAPED COTYLEDON (CUC)/NO APICAL MERISTEM (NAM) transcription factors and their microRNA regulator *miRNA164* plays a role in defining the boundary between the nucellus and chalaza that also appears to have been conserved since the MRCA of extant angiosperms (Jasinski et al. 2010; Vialette-Guiraud et al. 2011). It furthermore seems likely that expression of *miR164* may downregulate *CUC* genes to facilitate the fusion of carpel margins in ANA-grade angiosperms as it does in eudicots (Vialette-Guiraud et al. 2016b).

Many further classes of regulators have been analyzed using phylogenomic techniques to attempt to correlate changes in the structure of gene families (duplications, losses, etc.) with morphological evolutionary history (Pfannebecker et al. 2017a, b). The addition of expression studies to these phylogenomic data should provide stronger evidence for potential roles of the genes analyzed in early flowering plants. In the longer term, direct methods for functional genetic analysis in the ANA grade will be essential to a rigorous demonstration of the developmental roles of regulatory genes in these species (Scutt and Vandenbussche 2014). Such direct functional data in ANA-grade angiosperms should help to refine morphological reconstructions of the ancestral flower. For example, recent reconstruction work (Sauquet et al. 2017) has indicated with high confidence the presence of a whorled, trimerous perianth and androecium in the MRCA of euangiosperms and suggested, but with lower confidence, that these features were already present in the MRCA of all living angiosperms. It is clear that this latter conclusion must depend strongly on the presence of a whorled, trimerous arrangement in some taxa of Nymphaeales, in

Fig. 3 (continued) of gene duplications are indicated by dotted boxes or polytomies. Lighter shades indicate weaker regions of gene expression. Data were collated from Fourquin et al. (2005), Kim et al. (2005), Jasinski et al. (2010), Jiao et al. (2011), Yamada et al. (2011), Gramzow et al. (2014), Vialette-Guiraud et al. (2016a), and Moyroud et al. (2017). (**b**) Molecular models of reproductive development in the MRCAs of living seed plants and angiosperms, based on the (A)BC model of flower development proposed of Causier et al. (2010), modified to fit reconstructed ancestral gene expression patterns. MADS-box gene expression patterns in early angiosperms, as in some living ANA-grade taxa, may have had *fuzzy* boundaries, as indicated by color gradients, leading to the production of intermediate floral organ types, as indicated by asterisks

addition to euangiosperms. If the whorled, trimerous arrangements in Nymphaeales and euangiosperms prove to involve similar molecular mechanisms, brought about by orthologous sets of regulators, such a result would provide very strong support for the conclusion that a trimerous, whorled arrangement had evolved prior to the split between Nymphaeales and the common ancestral lineage of Austrobaileyales and euangiosperms.

Evo-Devo Hypotheses for the Origin of Angiosperm-Specific Reproductive Features

The Outer Integument and Carpel

The inner integument of angiosperms appears homologous to the single integument of gymnosperms and therefore can be concluded to have arisen over 300 MYA in a common ancestor of living seed plants. The outer integument and carpel, by contrast, are unique to angiosperms and therefore must have arisen along the angiosperm stem lineage, before the MRCA of living angiosperms that probably lived some 160 MYA. Distinct sets of genes control organ polarity in the inner and outer integument, at least in *Arabidopsis* (Kelley et al. 2009), reflecting the separate temporal origins of these organs.

Doyle (2008) summarizes several evolutionary hypotheses that could account for the approximately simultaneous evolution of the carpel and outer integument. One of these mechanisms postulates the cupule-bearing megasporphyll of a *Caytonia*-like gymnosperm as a progenitor of the female reproductive arrangement in angiosperms (Scenario 1 in Fig. 4). *Caytonia* cupules appear to be pinnately distributed along a radially symmetrical rachis, and each cupule contains 8–30 unitegmetic ovules that form on its adaxial surface. The outer integument may have evolved from the cupule wall by a reduction in ovule-number to one-per-cupule, while the carpel may then have been formed by the expansion and conduplicate folding of the rachis around the resulting bitegmetic ovules. This potential evolutionary mechanism leads elegantly to the anisotropous ovule, thought to have been present in early angiosperms (Endress and Doyle 2015).

Another cupule-bearing structure proposed as a potential progenitor of the carpel is the glossopterid-type sporophyll (Scenario 2 in Fig. 4). In this case, the carpel could be interpreted as a compound structure that includes both the cupulate sporophyll and its subtending bract. However, fossil evidence of glossopterids considerably predates the likely origin of the angiosperms, and these plants have also been suggested as possible ancestors of Caytoniales and therefore as possible indirect ancestors of the angiosperms. A further possibility is that the angiosperms evolved from Bennettitales (Scenario 3 in Fig. 4), in which case, the progenitor organs to the carpel and outer integument of angiosperms would remain unclear.

The origin of the outer integument and carpel probably took place at around the time of the epsilon whole genome duplication event (Jiao et al. 2011; Fig. 1). Numerous gene families involved in outer integument and/or carpel development

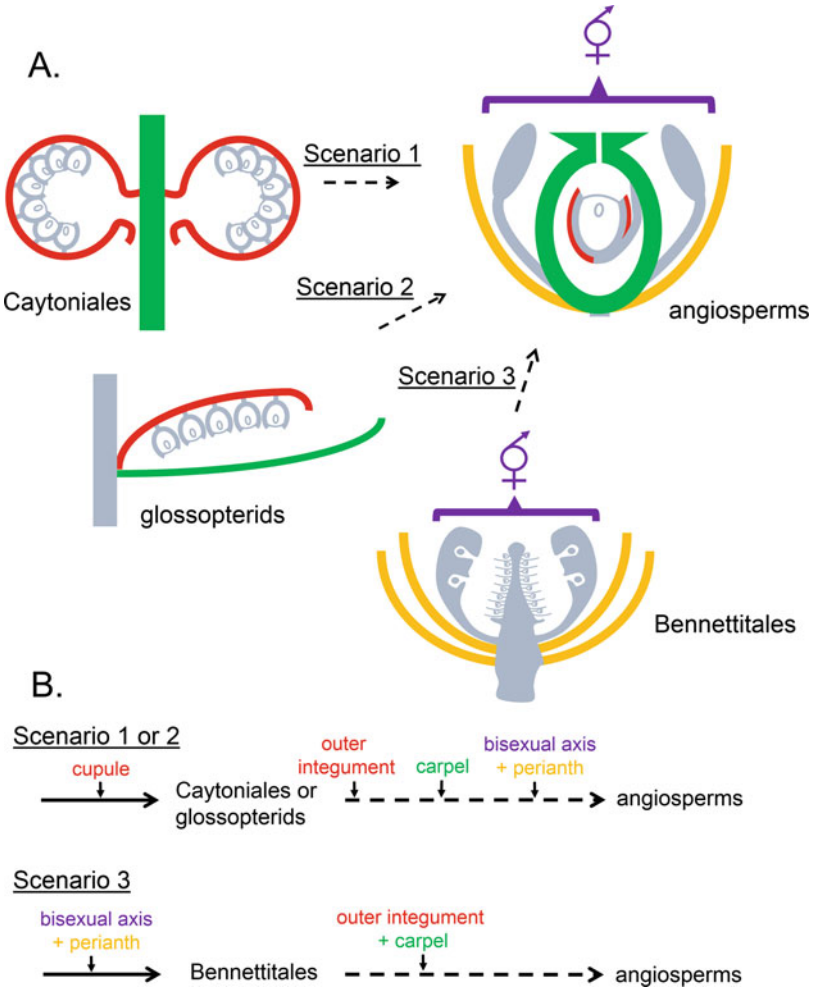


Fig. 4 (**a**) Three alternative evolutionary scenarios for the origin of major specific features of the flower (the outer integument, carpel, perianth, and bisexual axis) from ancestors resembling Caytoniales, glossopterids, and Bennettitales (also illustrated in Fig. 1), based in part on Doyle (2008, 2012). Potentially homologous features of stem-lineage relatives and angiosperms are indicated using colors. (**b**) Two alternative timelines for the acquisition of angiosperm-specific features, depending on the evolutionary scenario (from **a**) considered

contain paralogs that were apparently retained in this duplication (Fig. 3a) or in localized duplications that occurred at a similar evolutionary stage. Carpel development in model eudicots is under the control of C- and E-function MADS-box transcription factors, which are thought to act as heterotetramers, each composed of two C- and two E-function proteins (see chapter ▶ "Evolution of Floral Organ Identity"). The angiosperm C-lineage was derived from a duplication that took place along the angiosperm stem lineage and separated this from the angiosperm

D-lineage, whose genes play various roles in carpel and ovule development. The gymnosperm pro-orthologs of angiosperm C/D genes are also expressed in female tissues. Hence, neofunctionalization processes that followed the separation of the C and D MADS-box lineages may have been key events in the origin of the carpel, by adapting part of the molecular toolkit that previously specified megasporophyll and ovule development in a gymnospermous progenitor of the angiosperms.

The early evolution of the E-clade of MADS box genes, also involved in carpel development, is enigmatic. These genes are present only in angiosperms, but there were apparently already two separate E-class lineages in the MRCA of living angiosperms (Fig. 3a). The nearest relatives to E-function genes in gymnosperms are genes of the *AGAMOUS LIKE6 (AGL6)* lineage, which is also present in angiosperms. It is not currently clear whether an ancient E-clade was lost in gymnosperms or whether the angiosperm E-clade was derived by a duplication in the angiosperm *AGL6* lineage, followed by unequal rates of evolution in the resulting paralogous lineages (Fig. 3a). It is also not currently clear whether the gymnosperm orthologs of angiosperm MADS-box floral homeotic proteins form tetramers *in planta*, and if so, of what composition. If MADS-box protein tetramer formation proves to be unique to the angiosperms, then clearly the origin of this molecular feature may have played a key role in the origin of the carpel and other floral organs.

Bisexuality

Bisexuality is predominant in the ANA-grade, as in the angiosperms as a whole, and ancestral reconstruction suggests this character was present in the MRCA of living angiosperms (Sauquet et al. 2017; Fig. 2a). Bisexuality is not, however, completely specific to angiosperms, as flower-like bisexual reproductive axes were present in the extinct gymnosperm order Bennettitales (Figs. 1 and 4), while morphologically bisexual, though functionally unisexual, reproductive axes are present in Gnetales. In addition, mutations causing bisexuality have been reported in gymnosperms including *Picea, Pinus, Pseudotsuga, Juniperus, Sequoia, Abies,* and *Agathis* (Lanner 1966), suggesting that the transition from unisexual to bisexual reproductive axes may not require very numerous or complex genetic changes.

Despite the apparent genetic simplicity of transitions to bisexuality, a number of hypotheses for the origin of the angiosperms have focused particularly on the acquisition of this character along the angiosperm stem lineage. Most of these hypotheses, reviewed in detail in the chapter ▶ "Evolution of Floral Organ Identity," emphasize the role of the transcription factor LEAFY (LFY), a master controller of floral patterning in angiosperms. In *Arabidopsis, LFY* is a unique gene that is expressed specifically in the floral meristem and interacts with cofactors including *UNUSUAL FLORAL ORGANS* (*UFO,* F-box ubiquitinase family) and *WUSCHEL* (*WUS;* homeobox transcription factor family) to set up the correct expression of MADS floral homeotic genes in overlapping zones of the floral meristem. This includes the expression of B-, C- and E-function MADS genes in the zones that will give rise to the androecium and gynoecium. An ortholog of *LFY* is present in

gymnosperms, and most gymnosperms also possess a second *LFY*-like gene termed *NEEDLY (NLY),* whose angiosperm ortholog was apparently lost before the radiation of extant angiosperms. Recent biochemical work has shown that *LFY* in *Welwitschia* (Gnetales) is specifically able to bind to B-function MADS-box gene promoters, indicating the conservation of early steps in the patterning of the reproductive axis between angiosperms and gymnosperms (Moyroud et al. 2017, Fig. 3b).

According to the mostly male theory (MMT; Frohlich 2003), bisexuality in a common ancestor of living angiosperms first arose through the ectopic production of ovules on microsporophylls situated towards the apex of its cone-like axes. The basal microsporophylls of these cones would have remained unisexual, while the subsequent loss of the microsporangia from the now bisexual sporophylls at the apex would have led to a bisexual axis composed of apical megasporophylls and basal microsporophylls. These transitions were, it is hypothesized, accompanied by the loss of *NLY* and all downstream pathways uniquely regulated by this factor. The MMT thus proposes that all angiosperm reproductive tissues, other than those of the ovule itself, are derived from previously male developmental programs. However, *LFY* and *NLY* have not proven to show strictly respective male- and female-specific expression patterns in all gymnosperms (Vazquez-Lobo et al. 2007), as might be predicted from the MMT, while studies of the sex-specific expression patterns of orthologous genes in angiosperms and gymnosperms have also failed to support this hypothesis (Tavares et al. 2010).

Despite the above difficulties, the MMT remains a useful conceptual framework which has encouraged the development of further hypothesis for the origin of the bisexual flower. One such hypothesis by Baum and Hileman (2006) also proposes a central role for *LFY* but postulates that the origin of floral bisexuality was caused by a change in the relative binding affinity of this factor to B- and C-function MADS-box gene promoters. During the evolutionary origin of the flower, B-genes would, accordingly, have become less sensitive to LFY than C-genes, such that C-proteins would have predominated at the lower LFY concentrations found near the apex of the reproductive axis, giving rise to female organs. Meanwhile, B- and C-genes would have been transcribed at comparable levels under the higher LFY concentrations encountered lower down the axis, thus giving rise to an outer whorl of male organs.

The Perianth

Character state reconstructions indicate that a bract-derived perianth was likely present in the MRCA of flowering plants (Endress and Doyle 2015) and may have shown a whorled arrangement (Sauquet et al. 2017; Fig. 2a). However, perianth-like organs are not entirely specific to angiosperms as these were present in the flower-like reproductive axes of Bennettitales and also occur in the reproductive axes of all three extant genera of Gnetales: *Ephedra, Gnetum,* and *Welwitschia*. In addition, female cones of Pinaceae are made up of developmental modules in which each ovulate scale (or megasporophyll) is subtended by an outer or bract scale, which

might thus be considered to occupy an equivalent position to a perianth organ in angiosperms. Given all of these examples of perianth-like organs external to the flowering plants, and the lack of clearly assignable angiosperm ancestors in the fossil record, it is not currently clear at what stage the perianth arose, compared to other angiosperm synapomorphies such as the carpel, outer integument, and bisexual axis (Fig. 4b). Indeed, organs of potential homology to the carpel and outer integument are much clearer in *Caytonia* and glossopterids than in Bennettitales, whereas the latter group shares the other angiosperm characteristics of a bisexual reproductive axis and perianth. The accurate assignment of closest angiosperm stem-lineage relatives would therefore help enormously to elucidate the order of appearance of the major synapomorphies of the angiosperm flower.

It is important to note that inner perianth organs, which are often petaloid, may have a distinct evolutionary origin from outer perianth organs, particularly in later diverging angiosperm groups within the core eudicots. In these latter cases, petals are believed to be derived not from bracts, as in more basally diverging angiosperms, but from outer stamens that have become secondarily sterilized, as reviewed by Ronse de Craene and Brockington (2013).

The original ABC model for flower development has been modified recently to a more generally applicable (A)BC model, which emphasizes the role of A-clade MADS box genes in floral patterning, rather than in the identity of perianth organs (Causier et al. 2010; Fig. 3b). According to the (A)BC model, the specification of outer perianth organ (sepal) identity does not require MADS-box gene expression of the A, B, or C classes, while combined B- and E-function expression, in the absence of C-function expression, generates inner perianth organs, or petals, in the second floral whorl. As previously mentioned, gene expression studies in the ANA grade suggest the role of B- and E-function genes in the generation of petaloid perianth organs in eudicots has been conserved since the earliest stages of angiosperm evolution, even if a major switch from bract-derived *bracteopetals* to stamen-derived *andropetals* subsequently occurred within the core eudicots and other groups.

Embryo Sac Anatomy and Fertilization Mechanisms

Gymnosperm ovules consist of a haploid megagametophyte that develops from a functional megaspore within a diploid nucellus, which is, in turn, enclosed with a single integument (Linkies et al. 2010). Gymnosperm megagametophytes typically produce several archegonia, each containing an egg cell. Fertilization by sperm nuclei from pollen grains thus often leads to the development of several embryos within each ovule, though in most cases, only one of these will survive. In gymnosperms, therefore, fertilization essentially involves a single event between two haploid nuclei to generate a diploid zygote that will divide to form the embryo within the seed. The gymnosperm nucellus, which is a diploid maternal tissue, functions to store food reserves to support the growth of the embryo during and immediately after germination.

By contrast to gymnosperms, double fertilization, leading to the production of both an embryo and a biparental nutritive endosperm, is present in angiosperms (Friedman and Ryerson 2009). A distinct form of double fertilization is known in *Ephedra* and *Gnetum* of the gymnosperm order Gnetales, though this gives rise to two zygotes, one of which subsequently degrades. Due to its biparental origin and key role in the fitness of offspring, the angiosperm endosperm is believed to play an important role in parental conflict (as does the placenta in mammals), which reflects the different interests of male and female parents in the supply of resources to developing offspring. In most angiosperms, the embryo sac is of the *Polygonum* type, containing seven cells of which the central cell, which will give rise to the endosperm, is binucleate. The two nuclei of the central cell combine with one sperm nucleus following fertilization to generate a triploid endosperm. However, in the ANA-grade genera *Nuphar* and *Trithuria* (both in Nymphaeales) and *Illicium* (Austobaileyales), the embryo sac contains four cells, including a uninucleate central cell. Double fertilization in these taxa generates an embryo and endosperm, both of which are diploid. In *Amborella*, the only representative of the remaining ANA-grade order Amborellales, the embryo sac contains eight cells, including a binucleate central cell that produces a triploid endosperm after fertilization. The *Amborella* embryo sac arrangement is thus more similar to that of *Polygonum* and the majority of later-diverging angiosperms than to other members of the ANA grade.

Because of this distribution of characters, character state reconstruction by parsimony fails to formally resolve the embryo sac arrangement in the MRCA of living angiosperms between four-, seven-, and eight-celled types, or the ploidy of its endosperm, which might be either diploid or triploid. However, Friedman and Ryerson (2009) argue that the *Amborella* and *Polygonum* embryo sac arrangements represent variants on a doubled form of a basic module composed of four cells, which is still present in Nymphaeales and Austrobaileyales. The doubling of the embryo sac module, according to this view, occurred in parallel in the Amborellales and euangiosperm lineages but involved one extra cell division in Amborellales. Thus, according to Friedman and Ryerson (2009), the MRCA of living angiosperms would have contained a four-celled embryo sac and double fertilization leading to a zygote and endosperm, both of which were diploid. Interestingly, both of the hypothesized independent origins of a triploid biparental endosperm (in Amborellales and euangiosperms, respectively) may have had a role in increasing the female genetic component of the endosperm and thus biasing parental conflict in favor of female factors that promote an equal distribution of nutrients among sibling and/or half-sibling offspring.

Interestingly, an extensive perisperm is present in mature seeds of *Trithuria* (Nymphaeales), in addition to a diploid endosperm and a very small, underdeveloped embryo (Friedman 2008). Similarly to the endosperm, the perisperm is an embryo-nourishing tissue, though one which is derived exclusively from the maternal nucellus. The presence of a nutritive perisperm or nucellus is mainly associated with gymnosperms and the prominence of this tissue in *Trithuria* has been suggested to form a link with the gymnosperm-like ancestor of the flowering plants.

Technical Developments to Help Elucidate the Origin of Angiosperms

A number of recent developments provide much hope for efforts to understand the origin of the angiosperms. At present, complete genome sequence data, in draft form, is available only from one ANA-grade angiosperm (*Amborella trichopoda*) and two closely related gymnosperm taxa (*Pinus* spp. and *Picea abies*). However, increasing efficiency and decreasing costs of next generation sequencing (NGS) make it likely that we will soon have available a much more complete list of whole genome and transcriptome sequences. Certain recent NGS methods provide reads extending to tens of kilobases, thus simplifying the problem of genome assembly in the absence of physical linkage maps and genetic markers, etc. This will be particularly useful for the sequencing of basal angiosperms and gymnosperms, all of which, with the exceptions of Hydatellaceae and Nymphaeaceae (both in Nymphaeales), have moderately large genomes. In parallel, excellent progress has been made to develop methods, based on the combined analysis of synteny and phylogeny, to reconstruct the structure of ancestral genomes from those of their living descendants (Murat et al. 2017). Accordingly, we may soon be able to reconstruct, in considerable detail, the genome of the ancestor of living angiosperms, and even that of their more distant ancestor from before the epsilon whole genome duplication event.

To fill the current gaps in functional studies of developmental regulators in basal angiosperms and gymnosperms, a number of recent developments appear promising (Scutt and Vandenbussche 2014). First, the recently discovered *Nymphaea thermarum,* and possibly also certain *Trithuria* spp. (both from Nymphaeales), could form well-adapted model ANA-grade angiosperms, amenable to molecular-genetic studies. If transformation methods can be developed for these species, the exciting recent developments in gene editing should greatly facilitate functional studies of genes that were crucial to the origin of the angiosperms.

A second major approach that can help to elucidate the evolution of developmental regulatory mechanisms in non-model plants consists of using in vitro and heterogeneous in vivo methods to measure protein-DNA and protein-protein interactions (Vialette-Guiraud et al. 2016a). These methods can be combined with genomic-scale analyses and modeling approaches to describe the networks of posttranscriptional, transcriptional, and epigenetic interactions that control reproductive development in convenient angiosperm and gymnosperm species of importance for the study of angiosperm origin and other important evo-devo questions. Furthermore, the approach of ancestral sequence reconstruction, also known as *protein resurrection*, can be used to directly study the biophysical and biochemical properties of ancestral regulatory molecules from key stages in plant evolution.

In terms of paleobotany, hope for discoveries of further early angiosperms and stem lineage-relatives comes from mesofossils, which are fossils of up to a few millimeters in diameter, often preserved as coalified specimens that were generated by intense forest fires or similar events (Schoenenberger 2005). These coalified fossils often show anatomical details down to the cellular and even subcellular

level and can be examined thoroughly and nondestructively using recently developed tomographic methods. As the earliest angiosperms likely had small flowers and grew in the forest understory, the chances of their preservation as coalified mesofossils appear relatively high.

Integration of Data from Neo- and Paleobotany

The task of understanding the origin of angiosperms is considerable, and it is clear that evidence from both living and extinct groups will be needed to provide the fullest possible answer to this question (see chapter ▶ "Structural Fingerprints of Development at the Intersection of Evo-Devo and the Fossil Record"). There are several ways in which data from fossils and living plants can be combined to useful effect. For example, well characterized and accurately dated fossil divergences can be used to calibrate the molecular clock and thereby provide the best possible date for key points in molecular phylogenies, such as the MRCA of living angiosperms, which are not themselves directly represented in the current meso- or macrofossil record (Bell et al. 2005).

Paleobotanical data can also be used to support or refute given phylogenetic topologies generated from molecular data. For example, molecular phylogenies performed using various different data-sets and methods suggest four distinct possible topologies for the living seed plants (Doyle 2012). However, the fossil record indicates that Gnetales and angiosperms are of more recent origin than ginkophytes and cycads, and this information is only consistent with a subset of molecular topologies in which Gnetales arose recently, within or close to conifers, such as that shown in Fig. 1. Paleobotanical data thus refute alternative topologies in which Gnetales emerge in a basal position within extant gymnosperms.

Evo-devo hypotheses based principally on data from extant species can sometimes be reinforced by the use of data from fossil plants. Such an example is provided by the mostly male theory (MMT; Frohlich 2003), whose original formulation suggested corystosperms as a potential unisexual ancestor to the bisexual angiosperms. The MMT did not depend on the identification of corystosperms in this role, or provide a test of this group as a potential angiosperm ancestor; rather, it suggested a pair of hypotheses that were mutually consistent and thus appeared stronger than either hypothesis would have on its own.

A fourth and more ambitious combination of data can be envisaged in which molecular-developmental information from living species can be used to test evolutionary hypotheses based on paleobotanical data. Though no DNA is present in the fossils of potential relevance to the origin of the angiosperms, it is possible in many cases to *resurrect*, using ancestral reconstruction, the proteins encoded by genes that were present along the angiosperm stem lineage. The structure, activity, and molecular evolution of proteins that controlled plant development along the angiosperm stem lineage may be informative on the morphology and development of the species in which they functioned, and this information might be used to choose between potential angiosperm ancestors (or close stem-lineage relatives) from the fossil

record. For example, the timeline of neofunctionalization events in selected gene lineages could be compared with hypothesized developmental evolutionary sequences to support or refute hypotheses such as the origin of the angiosperm outer integument and carpel from female structures in cupulate gymnosperms, or the origin of the angiosperm perianth from preexisting structures in the flower-like reproductive axes of Bennettitales, thus testing the relative likelihood of the alternative scenarios shown in Fig. 4.

Cross-References

- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Evolution of Floral Organ Identity
- ▶ Structural Fingerprints of Development at the Intersection of Evo-Devo and the Fossil Record
- ▶ The Impact of Atmospheric Composition on the Evolutionary Development of Stomatal Control and Biochemistry of Photosynthesis over the Past 450 Ma

Acknowledgments Work on the origin of angiosperms in the author's laboratory is supported by grants from the *Agence Nationale de la Recherche* DS0502 *NGSexe* and ANR-13-BSV2-0009 *ORANGe*.

References

Bachelier JB, Friedman WE (2011) Female gamete competition in an ancient angiosperm lineage. Proc Natl Acad Sci U S A 108:12360–12365. https://doi.org/10.1073/pnas.1104697108

Baum DA, Hileman LC (2006) A developmental genetic model for the origin of the flower. In: Ainsworth C (ed) Flowering and its manipulation. Blackwell, Oxford, pp 3–27

Bell CD, Soltis DE, Soltis PS (2005) The age of the angiosperms: a molecular timescale without a clock. Evolution 59:1245–1258. https://doi.org/10.1111/j.0014-3820.2005.tb01775.x

Causier B, Schwarz-Sommer Z, Davies B (2010) Floral organ identity: 20 years of ABCs. Semin Cell Dev Biol 21:73–79. https://doi.org/10.1016/j.semcdb.2009.10.005

Doyle JA (2008) Integrating molecular phylogenetic and paleobotanical evidence on origin of the flower. Int J Plant Sci 169:816–843. https://doi.org/10.1086/589887

Doyle JA (2012) Molecular and fossil evidence on the origin of angiosperms. In: Jeanloz R (ed) Annual review of earth and planetary sciences, vol 40. Department of Evolution and Ecology, University of California, Davis, pp 301–326

Endress PK (2001) The flowers in extant basal angiosperms and inferences on ancestral flowers. Int J Plant Sci 162:1111–1140. https://doi.org/10.1086/321919

Endress PK, Doyle JA (2015) Ancestral traits and specializations in the flowers of the basal grade of living angiosperms. Taxon 64:1093–1116

Feild TS, Arens NC (2005) Form, function and environments of the early angiosperms: merging extant phylogeny and ecophysiology with fossils. New Phytol 166:383–408. https://doi.org/10.1111/j.1469-8137.2005.01333.x

Fogliani B, Gateble G, Villegente M, Fabre I, Klein N, Anger N, Baskin CC, Scutt CP (2017) The morphophysiological dormancy in Amborella trichopoda seeds is a pleisiomorphic trait in angiosperms. Ann Bot 119:581–590. https://doi.org/10.1093/aob/mcw244

Fourquin C, Vinauger-Douard M, Fogliani B, Dumas C, Scutt CP (2005) Evidence that CRABS CLAW and TOUSLED have conserved their roles in carpel development since the ancestor of

the extant angiosperms. Proc Natl Acad Sci U S A 102:4649–4654. https://doi.org/10.1073/pnas.0409577102
Friedman WE (2008) Hydatellaceae are water lilies with gymnospermous tendencies. Nature 453:94–97. https://doi.org/10.1038/nature06733
Friedman WE, Ryerson KC (2009) Reconstructing the ancestral female gametophyte of angiosperms: insights from Amborella and other ancient lineages of flowering plants. Am J Bot 96:129–143. https://doi.org/10.3732/ajb.0800311
Frohlich MW (2003) An evolutionary scenario for the origin of flowers. Nat Rev Genet 4:559–566. https://doi.org/10.1038/nrg1114
Gramzow L, Weilandt L, Theissen G (2014) MADS goes genomic in conifers: towards determining the ancestral set of MADS-box genes in seed plants. Ann Bot 114:1407–1429. https://doi.org/10.1093/aob/mcu066
Jasinski S, Vialette-Guiraud ACM, Scutt CP (2010) The evolutionary-developmental analysis of plant microRNAs. Philos Trans Royal Soc B-Biol Sci 365:469–476. https://doi.org/10.1098/rstb.2009.0246
Jiao Y, Wickett NJ, Ayyampalayam S, Chanderbali AS, Landherr L, Ralph PE, Tomsho LP, Hu Y, Liang H, Soltis PS, Soltis DE, Clifton SW, Schlarbaum SE, Schuster SC, Ma H, Leebens-Mack J, dePamphilis CW (2011) Ancestral polyploidy in seed plants and angiosperms. Nature 473:97–U113. https://doi.org/10.1038/nature09916
Kelley DR, Skinner JD, Gasser CS (2009) Roles of polarity determinants in ovule development. Plant J 57:1054–1064. https://doi.org/10.1111/j.1365-313X.2008.03752.x
Kim S, Koh J, Yoo MJ, Kong HZ, Hu Y, Ma H, Soltis PS, Soltis DE (2005) Expression of floral MADS-box genes in basal angiosperms: implications for the evolution of floral regulators. Plant J 43:724–744. https://doi.org/10.1111/j.1365-313X.2005.02487.x
Lanner R (1966) A unusual bisexual Agathis cone. Pac Sci 20:382–385
Linkies A, Graeber K, Knight C, Leubner-Metzger G (2010) The evolution of seeds. New Phytol 186:817–831. https://doi.org/10.1111/j.1469-8137.2010.03249.x
Moyroud E, Monniaux M, Thevenon E, Dumas R, Scutt CP, Frohlich MW, Parcy F (2017) A link between LEAFY and B-gene homologues in *Welwitschia mirabilis* sheds light on ancestral mechanisms prefiguring floral development. New Phytol 216:469–481. https://doi.org/10.1111/nph.14483
Murat F, Armero A, Pont C, Klopp C, Salse J (2017) Reconstructing the genome of the most recent common ancestor of flowering plants. Nat Genet 49:490. https://doi.org/10.1038/ng.3813
Pfannebecker KC, Lange M, Rupp O, Becker A (2017a) An evolutionary framework for carpel developmental control genes. Mol Biol Evol 34:330–348. https://doi.org/10.1093/molbev/msw229
Pfannebecker KC, Lange M, Rupp O, Becker A (2017b) Seed plant-specific gene lineages involved in carpel development. Mol Biol Evol 34:925–942. https://doi.org/10.1093/molbev/msw297
Ronse de Craene LPR, Brockington SF (2013) Origin and evolution of petals in angiosperms. Plant Ecol Evol 146:5–25. https://doi.org/10.5091/plecevo.2013.738
Sauquet H, von Balthazar M, Magallon S, Doyle JA, Endress PK, Bailes EJ, de Morais EB, Bull-Herenu K, Carrive L, Chartier M, Chomicki G, Coiro M, Cornette R, El Ottra JHL, Epicoco C, Foster CSP, Jabbour F, Haevermans A, Haevermans T, Hernandez R, Little SA, Loefstrand S, Luna JA, Massoni J, Nadot S, Pamperl S, Prieu C, Reyes E, dos Santos P, Schoonderwoerd KM, Sontag S, Soulebeau A, Staedler Y, Tschan GF, Leung AW-S, Schoenenberger J (2017) The ancestral flower of angiosperms and its early diversification. Nat Commun 8:16047. https://doi.org/10.1038/ncomms16047
Schoenenberger J (2005) Rise from the ashes – the reconstruction of charcoal fossil flowers. Trends Plant Sci 10:436–443. https://doi.org/10.1016/j.tplants.2005.07.006
Scutt CP, Vandenbussche M (2014) Current trends and future directions in flower development research. Ann Bot 114:1399–1406. https://doi.org/10.1093/aob/mcu224
Sokoloff DD, Remizowa MV, Bateman RM, Rudall PJ (2018) Was the ancestral angiosperm flower whorled throughout? Am J Bot 105:5–15. https://doi.org/10.1002/ajb2.1003
Tavares R, Cagnon M, Negrutiu I, Mouchiroud D (2010) Testing the recent theories for the origin of the hermaphrodite flower by comparison of the transcriptomes of gymnosperms and angiosperms. BMC Evol Biol 10:240. https://doi.org/10.1186/1471-2148-10-240

Thien LB, Bernhardt P, Devall MS, Chen Z-D, Luo Y-B, Fan J-H, Yuan L-C, Williams JH (2009) Pollination biology of basal angiosperms (ANITA grade). Am J Bot 96:166–182. https://doi.org/10.3732/ajb.0800016

Vazquez-Lobo A, Carlsbecker A, Vergara-Silva F, Alvarez-Buylla ER, Pinero D, Engstroem P (2007) Characterization of the expression patterns of LEAFY/FLORICAULA and NEEDLY orthologs in female and male cones of the conifer genera Picea, Podocarpus, and Taxus: implications for current evo-devo hypotheses for gymnosperms. Evol Dev 9:446–459. https://doi.org/10.1111/j.1525-142X.2007.00182.x

Vialette-Guiraud ACM, Adam H, Finet C, Jasinski S, Jouannic S, Scutt CP (2011) Insights from ANA-grade angiosperms into the early evolution of CUP-SHAPED COTYLEDON genes. Ann Bot 107:1511–1519. https://doi.org/10.1093/aob/mcr024

Vialette-Guiraud ACM, Andres-Robin A, Chambrier P, Tavares R, Scutt CP (2016a) The analysis of gene regulatory networks in plant evo-devo. J Exp Bot 67:2549–2563. https://doi.org/10.1093/jxb/erw119

Vialette-Guiraud ACM, Chauvet A, Gutierrez-Mazariegos J, Eschstruth A, Ratet P, Scutt CP (2016b) A conserved role for the NAM/miR164 developmental module reveals a common mechanism underlying carpel margin fusion in monocarpous and syncarpous eurosids. Front Plant Sci 6:1239. https://doi.org/10.3389/fpls.2015.01239

Willis K, McElwain J (2013) The evolution of plants, 2nd edn. Oxford University Press, Oxford

Willis CG, Baskin CC, Baskin JM, Auld JR, Venable DL, Cavender-Bares J, Donohue K, Rubio de Casas R (2014) The evolution of seed dormancy: environmental cues, evolutionary hubs, and diversification of the seed plants. New Phytol 203:300–309. https://doi.org/10.1111/nph.12782

Yamada T, Ito M, Kato M (2003) Expression pattern of INNER NO OUTER homologue in Nymphaea (water lily family, Nymphaeaceae). Dev Genes Evol 213:510–513. https://doi.org/10.1007/s00427-003-0350-8

Yamada T, Yokota S' y, Hirayama Y, Imaichi R, Kato M, Gasser CS (2011) Ancestral expression patterns and evolutionary diversification of YABBY genes in angiosperms. Plant J 67:26–36. https://doi.org/10.1111/j.1365-313X.2011.04570.x

The Evolution of Sex Determination in Plants

Cécile Fruchard and Gabriel A. B. Marais

Contents

Abstract

Separate sexes, i.e., the presence of male and female individuals in a species (= dioecy), do exist in flowering plants, despite being much less common than in animals. How becoming a male or a female (= sex determination) is achieved in dioecious plants is much less understood than it is in animals. On one hand, phylogenetic, ecological, and theoretical population genetics studies have provided a lot of information on what could be the evolutionary routes from hermaphroditism, the assumed ancestral sexual system in angiosperms, to dioecy, and what could be the genetics and the selective forces driving the evolution of males and females. On the other hand, genetic, molecular, and developmental data are scarce. Sex chromosomes have been described in a few dioecious species, and very recently two master sex-determining genes have been identified. We review here the theoretical findings on the evolution of dioecy and sex

C. Fruchard · G. A. B. Marais (✉)
Laboratoire de Biométrie et Biologie Evolutive (UMR 5558), Université Lyon 1, CNRS, Villeurbanne, France
e-mail: cecile.fruchard@univ-lyon1.fr; gabriel.marais@univ-lyon1.fr

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_168

determination in plants and also discuss recent work on the genetics of the evolution of dioecy and on the molecular characterization of the first master sex-determining genes found in plants.

Keywords

Angiosperms · Sex determination · Sex chromosomes · Sexual systems · Dioecy · Monoecy · Gynodioecy

Introduction

Albeit rare, dioecious plant species, that is, species with male and female individuals (as typically found in most animal species) exist in flowering plants and represent 15,600 species (i.e., 5–6% of all species, Renner 2014). Sexual systems are very diverse in plants, but the most common sexual system is hermaphroditism with bisexual flowers, which suggests that this is the ancestral sexual system (Barret 2002). It is thus assumed that dioecy has evolved from hermaphroditism and it has done so repeatedly, as dioecy is widespread in plants and found in 43% of all families (Renner 2014). A total of 871 to 5000 independent origins of dioecy in plants have been estimated (Renner 2014). In most cases, these events are very recent and sometimes how dioecy has evolved can be tracked. Why dioecy has remained at a low frequency in angiosperms, whereas its frequency is much higher in animals (>95%, see Table 1) and in other land plant lineages such as gymnosperms (36%), mosses (50%), and liverworts (75%) are not yet clear (Käfer et al. 2017).

In animals, a range of sex determination mechanisms have been described (Bachtrog et al. 2014): genetic (sex chromosomes, polygenic systems, single gene) or environmental (e.g., temperature-dependent), and some information on sex determination mechanism is known for >90% of animal species (Table 1). In plants, we know much less on sex determination. Sex chromosomes have been reported in ~40 species out of the 871 to 5000 independent dioecious systems (Ming et al. 2011; Renner 2014; Muyle et al. 2017). Dozens of master sex-determining genes such as *Sry*, the male-determining gene in mammals, have been identified in animals (Bachtrog et al. 2014), and the gene network for sex determination is well

Table 1 Frequency of species with separate male and female individuals and the current state of knowledge on sex determination in animals and flowering plants

	Animals	Flowering plants
% of species with separate sexes	95%	5–6%
% of species with rough information on sex determination mechanisms[a]	>90%	<1%

From (Weeks 2012; Barrett 2002; Ming et al. 2011; Renner 2014; Bachtrog et al. 2014; Muyle et al. 2017)

[a]Genetic or environmental, presence of sex chromosomes

characterized in several groups such as vertebrates (Matson and Zarkower 2012). By contrast in plants, only two master sex-determining genes have been recently identified (Agaki et al. 2014; Murase et al. 2017). We do not yet know how widespread sex chromosomes are in plants, or whether other genetic mechanisms and environmental sex determination have evolved in this taxon, and we have not yet identified the vast majority of the molecular players involved in plant sex determination.

This current lack of knowledge strongly limits our understanding of dioecy both at functional and evolutionary levels, which we will see is based on a few well-studied species and some elegant theories, but with little empirical evidence to support them. This problem has major implications for the understanding of dioecy in crops, as ~20% of all crop species are dioecious, or derive from a dioecious progenitor. Examples of dioecious crops are kiwi, asparagus, hop, cannabis, date-palm, and persimmons, while crops such as papaya, grapevine, and strawberries are dioecious-derived. In species where only one sex (female in general) has an agricultural utility, the lack of genetic markers for sexing to select out the useless sex can generate huge costs, especially in trees where sexual maturity (and the opportunity of sexing individuals by looking at flowers) is reached only after several years. Moreover, our lack of knowledge on sex-determining genes prevents us from controlling the sexual system of crops.

Here we will present the data and theories that are currently available for dioecious plants. The first part of our chapter concerns theoretical aspects of dioecy and sex determination in plants, which have been developed despite the paucity of concrete data in this field of research. The second part concerns the data that have been accumulating at a slow pace for a long time. However, this situation is changing and we will discuss recent important empirical findings in plant sex determination.

Theories on the Evolution of Dioecy

The Routes to Dioecy

By both looking at the sexual system of close relatives of dioecious species and reasoning on how dioecy could evolve using population genetics, it was suggested several decades ago that dioecy has probably not evolved directly from hermaphroditism, but indirectly along various routes via different intermediates with distinct sexual systems (all sexual systems found in angiosperms and their frequencies are shown in Table 2). Two main routes have been proposed (Barrett 2002, see Fig. 1A and B): the first of these involves a monoecious intermediate (monoecy = presence of separate male and female flowers on the same individual in a population, Renner and Ricklefs 1995), while the second involves a gynodioecious intermediate (gynodioecy = presence in a population of individuals with bisexual flowers and others with female flowers, Charlesworth and Charlesworth 1978).

The gynodioecy-dioecy pathway has been well studied theoretically (Charlesworth and Charlesworth 1978; Charlesworth 1999), and there is some

Table 2 Frequency of sexual systems in angiosperm species

	Dimorphic individuals (two or more distinct forms)	Monomorphic individuals (only one phenotype)
Bisexual flowers	Heterostyly <1%	Monocliny ~ 80%
Female and bisexual flowers	Gynodioecy <1%	Gynomonoecy 2–3%
Male and bisexual flowers	Androdioecy ≪1%	Andromonoecy 1–2%
Female and male unisexual flowers	Dioecy 5–6%	Monoecy 6–7%

Adapted from Käfer et al. (2017)

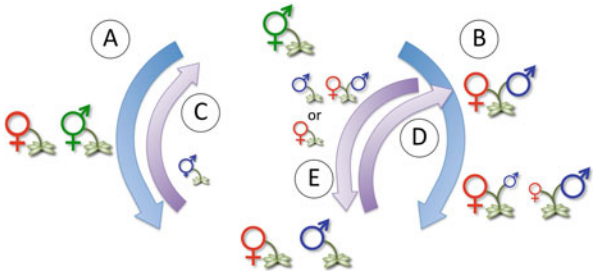


Fig. 1 The main evolutionary routes to dioecy. Two main routes from hermaphroditism (the assumed ancestral sexual system in angiosperms) to dioecy have been proposed: the gynodioecy-dioecy route (*A*) and the monoecy-dioecy route (*B*). It has been recently highlighted that dioecy may frequently revert to hermaphroditism (*C*, *D*), hence its rareness (Käfer et al. 2017). In the gynodioecy-dioecy route, inconstant males (males producing few viable seeds) may help reversions to hermaphroditism as drawn in C (Ehlers and Bataillon 2007). In the monoecy-dioecy route, there may be cycles between monoecy and dioecy (*D*, *E*). Some work suggests that in this case, androdioecy (males and monoecious individuals) and gynodioecy (females and monoecious individuals) may also evolve and may represent a situation of the re-evolution of dioecy after it has reverted to monoecy (e.g., Pannell et al. 2014). See text for details

phylogenetic evidence suggesting that many dioecious species may have evolved through a gynodioecious intermediate (Dufay et al. 2014). This pathway starts with a hermaphroditic population in which a male-sterility mutation producing females appears (Fig. 1A). Those females will reallocate all their reproductive energy to producing seeds and may produce more seeds than hermaphrodites. If the hermaphroditic population suffers from inbreeding depression, due for example to some self-fertilization, the females may not only produce more seeds but also seeds of better quality through obligate outcrossing. At some point, a female-sterility mutation producing males might appear. Those males will reallocate all their reproductive energy to producing pollen and will outcompete the hermaphrodites in fertilizing the females. The population has become dioecious.

A strong co-occurrence of monoecy and dioecy in the angiosperms phylogeny suggests that the monoecy-dioecy pathway is widespread among angiosperms

perhaps more widespread than the gynodioecy-dioecy pathway (Renner and Ricklefs 1995; Renner 2014). However, much less has been done on modeling the transition from hermaphroditism to monoecy and then to dioecy, and in particular, no population genetics model is available. In this pathway, the hermaphroditic population first evolves to a monoecious population in which both unisexual flower types can be found on the same individual (Fig. 1B). Then, it is assumed that disruptive selection (in which extreme values for a trait are favored over intermediate values) on the quantitative genetic variation in floral sex ratios within a monoecious population will gradually increase sex specialization, culminating in female and male individuals (Charnov 1982).

The androdioecy-dioecy pathway in which males would have evolved first in a hermaphroditic population is considered very unlikely (androdioecy = presence in a population of individuals with bisexual flowers and others with male flowers). First, contrary to monoecy and gynodioecy, androdioecy is extremely rare in angiosperms (Table 2). Second, in a hermaphroditic population where the individuals can self-fertilize, this pathway would require the production of a huge amount of pollen for the males to spread. The spread of females is much easier. A single example of androdioecy that may have evolved from hermaphroditism is found in *Phillyrea angustifolia* (Saumitou-Laprade et al. 2010), though for most androdioecious species, the scenario is believed to be different (Pannell et al. 2014). In *Mercurialis annua*, one of the best-studied androdioecious systems, it seems that androdioecy arose during cycles of evolution between dioecy and monoecy, and is an intermediate in populations evolving back to dioecy (Fig. 1D, E).

Reversions from dioecy, as shown in Fig. 1C and D, are probably much more frequent than we used to think and these could explain why dioecy is so rare in angiosperms (Käfer et al. 2017). Dioecy would be very easy to evolve (hence the numerous independent evolution of dioecy in angiosperms) but also easy to lose, in particular when population density drops and mates are difficult to find (Käfer et al. 2017).

The Genetics of the Transition to Dioecy

Only the genetics of the gynodioecy-dioecy pathway has been modeled. In the most popular model, both male-sterility and female-sterility mutations are nuclear (Charlesworth and Charlesworth 1978). If the male-fertility and female-fertility loci where these mutations appear are on the same chromosome, then selection will suppress recombination between these loci, which will create proto-sex chromosomes (Fig. 2a). A recessive male-sterility mutation and a dominant female-sterility mutation would typically create an XY system (Charlesworth and Charlesworth 1978), and once such a system is established, the sex chromosomes may then differentiate over time (Muyle et al. 2017).

Another possibility for the generation of sex chromosomes is Cytoplasmic Male Sterility (= CMS), which is well known in plants. Females carry a male-sterility mutation in the mitochondrial genome, the CMS mutation, and hermaphrodites

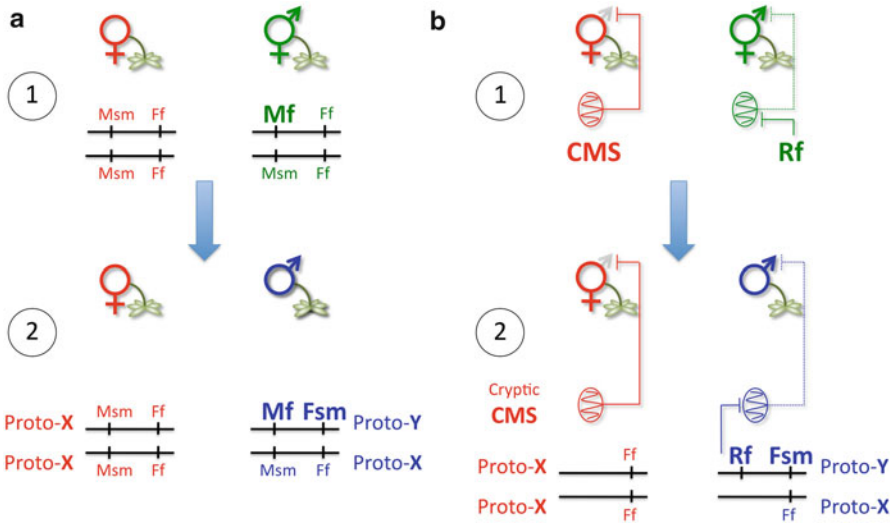


Fig. 2 The genetics of the evolution to dioecy from gynodioecy. The dominant model involves two nuclear mutations, which if on the same autosome will evolve into sex chromosomes (**a**). *Mf* male fertility gene (dominant), *Ff* female fertility gene (recessive), *Msm* male sterility mutation (recessive), *Fsm* female sterility mutation (dominant). Proto-X and proto-Y = nascent sex chromosomes. (**b**) An alternative model involves nucleo-cytoplasmic mutations, with gynodioecy being determined by a cytoplasmic-male-sterility factor (*CMS*) and a nuclear restorer of male fertility gene (*Rf*) system, in which case dioecy is determined by cryptic CMS, fixed in the population. If the Rf gene and a female fertility gene are on the same autosome, a proto-Y chromosome comprising the Rf gene (dominant) and a female sterility mutation (Fsm, dominant) may evolve

either have a male-fertile cytotype or carry a nuclear restorer-of-fertility (= Rf) gene that counteracts the CMS factor. It has been shown through modeling and computer simulations that the evolution of dioecy is less restrictive in nucleo-cytoplasmic than in nuclear gynodioecy (Schultz 1994; Maurice et al. 1994). CMS-Rf systems are engaged in evolutionary arms races. CMS-Rf systems arise from conflicts over what is the optimal reproductive system for mitochondrial genes versus nuclear genes. Mitochondrial genes are transmitted only through females, and a CMS mutation will clearly increase their transmission; females will spread in the population. Nuclear genes are transmitted through both males and females, and once CMS has evolved, there will be a strong selective pressure for Rf genes to evolve, increasing the frequency of the hermaphrodites in the population. Gynodioecy will be maintained through episodic invasions of a new CMS mutation followed by evolution of a new Rf gene and so on or through balanced polymorphism due to Rf genes having some costs that prevent their fixation (Delph et al. 2007). These dynamics can occasionally generate peaks of female frequency in the population, an ideal situation for males to spread, and simulations have shown that the evolution of dioecy from nucleo-cytoplasmic gynodioecy is, in fact, easier than from purely nuclear gynodioecy (Schultz 1994; Maurice et al. 1994).

This scenario too could create sex chromosomes (Fig. 2b). The genetics of the dioecy is expected to be different in the fully nuclear model (Fig. 2a) and in the nucleo-cytoplasmic model (Fig. 2b). If a dioecious species with an XY system evolved from a nucleo-cytoplasmic gynodioecious precursor, a CMS cytotype should be present in the dioecious species, in both sexes. CMS would generate the females; the males would be determined by a Y chromosome including an Rf gene (counteracting CMS) and a female-sterility gene (Fig. 2b). The CMS would be cryptic as the sex ratio would be balanced (not female-biased as in gynodioecy). However, some simulations suggest that dioecy might be unstable when arising from a nucleo-cytoplasmic context; males may disappear when a new unrestored CMS cytotype appears, if some inconstant males/hermaphrodites (still producing seeds) are present when this happens (Schultz 1994). This is why the evolution of dioecy through nucleo-cytoplasmic gynodioecy is usually considered less likely on theoretical ground (Charlesworth 1999), although empirical tests of these models are needed to tell how restricted/widespread they are.

Empirical Data on Dioecious Plants

Evolution of Dioecy Through Gynodioecy

Available data suggest that gynodioecy and dioecy are found associated in the same genus more than expected by chance in angiosperms (Dufay et al. 2014). Some case-studies have provided clear phylogenetic evidence that dioecy can evolve through gynodioecy, as in the *Silene* genus, for example (see below). The expected reallocation from female to male functions in hermaphrodites co-occurring with females has been documented in several subdioecious (anatomically cosexual, but functionally male or female) and gynodioecious species and suggests an ongoing transition to dioecy (Spigler and Ashman 2012).

There are two types of gynodioecy: nuclear and nucleo-cytoplasmic, on which the current models are based (see previous section and Fig. 2 and Table 3). Despite the fully nuclear model being the dominant model in the literature on dioecy, this type of gynodioecy is rare; nucleo-cytoplasmic gynodioecy is much more common (Delph et al. 2007). Species showing nucleo-cytoplasmic gynodioecy typically have a CMS-Rf system (Touzet and Meyer 2014).

Silene latifolia, a dioecious plant, is often presented as the typical example of the evolution of dioecy through gynodioecy (Fig. 3). It has indeed several gynodioecious relatives and dioecy has probably followed the gynodioecy pathway in the *Silene* genus (Desfeux et al. 1996). *Silene latifolia* has a X/Y chromosome pair, which is probably ~5 million years old (Rautenberg et al. 2010). These sex chromosomes are very large (X: 400 Mb, Y: 550 Mb) and still not fully sequenced (Papadopulos et al. 2015). Although sex-determining genes are still unknown in *S. latifolia*, three sex-determining regions of a few Mb have been identified on the Y chromosome using a mutant collection (Fig. 3c, Zluvova et al. 2007), among which

Table 3 Sex determination mechanisms in dioecy and in gynodioecious and monoecious intermediates

Sexual systems	Sex determination	Genes
Gynodioecy	Mostly nucleo-cytoplasmic	CMS: Chimeric mitochondrial genes Rf: Pentatricopeptide-repeat (PPR) family proteins
Monoecy	Phytohormonal	
	Cucurbits: Ethylene pathway	Cucurbits: Genes of the ethylene pathway (2 enzymes, 1 transcription factor)
	Maize: Gibberellic acid pathway	Maize: *Silkless* gene *sk1, Tasselseed* family genes *TS1* and *TS2, tasselseed4* microRNA
Dioecy	Sex chromosomes (XY, ZW)[a]	Persimmon: OGI small RNA/MeGI Asparagus: MYB transcription factor

See text for references

[a]In XY systems, the males are heterogametic (have a pair of nonidentical sex chromosomes), while in ZW systems, it the females that are heterogametic

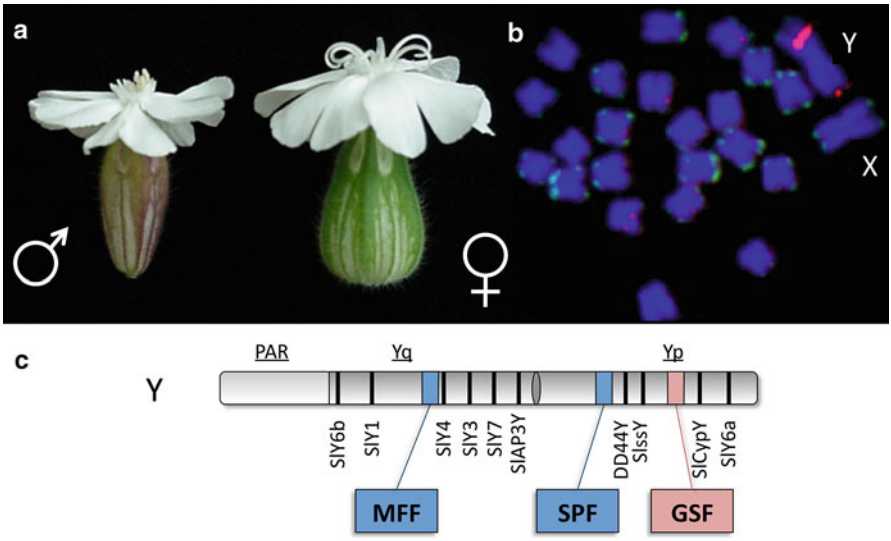


Fig. 3 The dioecious plant *Silene latifolia*. (**a**) Flowers from male and female individuals. (**b**) A karyotype of a *S. latifolia* male individual (from Hobza et al. 2007). (**c**) The *S. latifolia* Y chromosome and the three sex-determining regions identified using mutants. These regions comprise two male-fertility regions: *MFF* male fertility factor, *SPF* stamen promoting factor, and one female-sterility region: *GSF* gynoecium sterility factor. Well-known Y-linked genes are indicated. *PAR* pseudoautosomal region (X-Y recombining region)

are a female-sterility region and a male-fertility region, as would be expected from theory (Fig. 2).

The gynodioecious *Silene* species are of the nucleo-cytoplasmic type (e.g., *Silene vulgaris*, *Silene nutans, Silene acaulis*), but their CMS and Rf genes have not yet

been identified (Bernasconi et al. 2009). There are several indirect lines of evidence indicating cryptic CMS in *S. latifolia,* including biased sex ratios in crosses with close dioecious relatives (Taylor 1994). One of the sex-determining regions (MFF, see Fig. 3c), when deleted, gives a phenotype that closely resembles that of females in gynodioecious close relatives; they undergo anomalous anther development, with problems of cell proliferation in the tapetum (a cell layer feeding the developing pollen grains), which results in pollen-less anthers (Zluvova et al. 2007). Also, a cross of a *S. latifolia* female and a *Silene viscosa* hermaphrodite resulted in a 100%-female F1 (Zluvova et al. 2005), which can be easily explained by CMS transmitted by the *S. latifolia* mother for which the *S. viscosa* father did not have the corresponding Rf.

Fragaria (strawberry) is another genus in which dioecy probably evolved through the gynodioecy-dioecy pathway. The gynodioecious *Fragaria vesca* subsp. bracteata has a CMS-Rf system involving several Rf and Rf inhibiting loci (Ashman et al. 2015). Interestingly, one of the chromosomes harboring these loci has evolved into ZW chromosomes in the dioecious *F. virginiana* and *F. chiloensis*, which raises the possibility that these Rf loci are involved in sex determination in those dioecious relatives.

Evolution of Dioecy Through Monoecy

Phylogenetic evidence that monoecy and dioecy are associated is very strong as shown by angiosperm-wide, plant-family-focused and case studies (Renner and Ricklefs 1995; Barrett 2002; Renner 2014; Käfer et al. 2017). It is quite clear that the model developed for the gynodioecy-dioecy pathway does not apply to the monoecy-dioecy pathway (Golenberg and West 2013). In monoecious plants, male and female flowers do not develop randomly on the plant, instead the sex of flowers depends on their location. The sexual identity of flowers appears to result from gene networks that are responsive to information on the position of the flower in the plant, which are mediated by hormones. This is a starting point for evolving master sex-determining genes that is very different from that in the gynodioecy-dioecy pathway (Golenberg and West 2013). The genetics of monoecy is starting to be deciphered, in cucurbits and in grasses, and both groups also include dioecious species.

In melon (*Cucumis melo*) and cucumber (*Cucumis sativus*), three key genes, the *monoecious* (*M*), *androecious* (*A*) and *gynoecious* (*G*) genes, interact to determine the sexual identity of a given flower with respect to its position on the plant. Combinations of mutations of these genes produce female individuals, male individuals, and hermaphroditic individuals with bisexual flowers, and it was possible to create an artificially dioecious population in melon using these mutations (Boualem et al. 2015). The *M* gene inhibits the development of stamens (male organs), which results in a female flower. The *M* gene is inhibited by the *G* gene, which also suppresses the development of carpel (female organs). When expressed, the *G* gene turns a flower into a male one. The *A* gene inhibits the *G* gene and thus

produces female flowers. Its expression is probably under the influence of the information on the position of the flower in the plant. If the *M* gene is nonfunctional (and *A* and *G* are functional), hermaphroditic flowers are produced. Both *A* and *M* genes encode for enzymes (*ACS-11* and *ACS-7*, respectively) which are involved in ethylene biosynthesis, an important hormone that regulates many plant processes. The *G* gene encodes a C2H2 zinc finger transcription factor (*WIP1*). These three genes thus work coordinately to control the sexual phenotype of flowers in monoecious cucumis species (Boualem et al. 2015).

Maize (*Zea mays*) is monoecious with two types of unisexual inflorescences located, respectively, at the plant apex (tassels = male inflorescences) and leaf axils (ears = female inflorescences). Many mutants affecting the sexual phenotype of flowers have been identified, contributing to the choice of this species for studies of the molecular mechanisms of flower development in grasses (Li and Liu 2017). The *Silkless* gene *sk1* was identified as the master gene for pistil identity. This gene interacts with the *Tasselseed* family genes *TS1* and *TS2,* which are responsible for the arrest of pistil development. On the other hand, male flower development is controlled by the *tasselseed4* microRNA, a member of the miR172 family of microRNAs (miRNAs), which regulates the APETALA2 family of transcription factors and inhibits pistil development. A genetic hierarchy is beginning to emerge from the analysis of single and double mutants of these genes.

The Master Sex-Determining Genes in Dioecious Plants

The only sex determination mechanism known thus far in flowering plants is based on sex chromosomes, which have been reported in a minority of dioecious plants (<1%, Tables 1 and 3). For the vast majority of dioecious plants, the basis of sex determination, either genetic or environmental, is unknown. Both XY systems, in which males are heterogametic (have different sex chromosomes), and ZW systems, in which females are heterogametic, have been found, although the latter seem to be rarer than the former (Ming et al. 2011). Of the ~40 sex chromosome systems currently described, ~20 are heteromorphic (i.e., sex chromosomes are clearly distinguishable using cytogenetics). Examples of heterogametic systems are *S. latifolia* and *Coccinia grandis*, a dioecious cucurbit with an XY system, which happens to have the most heteromorphic sex chromosomes known in plants (Sousa et al. 2013). In these two species, the Y is larger than the X, a situation that has not been encountered in any animal species thus far. The remaining ~20 known plant sex chromosome systems are homomorphic (i.e., the sex chromosomes are indistinguishable using cytogenetics). One example is *Carica papaya* (papaya), the only plant species for which the sex chromosomes (XY as it happens) are fully sequenced and assembled (Wang et al. 2012). Interestingly, in this species a modified Yh determines XYh hermaphrodites in addition to XY males and XX females, and this Yh was probably selected during the domestication process of papaya (VanBuren et al. 2015). It is possible that many dioecious plants have yet unidentified homomorphic sex chromosomes, which are more difficult to detect, as in many cases dioecy has evolved recently and young sex chromosomes tend to be

homomorphic (Ming et al. 2011; Muyle et al. 2017). Importantly, among the ~40 plant species with known sex chromosomes, in almost all cases the master sex determining genes have not yet been identified.

The only dioecious plant species for which master sex determining genes have been identified are persimmons (Akagi et al. 2014) and possibly asparagus (Murase et al. 2017) (Table 3). In persimmons (*Diospyros lotus*), sex determination is driven by a Y-specific sex determinant, *OGI* (Japanese for "male tree"), which encodes a small RNA, and its target, the autosomal *MeGI* gene (Japanese for "female tree"), a homeodomain transcription factor that regulates anther fertility. Transformed tobacco and *Arabidopsis thaliana* plants clearly suggest that *MeGI* is a feminizing gene and *OGI* acts by suppressing *MeGI* expression. In XY individuals, *OGI* is present and inhibits the expression of *MeGI*, which results in anther development and the arrest of carpel development, and the individuals are consequently male. In XX individuals, *MeGI* is expressed, carpel development proceeds, and anther development is repressed, resulting in female individuals. The *OGi-MeGI* system seems to be conserved in several *Diospyros* species and could be >50 million years old.

In asparagus (*Asparagus officinalis*), a plant with X and Y chromosomes, sex determination is controlled by a single locus, the *Mating* (M) locus. Transcriptome and RT-PCR analysis showed that a *myeloblastosis-like* (*MYB*) gene, *Male Specific Expression 1* (*MSE1*), is specifically expressed in males during early anther development and exhibits tight linkage with the Y chromosome, as well as loss-of-function on the X chromosome (Murase et al. 2017). Knockout of *MSE1* orthologue in *A. thaliana* produces female plants, as expected for a male-determining gene. *MSE1* has a male-specific expression pattern in several dioecious asparagus species, which suggests it plays a master male-determining role in these species too. It is also present in hermaphroditic species, which suggests that another master sex-determining gene, suppressing the female development program, is yet to be discovered in asparagus.

In asparagus, dioecy may have evolved through gynodioecy (Dufay et al. 2014) and current data are consistent with more than one master sex determining gene, which is expected from models for the genetics of the gynodioecy-dioecy pathway. In persimmons, in which dioecy may have evolved through the monoecy-dioecy pathway (Renner 2014), a Y-autosomal gene duet seems to be enough to determine male and female, and there seems to be a single master sex determining gene on the Y, as is the case for mammals, in which *Sry* is the master control gene. These considerations suggest that the genetics of the monoecy-dioecy and gynodioecy-dioecy pathways may be different (Renner 2016).

Conclusions and Perspectives

At this stage, it is of course difficult to draw general conclusions about sex determination in plants as genetic, molecular, and developmental data are too scarce. This scarcity is partly explained by the difficulty in studying sex chromosomes in plants. First, it is likely that many dioecious plants have young and homomorphic sex

chromosomes, which cannot be identified simply looking at female/male karyotypes and requires more sophisticated methods. Second, sequencing sex chromosomes is notoriously difficult, even in animals (Muyle et al. 2017). However, new approaches to sequence sex chromosomes have been developed recently, and this could boost the field of plant sex determination (Muyle et al. 2017).

To understand better the gynodioecy-dioecy pathway, more master sex-determining genes of dioecious species that evolved from this pathway should be identified, in particular to understand whether fully nuclear or nucleo-cytoplasmic mutations have been more common. The monoecy-dioecy route is well established, although monoecy could also evolve from dioecy. More theoretical work is needed to understand the evolutionary forces underlying the monoecy-dioecy pathway. The genetics of this pathway is not clear either, but recent findings on genes controlling monoecy will surely give a boost to this field of research. Also, some dioecious plants might have evolved via minor pathways, i.e., androdioecy or heterodistyly. Study of these cases will certainly be needed to form a global picture of the evolution of dioecy and sex determination in plants.

Dioecy is rare in angiosperms, but this is not the case in other land plant lineages such as gymnosperms, mosses and liverworts. In haplo-diploid plants, males and females can be haploid and carry a third type of sex chromosomes called UV (Muyle et al. 2017). In order to gain an even wider picture of sex determination in plants, these groups must also be studied.

Cross-References

► Alternation of Generations in Plants and Algae

Acknowledgments The authors thank Jos Käfer and Editor Charlie Scutt for comments and suggestions for improving this manuscript. We apologize to all colleagues whom work could not be cited due to number of references restrictions.

References

Akagi T, Henry IM, Tao R, Comai L (2014) Plant genetics. A Y-chromosome-encoded small RNA acts as a sex determinant in persimmons. Science 346(6209):646–650

Ashman TL, Tennessen JA, Dalton RM, Govindarajulu R, Koski MH, Liston A (2015) Multilocus sex determination revealed in two populations of Gynodioecious wild strawberry, *Fragaria vesca* subsp. bracteata. G3 (Bethesda) 5(12):2759–2773

Bachtrog D, Mank JE, Peichel CL, Kirkpatrick M, Otto SP, Ashman TL, Hahn MW, Kitano J, Mayrose I, Ming R, Perrin N, Ross L, Valenzuela N, Vamosi JC, Tree of Sex Consortium (2014) Sex determination: why so many ways of doing it? PLoS Biol 12(7):e1001899

Barrett SC (2002) The evolution of plant sexual diversity. Nat Rev Genet 3(4):274–284

Bernasconi G, Antonovics J, Biere A, Charlesworth D, Delph LF, Filatov D, Giraud T, Hood ME, Marais GA, McCauley D, Pannell JR, Shykoff JA, Vyskot B, Wolfe LM, Widmer A (2009) Silene as a model system in ecology and evolution. Heredity (Edinb) 103(1):5–14

Boualem A, Troadec C, Camps C, Lemhemdi A, Morin H, Sari MA, Fraenkel-Zagouri R, Kovalski I, Dogimont C, Perl-Treves R, Bendahmane A (2015) A cucurbit androecy gene reveals how unisexual flowers develop and dioecy emerges. Science 350(6261):688–691

Charlesworth D (1999) Theories of the evolution of dioecy. In: Dawson T, Geber MA, Delph LF (eds) Gender and sexual dimorphism in flowering plants. Springer, Berlin/Heidelberg, pp 33–60

Charlesworth B, Charlesworth D (1978) A model for the evolution of dioecy and gynodioecy. Am Nat 112:975–997

Charnov EL (1982) The theory of sex allocation. Princeton University Press, Princeton

Delph LF, Touzet P, Bailey MF (2007) Merging theory and mechanism in studies of gynodioecy. Trends Ecol Evol 22(1):17–24

Desfeux C, Maurice S, Henry JP, Lejeune B, Gouyon PH (1996) Evolution of reproductive systems in the genus Silene. Proc R Soc Lond B Biol Sci 263(1369):409–414

Dufay M, Champelovier P, Käfer J, Henry JP, Mousset S, Marais GA (2014) An angiosperm-wide analysis of the gynodioecy-dioecy pathway. Ann Bot 114(3):539–548

Ehlers BK, Bataillon T (2007) 'Inconstant males' and the maintenance of labile sex expression in subdioecious plants. New Phytol 174(1):194–211

Golenberg EM, West NW (2013) Hormonal interactions and gene regulation can link monoecy and environmental plasticity to the evolution of dioecy in plants. Am J Bot 100(6):1022–1037

Hobza R, Kejnovsky E, Vyskot B, Widmer A (2007) The role of chromosomal rearrangements in the evolution of *Silene latifolia* sex chromosomes. Mol Gen Genomics 278(6):633–638

Käfer J, Marais GAB, Pannell JR (2017) On the rarity of dioecy in flowering plants. Mol Ecol 26(5):1225–1241

Li Q, Liu B (2017) Genetic regulation of maize flower development and sex determination. Planta 245(1):1–14

Matson CK, Zarkower D (2012) Sex and the singular DM domain: insights into sexual regulation, evolution and plasticity. Nat Rev Genet 13(3):163–174

Maurice S, Belhassen E, Couvet D, Gouyon PH (1994) Evolution of dioecy: can nuclear-cytoplasmic interactions select for maleness? Heredity (Edinb) 73(Pt 4):346–354

Ming R, Bendahmane A, Renner SS (2011) Sex chromosomes in land plants. Ann Rev Plant Biol 62:485–514

Murase K, Shigenobu S, Fujii S, Ueda K, Murata T, Sakamoto A, Wada Y, Yamaguchi K, Osakabe Y, Osakabe K, Kanno A, Ozaki Y, Takayama S (2017) MYB transcription factor gene involved in sex determination in *Asparagus officinalis*. Genes Cells 22(1):115–123

Muyle A, Shearn R, Marais GAB (2017) The evolution of sex chromosomes and dosage compensation in plants. Genome Biol Evol 9(3):627–645

Pannell JR, Eppley SM, Dorken ME, Berjano R (2014) Regional variation in sex ratios and sex allocation in androdioecious Mercurialis annua. J Evol Biol 27(7):1467–77. doi:10.1111/jeb.12352

Papadopulos AS, Chester M, Ridout K, Filatov DA (2015) Rapid Y degeneration and dosage compensation in plant sex chromosomes. Proc Natl Acad Sci U S A 112(42):13021–13026

Rautenberg A, Hathaway L, Oxelman B, Prentice HC (2010) Geographic and phylogenetic patterns in Silene section Melandrium (Caryophyllaceae) as inferred from chloroplast and nuclear DNA sequences. Mol Phylogenet Evol 57(3):978–991

Renner SS (2014) The relative and absolute frequencies of angiosperm sexual systems: dioecy, monoecy, gynodioecy, and an updated online database. Am J Bot 101(10):1588–1596

Renner SS (2016) Pathways for making unisexual flowers and unisexual plants: moving beyond the "two mutations linked on one chromosome" model. Am J Bot 103(4):587–589

Renner SS, Ricklefs RE (1995) Dioecy and its correlates in the flowering plants. Am J Bot 82(5):596–606

Saumitou-Laprade P, Vernet P, Vassiliadis C, Hoareau Y, de Magny G, Dommée and B, Lepart J (2010) A self-incompatibility system explains high male frequencies in an androdioecious plant. Science 327:1648–1650

Schultz S (1994) Nucleo-cytoplasmic male sterility and alternative routes to dioecy. Evolution 48:1933–1945

Sousa A, Fuchs J, Renner SS (2013) Molecular cytogenetics (FISH, GISH) of *Coccinia grandis*: a ca. 3 myr-old species of cucurbitaceae with the largest Y/autosome divergence in flowering plants. Cytogenet Genome Res 139(2):107–118

Spigler RB, Ashman TL (2012) Gynodioecy to dioecy: are we there yet? Ann Bot 109:531–543

Taylor DR (1994) The genetic basis of sex ratio in Silene alba (= *S. latifolia*). Genetics 136(2):641–651

Touzet P, Meyer EH (2014) Cytoplasmic male sterility and mitochondrial metabolism in plants. Mitochondrion 19(Pt B):166–171

VanBuren R, Zeng F, Chen C, Zhang J, Wai CM, Han J, Aryal R, Gschwend AR, Wang J, Na JK, Huang L, Zhang L, Miao W, Gou J, Arro J, Guyot R, Moore RC, Wang ML, Zee F, Charlesworth D, Moore PH, Yu Q, Ming R (2015) Origin and domestication of papaya Yh chromosome. Genome Res 25(4):524–533

Wang J, Na JK, Yu Q, Gschwend AR, Han J, Zeng F, Aryal R, VanBuren R, Murray JE, Zhang W, Navajas-Pérez R, Feltus FA, Lemke C, Tong EJ, Chen C, Wai CM, Singh R, Wang ML, Min XJ, Alam M, Charlesworth D, Moore PH, Jiang J, Paterson AH, Ming R (2012) Sequencing papaya X and Yh chromosomes reveals molecular basis of incipient sex chromosome evolution. Proc Natl Acad Sci U S A 109(34):13710–13715

Weeks SC (2012) The role of androdioecy and gynodioecy in mediating evolutionary transitions between dioecy and hermaphroditism in the animalia. Evolution 66(12):3670–3686

Zluvova J, Lengerova M, Markova M, Hobza R, Nicolas M, Vyskot B, Charlesworth D, Negrutiu I, Janousek B (2005) The inter-specific hybrid *Silene latifolia* x *S. viscosa* reveals early events of sex chromosome evolution. Evol Dev 7(4):327–336

Zluvova J, Georgiev S, Janousek B, Charlesworth D, Vyskot B, Negrutiu I (2007) Early events in the evolution of the *Silene latifolia* Y chromosome: male specialization and recombination arrest. Genetics 177(1):375–386

Evolution of Floral Organ Identity

Günter Theißen and Florian Rümpler

Contents

Abstract

Understanding the origin and rapid diversification of the angiosperm flower is a long-standing problem of evolutionary biology. A poor fossil record and a large morphological gap between extant angiosperms and their closest living relatives, extant gymnosperms, are major reasons that make reconstruction of flower origin such a hard problem. In recent years, however, significant progress has been made, especially in our understanding of the evolution of floral organ identity, by the

G. Theißen (✉) · F. Rümpler
Department of Genetics, Friedrich Schiller University Jena, Jena, Germany
e-mail: guenter.theissen@uni-jena.de; florian.ruempler@uni-jena.de

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_163

application of the evo-devo rationale. Quite useful as guiding concepts for understanding floral organ identity development and evolution proved genetic and molecular models, such as diverse ABC models and the floral quartet model. These models highlight the importance of organ identity genes and the interaction of the transcription factors they encode for a better understanding of evolutionary novelties such as flowers. Comparative studies on the MIKC-type genes and proteins underlying the ABC and floral quartet models throughout the seed plants suggest that gene regulatory networks controlling reproductive organ identity quite similar to those of angiosperms existed already in the most recent common ancestor of extant seed plants about 300 million years ago. These networks were probably just recruited and slightly modified to control diverse floral organ identities. Among the major floral organs the carpel, a key character of angiosperms, is arguably least well understood in terms of its origin. This applies even more so to the ovule, which is an important component of the carpel, but has much deeper evolutionary roots.

Keywords

ABC model · Angiosperm · Evo-devo · Fading borders · Floral quartet · Gymnosperm · MADS-box gene · MIKC-type gene · Shifting boundaries · Transcription factor

Introduction

Explaining the modes and mechanisms that bring about evolutionary novelties is a key goal of evolutionary developmental biology (evo-devo, for short) and arguably one of the most ambitious and long-standing goals of all of biology. A famous example is provided by the characteristic reproductive structure of the angiosperms (flowering plants), the flower. Despite decades of research and diverse, complementary approaches, it is still quite unclear how exactly the angiosperm flower originated (Bateman et al. 2006; Frohlich and Chase 2007; Theißen and Melzer 2007; Specht and Bartlett 2009; Sauquet et al. 2017). Major reasons for this state of affairs are a quite uninformative fossil record and a great morphological gap between the reproductive structures of living (extant) angiosperms and its closest relatives. It is clear that angiosperms originated from some gymnosperms, but not which gymnosperms are the closest relatives of angiosperms. Even worse, although extant gymnosperms are a very diverse group, comprising conifers (such as pine and spruce species), gnetophytes, cycads, and Ginkgo, they are probably monophyletic (meaning that they share a common ancestor that is not also a common ancestor of any other group of organisms). The same also applies for angiosperms. There are thus no living species that would link extant gymnosperms and angiosperms and would provide transitional forms of flower evolution. The lineages which led to extant gymnosperms and angiosperms separated about 300–350 million years ago (MYA), whereas the most recent common ancestor (MRCA) of extant angiosperms existed 140–250 MYA; unambiguous angiosperm fossils are not older than 130 million

years (Sauquet et al. 2017). Moreover, extant gymnosperm diversity is only a poor representation of gymnosperms as a whole, including also diverse extinct taxa. Even though some of them, such as Bennettitales, Cordaitales, Corystosperms, and Glossopterids, have been considered as close relatives of angiosperms, we are far away from a consensus on this topic (Frohlich and Chase 2007; Specht and Bartlett 2009). All in all this means that we are quite ignorant about more than 100 million years of evolution of the lineage that led to extant flowering plants.

The uninformative fossil record and large morphological gap between gymnosperms and angiosperms lead to problems with assignments of homology (meaning that two organs or other biological entities in different taxa are derived from a common ancestor). This applies especially to reproductive organs from angiosperms and gymnosperms, i.e., floral organs and their precursors (Frohlich and Chase 2007).

For classical paleo- and neobotany explaining the origin of the angiosperm flower thus may appear as difficult as ever. That is why complementary approaches, such as evo-devo, have gained ground. The rationale of evo-devo in the context of flower evolution has already been outlined in quite some detail elsewhere (see, for example, Theißen et al. 2002; Kramer and Jamarillo 2005; Irish 2009, to mention but a few). Briefly, since multicellular organisms usually develop from single cells (zygotes, i.e., fertilized egg cells) in each generation anew, morphological changes during evolution occur by changes in developmental processes. Since these processes are under strong genetic control, morphological novelties in evolution are based on changes in developmental control genes. These genes often encode relatively conserved transcription factors, typically constituting large protein families such as homeodomain or MADS-domain proteins. Thus studying all aspects of the evolution of developmental control genes (changes in gene sequence, expression pattern, activity and interaction of the encoded gene product, etc.) may tell us a great deal about the evolution of the structures whose development is controlled by these genes. The genes that control floral organ identity are a good case in point.

Dear reader, please note that, in accordance with the instructions for authors provided by the Publisher, this chapter is neither primary research literature nor a classical review citing primary literature, but rather work of tertiary literature that contains digested knowledge in an (hopefully) easily accessible format. As such it cites, whenever possible, secondary literature (reviews), except for recent publications that have not been reviewed yet. Moreover, the number of references is limited to about 20. We apologize to all authors whose work could hence not be cited.

The content of this chapter is expected to consist of "established information in the particular field." Given that the evolution of floral organ identity represents historical events that reach back up to 350 million years, this goal is difficult to achieve. Only circumstantial evidence such as fossil remains, and reconstruction of the evolution of plant morphology and developmental control genes based on the morphology of extant plants and genes, respectively, can be brought to bear on the problem. Such kind of data leaves quite a wide room for interpretation. Therefore, all this chapter can do is to summarize some major current views on the subject as available in the literature. The reader should not be too surprised, therefore, if some scenarios outlined here are subject to considerable change in the years to come.

A Flower Is a Flower Is a Flower: A Primer on Terminology

Considerations on the evolution of the flower are fraught with some terms that appear more simple than they actually are. Some clarification thus might be useful.

What Is a Flower?

Even though most people will have some intuitive ideas about what a flower actually is, providing a useful scientific definition is easier said than done. Rather than being single organs (such as leaves or roots), flowers are composite structures, composed of a number of organs that are arranged in an ordered pattern (Endress 2001). Depending on the flower type, the number (merosity) as well as the arrangement (phyllotaxy) of floral organs can vary. In a type of flower often considered in the literature the organs are arranged in four whorls, with sepals in the first whorl, petals in the second whorl, stamens in the third whorl, and carpels in the fourth whorl. Sepals are usually green organs that resemble vegetative leaves and protect the flower during early stages of development. Petals are usually showy, often colorful or white organs that often attract pollinators. Stamens are quite complex male organs that produce pollen within the microsporangia of their anthers. Carpels are the female reproductive organs, which are often fused and inside of which, after fertilization, the ovules develop into seeds.

An ordered structure of sepals, petals, stamens, and carpels reflects pretty well the flower type usually found in the largest group of angiosperms, the eudicotyledonous plants (eudicots), even though already here quite a number of exceptions, such as unisexual flowers that lack either stamens or carpels, exist.

If we look at other types of angiosperms, such as early diverging ("basal") eudicots, the monocotyledonous plants (monocots), or early diverging ("basal") angiosperms, even more deviations from this simple plan become apparent, such as the arrangement of organs in spirals, or the existence of alternative or additional types of organs, such as lodicules or staminodes. Trying to find a comprehensive definition of the angiosperm flower, therefore, proved difficult. In fact, Bateman et al. (2006) outlined about a dozen of historical definitions, and these authors as well as Baum and Hileman (2006) also provided their own definitions.

Trying to find a kind of consensus, one could define a flower as a determinate, compressed, bisexual reproductive axis composed of carpels (representing megasporangia), stamens (representing microsporangia), and a sterile perianth composed of at least one sterile laminar organ (Theißen and Melzer 2007). But even this is obviously not a comprehensive definition of a flower, since there are numerous types of flowers that lack one or more of these organ types, probably due to secondary simplification, for example, unisexual flowers with either stamens or carpels such as in white campion (*Silene latifolia*), and flowers that lack a perianth completely, such as in case of species of the genus *Chloranthus*.

So if flowers are defined just in a somewhat fuzzy way, one may ask as to which features represent their key characters. Also here a perfect consensus does not exist

in the literature, but the presence of carpels enclosing the ovules is generally considered an essential character that distinguishes typical angiosperm flowers from gymnosperms' reproductive cones (Endress 2001; Stuessy 2004; Scutt et al. 2006).

Another key feature of angiosperm flowers is seen in the fact that male and female reproductive organs are usually united in one structure (or secondarily separated, as in the unisexual flowers of monoecious and dioecious angiosperms), a trait termed "hermaphroditism," or "bisexuality" (Baum and Hileman 2006; Theißen and Melzer 2007). Note that male and female reproductive structures are probably ancestrally separated in the diverse reproductive structures of extant gymnosperms. Moreover, while gymnosperms have elongate reproductive axes, the floral axis is compressed (Baum and Hileman 2006). Another feature characteristically distinguishing the vast majority of angiosperm flowers from gymnosperm cones is the presence of a perianth surrounding the reproductive organs, often including showy, attractive organs (petals or petaloid tepals). Often double fertilization yielding a nutritive endosperm is also considered an important angiosperm feature (Stuessy 2004; Kramer and Jamarillo 2005). Hence also from an evolutionary point of view the angiosperm flower is not a simple characteristic but a complex of innovations (Baum and Hileman 2006).

What Is Floral Organ Identity?

Floral organs may differ in their position within a flower, and by specific characteristics, or their "identity" – both are important criteria of homology. (Note that functional similarity is not a valid criterion of homology!) Usually different homology criteria point in the same direction. However, angiosperms, much more than animals, are "masters of modularity and homeosis," meaning that their organs can change their identity, so that organs of different identities can appear at the same position within the body plan of different individuals (Kramer and Jamarillo 2005; Theißen 2005). Obviously, homeosis can give conflicting results in homology assignments, depending on whether position or organ identity is used. To properly describe developmental and evolutionary events it is important, therefore, to recognize the identity of organs in the framework of plant body plans.

Like "flower," however, also the term "floral organ identity" does not refer to a simple character, but rather to a syndrome of features. This has been exemplified in detail especially for petals (Irish 2009). Besides being defined by their position surrounding the stamens, petals are also defined by their special morphology (identity) thought to have evolved to attract pollinators. Specifically, petals are lateral organs that generally have a narrow base, one vascular trace and typically lag behind the stamens in their development. The blade of petals is often relatively large and showy, and often pigmented or white rather than green, due to the presence of chromoplasts or leucoplasts rather than chloroplasts in the cells of petals. Volatile substances such as terpenoids and benzenoids are often produced in petal epidermal cells giving flowers their characteristic scents. The adaxial epidermal cells are

usually conical or elongate, providing petals with a characteristic velved sheen (reviewed by Irish 2009).

Note that numerous angiosperms produce organs with morphological attributes of petals but that are not considered true petals. For example, the leaves subtending flowers or inflorescences (bracts) are often showy and petal-like (petaloid), for example, in case of flowering dogwood (*Cornus florida*), shrimp plant (*Justicia brandegeeana*), flamingo flower (*Anthurium* species), and many more (Kramer and Jamarillo 2005; Irish 2009).

In a Nutshell: Genetic and Gene Regulatory Models of Floral Organ Identity

During the last three decades much has been learned about the genetic and molecular mechanisms underlying flower development, mainly due to studies in a few major eudicot model plant species such as thale cress (*Arabidopsis thaliana*), snapdragon (*Antirrhinum majus*), and petunia (*Petunia* x *hybrida*). The insights gained with these models provide a perfect starting point for investigations on the evolution of floral organ identity.

The ABC Model and Its Derivatives

Eudicots have relatively standardized flowers that typically consist of four different classes of organs, sepals, petals, stamens, and carpels, which are arranged in four (or more) whorls (Fig. 1). Three classes of mutants, termed A, B, and C, helped to develop genetic models that explain how the different floral organs acquire their specific "identities" during development (reviewed by Theißen et al. 2016). In these mutants the identity of floral organs in two adjacent whorls is changed in a systematic way. The phenotypes of class A, B, and C mutants revealed that flower development is sculpted by "floral organ identity genes" (also termed "homeotic selector genes"). These genes act as major developmental switches that control the entire genetic program leading to the development of a particular organ. The activities and interactions of floral homeotic genes have been described in genetic models that have been extended and refined over time, starting with different "ABC models." The "classical ABC model" maintains that organ identity in each floral whorl is determined by a unique combination of three organ identity gene activities, called A, B, and C. Expression of class A genes alone specifies the formation of sepals, the combination A + B specifies the development of petals, the combination B + C specifies stamen formation, and the expression of C alone determines the development of carpels. Furthermore, the ABC model proposes that the class A and class C genes negatively regulate each other, so that the class C gene activity is expressed throughout the developing flower when the class A gene function is compromised, and vice versa (for a review see Theißen et al. 2016).

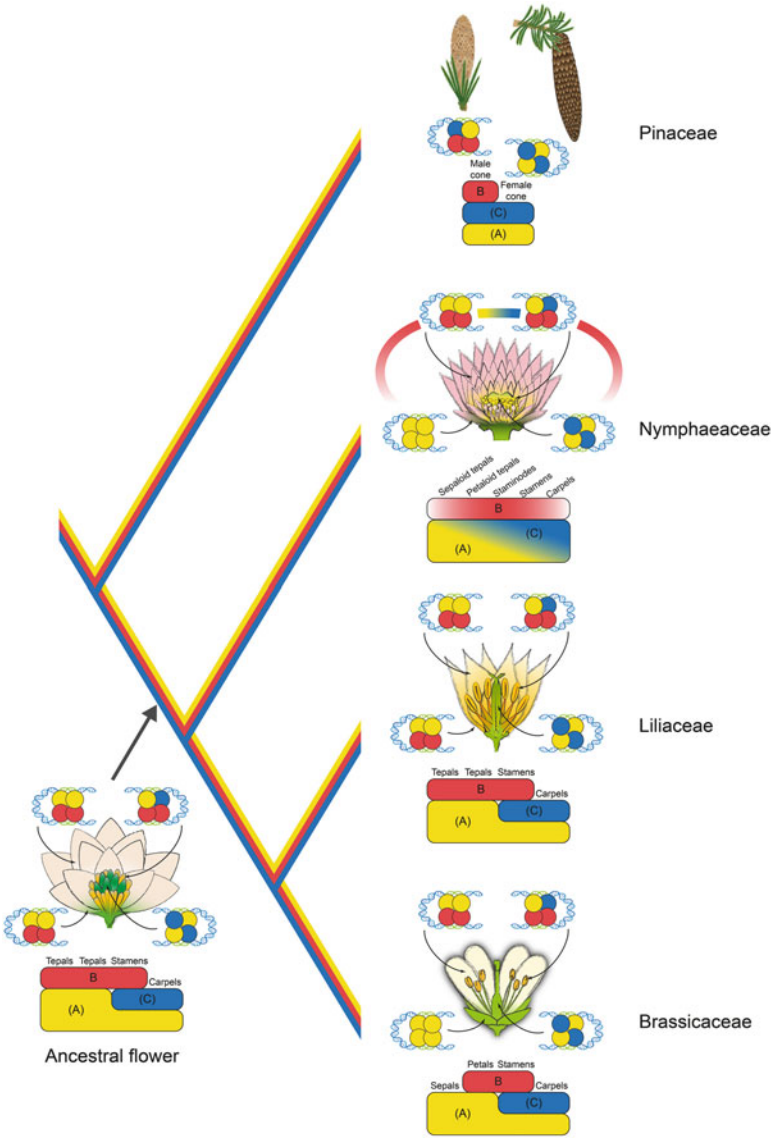


Fig. 1 Evolution of floral organ identity in seed plants. The floral structures of four extant and one extinct species are plotted on a phylogenetic tree of floral organ identity genes, with yellow, red, and blue symbolizing class (A), B, and (C) genes, respectively. A schematic example of Pinaceae, Nymphaeaceae, Liliaceae, and Brassicaceae represents gymnosperms, basal angiosperms, petaloid monocots, and core eudicots, respectively. Next to the flower pictures corresponding (A)B(C) and floral quartet models are shown (according to Theißen et al. 2016), using the same color code as explained above. The Nymphaeaceae exemplify the "fading borders" modification of the (A)B (C) and floral quartet model, the Liliaceae the "shifting boundaries" version. The ancestral flower is according to Sauquet et al. (2017); whether its tepals are sepal-like or petal-like remains unclear, so that the picture shows just one possible case, petaloid tepals expressing (A) and B genes and proteins

After inception of the ABC model several lines of evidence soon revealed that the ABC genes are not sufficient for the specification of floral organ identity, so that the ABC model was extended by class D genes involved in specifying ovule identity, and by class E genes involved in the specification of all types of floral organs. The corresponding "ABCDE model" proposes that class A + E genes specify sepals, A + B + E genes petals, B + C + E genes stamens, C + E genes carpels, and C + D + E genes ovules (Theißen et al. 2016).

In contrast to the genetically and developmentally quite well-defined class B, C, and E floral homeotic gene functions, the concepts of class A and D genes have been discussed controversially for quite a long time. For example, a function of class A genes in specifying sepal and petal identity and antagonizing the class C gene function is difficult to separate genetically from a more fundamental function in specifying floral meristem identity. Furthermore, in angiosperms ovules are part of the carpels, which is one (of several) reasons that makes a distinction between class C and class D genes difficult. Moreover, phylogeny reconstructions revealed that class A and class E function genes are relatively closely related and trace back to a common gene class that may have existed already in the stem group of extant seed plants; and the same applies to class C and class D function genes (Gramzow et al. 2014; Theißen et al. 2016). Based on such findings, the ABCDE model was recently developed into an (A) B(C) model (Fig. 1) (Theißen et al. 2016). This model proposes that a class (A) gene function controls both floral meristem identity and floral organ identity in the first two floral whorls; the (A) function comprises the class A and class E gene functions of the ABCDE model, i.e., (A) = A + E. Similarly, the class (C) gene function comprises the C and D functions, i.e., (C) = C + D. Note that the (A)B(C) model is a generic model; in the diverse species of angiosperms, the genes contributing to these functions may have been differentially sub- and neofunctionalized in some ways (Theißen et al. 2016).

Several lines of evidence reveal that the (A)B(C) genes are not only required but also sufficient to superimpose floral organ identity upon vegetative developmental programs of angiosperms. This makes these genes key factors also in investigations on the origin of floral organ identity in evolution (Theißen et al. 2016).

In *A. thaliana* all in all a dozen canonical floral organ identity genes were identified, namely the class A genes *APETALA1* (*AP1*) and *APETALA2* (*AP2*); the class B genes *APETALA3* (*AP3*) and *PISTILLATA* (*PI*); the class C gene *AGAMOUS* (*AG*); the class D genes *SEEDSTICK* (*STK*), *SHATTERPROOF1* (*SHP1*) and *SHP2*; and the class E genes *SEPALLATA1* (*SEP1*), *SEP2*, *SEP3*, and *SEP4*. Closely related, if not orthologous genes were found throughout the angiosperms (Gramzow et al. 2014). All of these genes encode transcription factors, and except for *AP2*, all encode MIKC-type MADS-domain proteins (reviewed by Theißen et al. 2016). In species other than *Arabidopsis thaliana AGL6*-like genes rather than or in addition to *SEP*-like gene may provide class E gene activity.

The Floral Quartet Model

The (A)B(C) model and its precursors mainly describe genetic functions and their interactions. But what are the molecular mechanisms by which the different floral

homeotic genes interact and exert their function while specifying the identity of the different floral organs?

As transcription factors, the products of the (A)B(C) genes control the transcription of other genes ("target genes") whose products are directly or indirectly involved in the formation or function of floral organs. Especially the MIKC-type proteins do that in a remarkable way, involving the formation of tetrameric complexes that bind to DNA, termed "floral quartets." Floral quartet formation is enabled by the characteristic, name giving domain structure of MIKC-type proteins, including a MADS (M-), Intervening (I-), Keratin-like (K-) and C-terminal (C-) domain. DNA binding is brought about by the MADS domain, which prefers DNA sites with similarity to the sequence 5′-CC(A/T)$_6$GG-3′, termed "CArG-box." Tetramer formation, but also dimerization of MIKC-type transcription factors depends on structural features of the K-domain (Theißen et al. 2016).

The floral quartet model proposes that quaternary complexes of different, paralogous MIKC-type proteins control the expression of the target genes relevant for the development of specific floral organ identities. Two protein dimers of each tetramer recognize two different CArG-boxes involving bending of the DNA between the CArG-boxes, so that the protein quartet (or tetramer) can be considered as a dimer of dimers. At least one unique floral quartet for each type of the different floral organs has to be assumed (Fig. 1) (Theißen et al. 2016). Like the ABC model, also the floral quartet model has been updated over time. Based on the (A)B(C) model, the original floral quartet model was recently refined to a generic model that fits well to the situation in many eudicots such as Brassicaceae. In this model there are floral quartets with four class (A) proteins specifying the identity of floral meristems and sepals, two class (A) and two class B proteins specifying petals, one class (A) plus two class B plus one class (C) proteins specifying the identity of stamens, and two class (A) plus two class (C) proteins specifying carpels (including ovules) (Fig. 1) (Theißen et al. 2016).

Given the importance of floral organ identity genes and floral quartets for the development of floral organ identity, the evolution of these entities is of obvious interest for a better understanding of the evolution of floral organ identity.

Phylogeny of Floral Homeotic Genes and Floral Quartets: A MIKC Blessing

Mutant analyses revealed that without the proper activity of floral homeotic genes lateral organs of angiosperms do not develop floral organ identity. The same applies quite likely to statements about the activity of floral quartets. Investigating the evolution of the transcription factors that control floral organ identity, especially the (A)B(C) genes of the MIKC-type, and of floral quartets may thus provide a key to better understand the origin of the angiosperm flower. Indeed, the origin of some novel clades of MIKC-type genes and of some evolutionary novelties, especially reproductive organs sensu *lato* in land plants, are strongly correlated (reviewed by Gramzow et al. 2014; Theißen et al. 2016). In addition to the usual frequent gene losses the MIKC-type gene family mainly evolved by the formation of ancient paralogs, many of which originated by whole genome duplications, preferential

retention after gene duplication, and sequence divergence often resulting in sub- and neofunctionalization (Gramzow et al. 2014).

Due to the fact that, except for monilophytes (ferns and their allies, such as horsetails), whole genome sequences are currently available for all major groups of land plants, including gymnosperms, the phylogeny of MIKC-type genes can now be reconstructed with unprecedented accuracy. According to recent analyses, radiations of MIKC-type genes occurred independently in different groups of land plants such as mosses, lycophytes, monilophytes, and seed plants. All MIKC-type genes of seed plants are members of 11 seed plant-specific gene clades that existed already in the MRCA of extant seed plants about 300 million years ago (MYA), but were not yet present in the MRCA of extant monilophytes and seed plants about 400 MYA (Gramzow et al. 2014). Among those clades are those containing, besides other genes, genes providing the floral homeotic class (A), B, and (C) gene functions. Based on gene or even whole genome duplications in the stem group of angiosperms, these 11 clades evolved into 17 clades that had already been established in the MRCA of living angiosperms, including distinct *DEF*- (*AP3*-) and *GLO*- (*PI*-) like genes (class B), *AG*-like and *STK*-like genes (class C and D), *AGL2*-like (*SEP*-like) and *AGL6*-like genes (class E), and *SQUA*- (*AP1*-) like genes (class A) (Gramzow et al. 2014). Thus the floral organ identity genes are all members of gene clades that are seed plant- or flowering plant-specific.

Several lines of circumstantial evidence suggest that floral quartet-like complexes quite similar to those of floral quartets in living angiosperms also exist in extant gymnosperms, and had already been established in the MRCA of extant seed plants (Fig. 1) (Theißen et al. 2016). Thus, besides possible refinements in the composition of these protein-DNA-complexes changes in the sets of target genes of some tetramers of MIKC-type transcription factors may have played a major role in the origin of organs with floral identity. Changes in the DNA-binding domain or the interaction pattern of these trans-acting proteins, or of *cis*-regulatory elements of target genes all may well have contributed to these target gene changes.

On the Origin of Floral Organ Identity: From Gymnosperm Cones to the Ancestral Flower

Floral organ identity in a strict sense must have originated in parallel to flowers. Monophyly of extant gymnosperms and angiosperms, the large morphological gap between the reproductive structures of extant angiosperms and gymnosperms, and the fact that unambiguous fossils from the stem group of angiosperms are unknown hampers a detailed understanding of as to how the different floral organs originated from organs of gymnosperm reproductive cones.

However, by identification of the "ANA grade" (*Amborella trichopoda* followed by Nymphaeales (including Hydatellaceae) followed by Austrobaileyales) as the most early diverging extant angiosperms (reviewed by Specht and Bartlett 2009) quite some progress could be made in recent years concerning the structure of the flower of the MRCA of extant angiosperms, henceforth termed "ancestral flower." This means

that we now can better estimate what structural changes had to have occurred during the transition from whatever kind of gymnosperm cone to the ancestral flower.

Quite a broad consensus has been achieved that the ancestral flower was bisexual and had already a perianth. The most comprehensive and sophisticated reconstruction so far postulates that the ancestral flower was bisexual, radially symmetric; that it had more than two whorls of three separate perianth organs each, and that these were undifferentiated tepals; finally, that it contained also more than two whorls of three separate stamens each, and more than five spirally arranged carpels (Fig. 1) (Sauquet et al. 2017). Of course, different degrees of unavoidable uncertainty remain for each of these characters. Moreover, several interesting aspects could not be clarified, for example, whether the ancestral tepals were sepal-like or petal-like.

How this "complex of innovations" called the flower was assembled during evolution is still quite speculative. A comprehensive overview about current models has been provided by Specht and Bartlett (2009). In any case it seems likely that the assembly of the flower bauplan occurred in several steps. Several authors have argued that, starting from unisexual axes in a gymnosperm, bisexual (hermaphrodite) structures evolved first (Theißen et al. 2002; Theißen and Becker 2004; Baum and Hileman 2006). Circumstantial evidence suggests that an ancestral sex determination mechanism has been working in gymnosperms, with class (C) gene expression distinguishing reproductive from vegetative organs, and class B gene expression distinguishing male from female organs. Based on this hypothesis the "Out of Male" (OOM) scenario hypothesizes that hermaphroditic flowers originated from a male gymnosperm cone, and that reduction of class B gene expression in the upper region of the male cone led to the development of female rather than male organs (Theißen et al. 2002; Theißen and Becker 2004). An alternative "Out of Female" (OOF) scenario has also been described. In any case a flower-like structure with male organs in the basal region and female ones in the apical region would have been established by homeosis (changes in organ identity). The resulting structure would not have had a perianth yet, and the female reproductive organs may not have been transformed into carpels yet. Nevertheless, the structure could well have enabled outcrossing by attracting pollinators with, for example, the color of the pollen, nectaries, scent, or by pollination droplets that were secreted by the ovules. Thus hermaphroditism may have provided an immediate selective advantage. An alternative "Mostly Male" (MM) hypothesis proposed that ovules developed ectopically in male reproductive organs (Frohlich and Chase 2007). A test of these hypotheses using comparative transcriptomics appears to support the OOM/OOF more than the MM theory (Tavares et al. 2010).

The gene regulatory events that may have led to the origin of hermaphrodite structures are unknown. Specific hypotheses involving apical-basal gradients of substances such as phytohormones or the floral meristem identity transcription factor LEAFY have been described in the literature (Theißen and Becker 2004; Baum and Hileman 2006). These gradients then may cause a gradient of class (C) proteins in reproductive axes, so that towards the apical region class (C) proteins would outcompete class B proteins in floral quartets, leading to female organ (megasporophyll) development, whereas in more basal regions, under the influence of floral quartets involving both class B and class (C) proteins, male organs (microsporophylls) develop (Baum and Hileman 2006).

The next steps of early flower evolution are even more speculative, but Baum and Hileman (2006) have outlined some inspiring suggestions. According to these authors, floral axis compression and determinacy may have originated in the next step. This was possibly achieved when class (C) genes became negative regulators of an ortholog and functional equivalent of the meristem maintenance gene *WUSCHEL* (*WUS*), which encodes a homeodomain transcription factor in *Arabidopsis thaliana*. The third step may then have been the origin of a petaloid perianth by sterilization of the outer stamens, possibly when the WUS protein was recruited as a coregulator of class (C) genes. Finally, the dimorphic perianth, as typically established in core eudicots, may have originated when an ortholog and functional equivalent of the *Arabidopsis thaliana* gene *UNUSUAL FLORAL ORGANS* (*UFO*) became a requirement for class B gene expression.

In this scenario petals are considered as sterilized stamens, and sepals are derived from petaloid organs. It does not explain, however, how the microsporophylls and megasporophylls found in gymnosperms turned into stamens and carpels, respectively, with their specific morphologies.

The origin of the carpel is still an open key question of flower origin. It may have originated from a megasporophyll that enrolled and surrounded the ovules, or from an ovule-bearing axis surrounded by a bract-like structure; thus even its homology to other structures is unclear (Specht and Bartlett 2009).

Ovules may be considered as parts of the carpel, but since they characterize seed plants and are present in all gymnosperms, they are evolutionary much older. From a developmental genetic point of view, they are also even more mysterious than the carpel and the other floral organs, since close relatives of (A)B(C) genes existed already in the MRCA of extant seed plants and hence could have been recruited to specify floral organ identity. In contrast, the MRCA of monilophytes and seed plants contained MIKC-type genes, but no close relatives of the (A)B(C) genes known from seed plants (Scutt et al. 2006; Gramzow et al. 2014). Ovules, like carpels, are specified by class (C) genes, thus these genes could not be recruited but first had to originate from other MIKC-type genes, probably by gene duplication and divergence in sequence, expression, and function. From a developmental genetic point of view, ovule origin has thus been more “demanding” than the origin of floral organs.

Variations on a Theme: The Diversification of Flowers and Floral Organs

Once established, the ancestral flower has been considerably conserved, but not fixed. Rather, the species that developed it gave rise to roughly 300,000 or so different species of extant angiosperms with flowers of impressive diversity concerning the number, arrangement, identity, and shape of floral organs, and the symmetry of the flower as a whole. Evolution of floral organ identity, including homeotic transitions, played a considerable role in the generation of that diversity.

Fuzzy Floral Organ Identity by Fading Borders

The "classical ABC model" was developed based on studies in the model plants *Antirrhinum majus* and *Arabidopsis thaliana*, which are both core eudicots. It is well established now that the situation described by this model and its derivatives, including the corresponding floral quartet model (Fig. 1), represents a derived rather than ancestral situation within angiosperms (Theißen and Melzer 2007; Soltis et al. 2007; Chanderbali et al. 2016). Studies in early diverging ("basal") angiosperms of the ANA grade corroborate that view. Even though the ANA species represent only a few percent of the angiosperms, they represent the greatest diversity in floral structure and form. One reason is that the bauplan of their flowers is not so fixed yet, and their flower development not as robust as in core eudicots. In consequence the flowers of basal angiosperms vary considerably in size, the number of floral parts, and the arrangement of floral organs in spirals or whorls (Soltis et al. 2007).

In a number of early diverging angiosperms, such as water lilies (*Nymphaea* species), gradual transitions in organ identity are found, for example, from sepaloid tepals via petaloid tepals and staminodes to stamens (from outer to inner) (Fig. 1). Comparative gene expression studies indicated that such gradual changes in organ identity result from gradients in levels of expression of (A)B(C) genes across the floral meristem, as outlined by the "Fading Borders Model" (Fig. 1) (Soltis et al. 2007). According to this model, relatively weak expression at the edge of each gene's expression domain overlaps with expression of another floral homeotic gene, while strong expression of each floral organ identity gene occurs distant from the adjacent gene's center of strong expression. This fuzzy pattern of (A)B(C) gene expression imposes some features of adjacent organs onto each other and thus leads to morphologically intergrading rather than distinct floral organs.

The (A)B(C) system with fading borders has been discussed as the ancestral state of angiosperm flower evolution (Theißen and Melzer 2007). However, a recent reconstruction suggests that the ancestral flower had distinct organ identities (Sauquet et al. 2017). It is also conceivable, therefore, that fading borders of (A)B(C) gene expression and gradual changes in organ identity are derived states that evolved in some lineages of basal angiosperms.

Perianth Diversity by Sliding Boundaries or Loss of Whorls

Irrespective of whether sharp borders of (A)B(C) gene expression and hence unambiguous floral organ identities had been established already in the ancestral flower, or occurred later after an initial phase with fading borders, differences in organ identity in different whorls of the flower contribute significantly to floral diversity. In frequently considered cases the organs (tepals) of the first floral whorl are petaloid rather than sepaloid, or organs of the second whorl are sepaloid rather than petaloid. Examples for the first case are represented by the petaloid monocots such as lilies (*Lilium*), toad lilies (*Tricyrtis*), tulips (*Tulipa*), and the numerous species of orchids (family Orchidaceae). Outside of the monocots a similar condition is found in some

early diverging eudicots such as columbine (*Aquilegia*) and other Ranunculaceae (Kramer and Jamarillo 2005; Sharma et al. 2014).

Petaloid organs in the first whorl have been easily explained and experimentally corroborated by a "modified ABC model" in that class B genes are also active in the organ primordia of the first floral whorl (Fig. 1) (Theißen and Melzer 2007; Dodsworth 2017).

A contrasting case is given in flowers with sepaloid rather petaloid organs in the second (and first) whorl, as found, for example, in the wind-pollinated sorrel (*Rumex acetosa*), where only the stamens, but not the perianth organs express class B floral homeotic genes (reviewed by Theißen and Melzer 2007).

Such cases were taken as evidence that floral diversity can be achieved by outward or inward shifts of class B floral organ identity gene expression. Shifts in class A and class C gene expression may also have occurred during evolution, albeit possibly with less overall importance for floral diversity (reviewed by Theißen and Melzer 2007).

Scenarios that explain floral diversity by changes in the expression domain of floral homeotic genes have been proposed by several authors and are now known as "Shifting Boundary" or "Sliding Boundary Model" (SBM) (Kramer and Jamarillo 2005; Theißen and Melzer 2007). However, the recent reconstruction of the ancestral flower by Sauquet et al. (2017) would also be compatible with an alternative mechanism. This flower had more than two whorls of three tepals and more than two whorls of three stamens (Fig. 1). It is hence conceivable that a reduction of the number of whorls, either by loss of entire whorls or by merging of whorls concomitant with an increase in the number of organs per whorl, played a major role during early flower diversification. Therefore, it has been suggested that, for example, the two perianth whorls of petaloid monocots such as lily, tulip, and orchids are homologous with the inner perianth (petal) whorl of core eudicots such as Brassicaceae (Sauquet et al. 2017). Thus not a shift in class B floral homeotic gene expression towards the first floral whorl but a selective reduction of floral whorls may explain the difference in the expression of class B genes with respect to floral whorls. Of course, both the SBM and the reduction of whorls may have contributed to floral diversity during evolution. More research is needed to determine which scenario explains what kind of floral diversity in which lineage of flowering plants more accurately.

New Types of Organs by Changes in Downstream Genes

In a number of cases organs with a very different structure and maybe even identity occurred during evolution. They may nevertheless be specified by very similar class (A)B(C) gene systems, and often might be homologs of the canonical floral organs sepals, petals, stamens, or carpels. The flowers of grasses (Poaceae, such as rice, *Oryza sativa*, and maize, *Zea mays* ssp. *mays*) provide good examples. The most impressive difference arguably concerns lodicules vs. petals. Lodicules are small, grass-specific, glandular-like organs that swell at anthesis to spread the perianth organs lemma and palea apart, so that eventually the wind can better disperse the pollen. Even though lodicules are very different from the usually large and showy

petals, their position within the florets of grasses and the fact that they are specified by class (A) and B organ identity genes strongly suggest that they are homologs of petals (reviewed by Theißen and Melzer 2007).

Another interesting case is provided by the staminodia of columbines (*Aquilegia*), which have been described to possess a "fifth distinct organ identity," even though they clearly have been derived from stamens during evolution (Sharma et al. 2014).

The development of very different, yet homologous floral organs under the control of orthologous homeotic genes is most plausibly explained, at least in part, by changes in target genes of floral homeotic genes during evolution, possibly following gene duplications, thus representing interesting cases where sub- and neofunctionalization are intermingled (Theißen and Melzer 2007; Sharma et al. 2014).

Different Identities Within One Whorl: Zygomorphy and the Orchid Code of Perianth Diversification

Even the organs within one whorl may adopt different identities, as in case of zygomorphic flowers. In contrast to actinomorphic (radially symmetrical) flowers, which have at least two planes of symmetry, zygomorphic (bilateral) flowers have only one (mirror symmetry). One can thus distinguish adaxial (dorsal) from abaxial (ventral) organs. Zygomorphic flowers originated several times independently during evolution, very likely to better attract insect pollinators. A spectacular case is provided by orchids. Here, three types of perianth organs can be distinguished: three outer tepals in the first floral whorl (often termed "sepals"), and two lateral inner tepals ("petals"), as well as a median inner tepal called lip (or labellum) in the second floral whorl. All these tepals are usually of petaloid appearance and express class B floral homeotic genes. However, in contrast to many eudicots where only one *AP3*-like gene is found, orchids typically have four *AP3*-like class B floral organ identity genes. According to the "orchid code hypothesis," sub- and neofunctionalization involving differential expression of the four paralogous *AP3*-like genes led to a combinatorial system that specifies the different identities of the distinct petaloid tepals (Mondragón-Palomino and Theißen 2009). There is evidence that competition between two different floral quartets decides whether outer and lateral inner tepals or lips develop (Hsu et al. 2015), providing a molecular explanation for differences in organ identity within the second whorl. Positional information for the distinction between more dorsal and ventral organs (such as lateral inner tepals and lip of orchids) might be manifested by gradients of TCP-type transcription factors, not only in orchids (Mondragón-Palomino and Theißen 2009).

Outlook

Even though we have learned a lot about the evolution of flowering plants, flowers, floral organs, and floral organ identity genes in recent years, some of this knowledge is fragmented, and from these pieces a comprehensive picture of the jigsaw puzzle of

flower origin has not been achieved yet. Future progress will be certainly achieved by making use of next-generation tools that can be applied genome wide (e.g., sequencing, determination of target genes of transcription factors, CRISPR/Cas), the establishment of new model species or clades among the gymnosperms and basal angiosperms, and, last but not least, a continuous application of the evo-devo rationale (Vialette-Guiraud et al. 2016; Chanderbali et al. 2016).

Cross-References

- ▶ Developmental Homology
- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Evolution of Symmetry in Plants
- ▶ Macroevolution
- ▶ Modeling Evolution of Developmental Gene Regulatory Networks
- ▶ The Evolution of Sex Determination in Plants

Acknowledgments GT thanks Charlie P. Scutt for his kind invitation to write this chapter and for helpful comments on the manuscript.

References

Bateman RM, Hilton J, Rudall PJ (2006) Morphological and molecular phylogenetic context of the angiosperms: contrasting the 'top-down' and 'bottom-up' approaches used to infer the likely characteristics of the first flowers. J Exp Bot 57:3471–3503

Baum DA, Hileman LC (2006) A developmental genetic model for the origin of the flower. In: Ainsworth C (ed) Flowering and its manipulation. Blackwell Publishing, Sheffield, pp 3–27

Chanderbali AS, Berger BA, Howarth DG, Soltis PS, Soltis DE (2016) Evolving ideas on the origin and evolution of flowers: new perspectives in the genomic era. Genetics 202:1255–1265

Dodsworth S (2017) Petal, sepal, or tepal? B-genes and monocot flowers. Trends Plant Sci. 22:8–10

Endress PK (2001) Origins of flower morphology. J Exp Zool (Mol Dev Evol) 291:105–115

Frohlich MW, Chase MW (2007) After a dozen years of progress the origin of angiosperms is still a great mystery. Nat Rev Genet 450:1184–1189

Gramzow L, Weilandt L, Theißen G (2014) MADS goes genomic in conifers: towards determining the ancestral set of MADS-box genes in seed plants. Ann Bot 114:1407–1429

Hsu HF, Hsu WH, Lee YI, Mao WT, Yang JY, Li YY, Yang CH (2015) Model for perianth formation in orchids. Nat Plants 1:15046

Irish VF (2009) Evolution of petal identity. J Exp Bot 60:2517–2517

Kramer EM, Jamarillo MA (2005) Genetic basis for innovations in floral organ identity. J Exp Zool 304B:526–535

Mondragón-Palomino M, Theißen G (2009) Why are orchid flowers so diverse? Reduction of evolutionary constraints by paralogues of class B floral homeotic genes. Ann Bot 104:583–594

Sauquet H, von Balthazar M, Magallón S, Doyle JA et al (2017) The ancestral flower of angiosperms and its early diversification. Nat Commun 8:16047

Scutt CP, Vinauger-Douard M, Fourquin C, Cedric Finet C, Dumas C (2006) An evolutionary perspective on the regulation of carpel development. J Exp Bot 57:2143–2152

Sharma B, Yant L, Hodges SA, Kramer EM (2014) Understanding the development and evolution of novel floral form in *Aquilegia*. Curr Opin Plant Biol 17:22–27

Soltis DE, Chanderbali AS, Kim S, Buzgo M, Soltis PS (2007) The ABC model and its application to basal angiosperms. Ann Bot 100:155–163

Specht CD, Bartlett ME (2009) Flower evolution: the origin and subsequent diversification of the angiosperm flower. Annu Rev Ecol Evol Syst 40:217–243

Stuessy TF (2004) A transitional-combinational theory for the origin of angiosperms. Taxon 53:3–16

Tavares R, Cagnon M, Negrutiu I, Mouchiroud D (2010) Testing the recent theories for the origin of the hermaphrodite flower by comparison of the transcriptomes of gymnosperms and angiosperms. BMC Evol Biol 10:240

Theißen G (2005) Birth, life and death of developmental control genes: new challenges for the homology concept. Theory Biosci 124:199–212

Theißen G, Becker A (2004) Gymnosperm orthologues of class B floral homeotic genes and their impact on understanding flower origin. Crit Rev Plant Sci 23:129–148

Theißen G, Melzer R (2007) Molecular mechanisms underlying origin and diversification of the angiosperm flower. Ann Bot 100:603–619

Theißen G, Becker A, Winter KU, Muenster T, Kirchner C, Saedler H (2002) How the land plants learned their floral ABCs: the role of MADS box genes in the evolutionary origin of flowers. In: Cronk QC, Bateman RM, Hawkins JM (eds) Developmental genetics and plant evolution. Taylor & Francis, London, pp 173–206

Theißen G, Melzer R, Rümpler F (2016) MADS-domain transcription factors and the floral quartet model of flower development: linking plant development and evolution. Development 143:3259–3271

Vialette-Guiraud ACM, Andres-Robin A, Chambrier P, Tavares R, Scutt CP (2016) The analysis of Gene Regulatory Networks in plant evo-devo. J Exp Bot 67:2549–2563

Evolution of Symmetry in Plants

Catherine Damerval, Florian Jabbour, Sophie Nadot, and Hélène L. Citerne

Contents

Abstract

Symmetry provides organisms with an efficient means to cope with physical constraints and explore three-dimensional space. We describe the diversity and

C. Damerval (✉) ·
H. L. Citerne
GQE – Le Moulon, INRA, Univ. Paris-Sud, CNRS, AgroParisTech, Université Paris-Saclay, Gif-sur-Yvette, France
e-mail: damerval@moulon.inra.fr; h_citerne@hotmail.com

F. Jabbour
Muséum national d'Histoire naturelle, Institut de Systématique, Evolution, Biodiversité, UMR 7205 ISYEB MNHN/CNRS/UPMC/EPHE, Sorbonne Universités, Paris, France
e-mail: fjabbour@mnhn.fr

S. Nadot
Laboratoire Ecologie, Systématique et Evolution, UMR 8079 Université Paris-Sud/CNRS/AgroParisTech, Orsay, France
e-mail: sophie.nadot@u-psud.fr

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_59

evolution of symmetry types in the aerial parts of the major group of land plants, the angiosperms. Two main types of symmetry occur: bilateral symmetry, where structures can be divided into two mirror halves, and radial symmetry, with multiple planes of symmetry. Different organ arrangements or phyllotactic patterns produce different types of symmetry, which may vary within a plant's life span. Leaves are usually flat bilaterally symmetrical organs with a bifacial organization resulting from an abaxial-adaxial differentiation associated with photosynthetic activity. Alterations in the genetic pathway underlying this asymmetry are thought to play a role in the repeated evolution of unifacial leaves. Flowers are composed of a series of organs that are considered to be highly modified leaves on a short compact axis. The symmetry of flowers as a whole is one of the most studied traits in plant evolutionary developmental genetics. Bilateral symmetry is derived from radial symmetry, probably from coevolution with specialized pollinators. Nearly 200 transitions in floral symmetry types have been recorded over the course of angiosperm evolution. Symmetry can change during flower development, and the timing of this change can vary between species. *CYCLOIDEA*-like transcription factors have been recruited repeatedly for the control of floral bilateral symmetry in angiosperms. The establishment of bilateral symmetry in leaves and flowers thus relies on different growth processes and gene networks.

Keywords

Symmetry · Evo-devo · Leaf · Flower · *CYCLOIDEA*

Introduction

The term symmetry comes from two Greek words: σύν (*sún,* meaning "with") and μέτρον (*métron*, meaning "measure") and originally indicated a relation of commensurability. It quickly took on the more general meaning of equilibrium of proportions, qualifying the harmony of the different elements of a unitary whole, thus becoming closely related to the idea of beauty and regularity. It is a widely used concept in physics and mathematics. In its current meaning, symmetry is defined in terms of the invariance of an object under specified groups of rotations and reflections. Among the various types of symmetry that are mathematically defined, two types of symmetry are appropriate for describing the phenotypes we consider in this chapter: radial symmetry, corresponding to the repetition of a same structure around a single axis of symmetry (n-fold rotational symmetry), and bilateral symmetry, where a single symmetry plane divides the organism or structure in two mirror images. An organism or a structure is asymmetrical when neither an axis nor a plane of symmetry can be defined.

In living organisms, symmetry can theoretically be examined at every level of complexity from cells to tissues, organs, or whole organisms. Symmetry is present in most body plans, and may be a convenient and adaptive means for organisms to

better explore the three-dimensional space they live in. In biology, however, symmetry is approximate. For instance, bilaterally symmetrical bodies or organs do not have exactly identical mirror halves; indeed, the degree of difference between both halves is often considered an indicator of the stability of development.

This chapter focuses on angiosperms (flowering plants), a group that represents 90% of extant land plant biodiversity. Angiosperm stem and root axes are usually radially symmetrical and terminated by meristems (respectively, the shoot apical meristem (SAM) and the root apical meristem) that ensure continuous growth and organ formation. Three aspects of symmetry in the aerial parts of this group are covered here: (1) phyllotaxis, the arrangement of plant organs, (2) leaf symmetry (independently of leaf form, which is very diverse among angiosperms and beyond the scope of this review), and (3) flower symmetry, the study of which has produced the most abundant literature devoted to evolutionary developmental studies of symmetry in plants.

Phyllotaxis

The SAM generates, in a sequential and regular order, units called phytomers (Fig. 1a), each composed of a leaf, an axillary bud (which together form the node), and a portion of the stem (the internode). In the same way, inflorescence meristems generate flowers, and floral meristems generate floral organs. The resulting pattern of arrangement is called phyllotaxis. The most common arrangements of leaves are spiral (Fig. 2a), distichous (Fig. 2b–c), opposite (two leaves at a node), and verticillate (more than two leaves at a node). In the flower, the insertion of floral organs is either spiral or verticillate (i.e., whorled), the latter being the most widespread. When phyllotaxis is spiral, the divergence angle at which consecutive primordia are generated by the SAM generally approaches 137.5° (the so-called "golden angle"). Organs appear inserted on parastichies (i.e., secondary clockwise and anticlockwise spirals obtained by linking the positions of adjacent organs), and the number of parastichies matches two consecutive numbers of the Fibonacci series (Fig. 2a, 13 clockwise and 8 anticlockwise spirals). From a top view, the different phyllotactic patterns can either be described as bilateral (for distichous phyllotaxis, Fig. 2b–c) or radial (n-fold rotational symmetry with $n =$ the number of parastichies for spiral phyllotaxis, $n = 2$ for opposite phyllotaxis, and $n =$ the number of organs for verticillate/whorled phyllotaxis). The type of phyllotaxis is species specific, but patterns of organ insertion may change during the life of the plant and with the type of organ. For example, cotyledon and early leaf phyllotaxis are not necessarily the same as adult leaf phyllotaxis, and the phyllotaxis of flowers in an inflorescence is not necessarily in continuity with vegetative phyllotaxis.

Such regular arrangements have long fascinated not only botanists but also mathematicians and physicists, and various models have been put forward to account for the phyllotactic patterns found in plants. The hormone auxin has been shown to

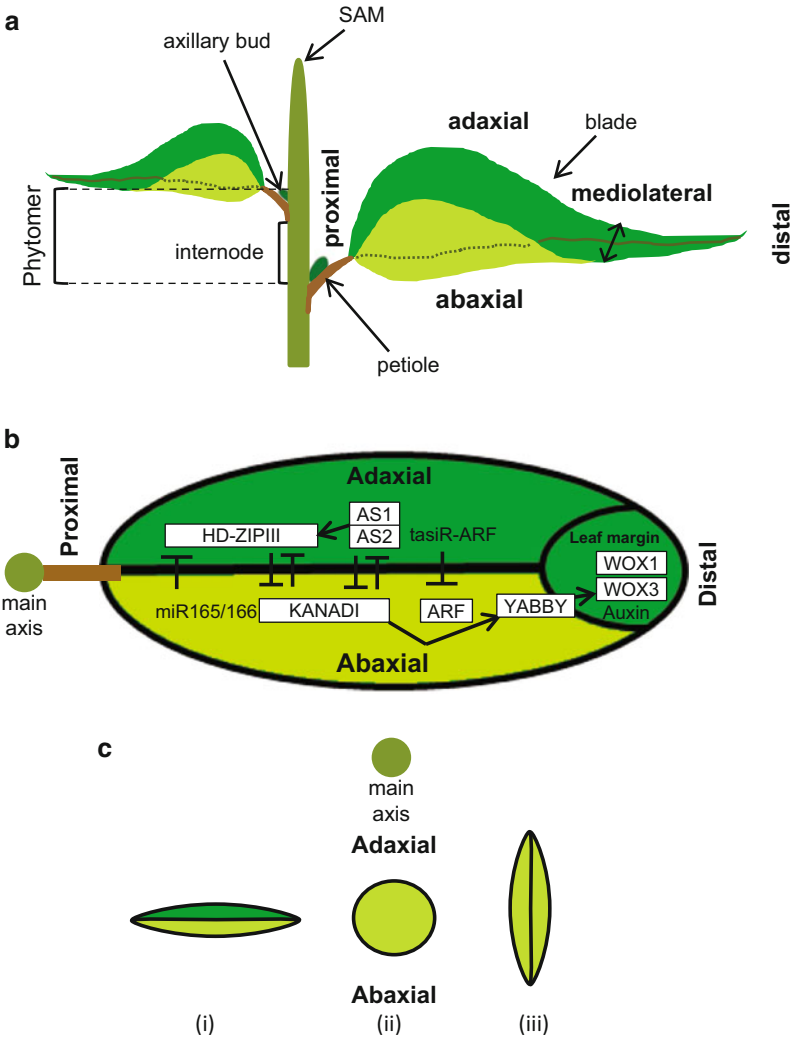


Fig. 1 Schematic representation of vegetative shoot structure and leaf abaxial-adaxial symmetry types and underlying gene regulatory network of leaf polarity. For details concerning gene names and interactions, see text. (**a**) Main components of a vegetative shoot (SAM = shoot apical meristem). The three symmetry axes of a leaf are shown (in bold). (**b**) Cross section of the leaf blade along the proximo-distal axis, showing the main genetic determinants and their interactions (activation: *pointed arrows*, repression: *T-shaped arrows*) involved in abaxial-adaxial polarity and blade outgrowth in *Arabidopsis*. (**c**) Changes in leaf symmetry displayed in cross section along the mediolateral axis; *i*) conventional bifacial leaf with abaxial-adaxial differentiation and bilateral symmetry along the mediolateral axis; (*ii*) unifacial abaxialized leaf with radial symmetry; (*iii*) unifacial abaxialized leaf with bilateral symmetry along the abaxial-adaxial axis

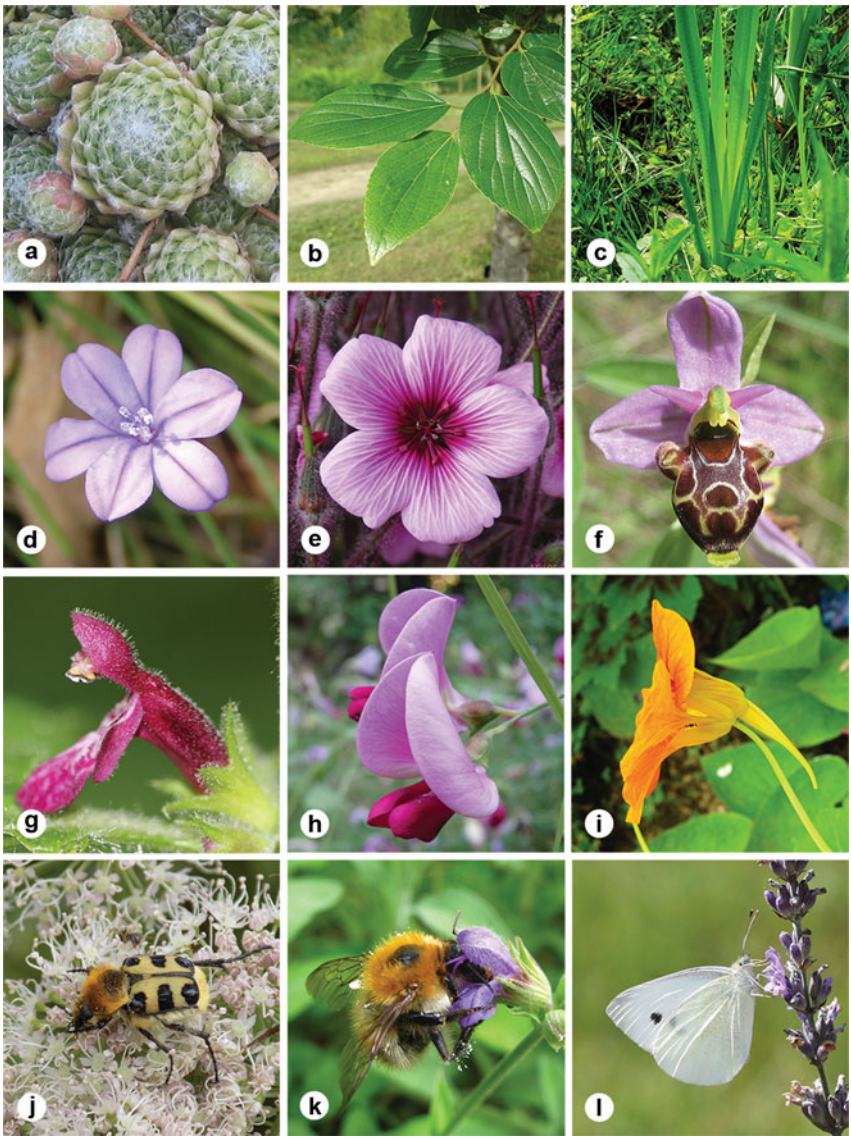


Fig. 2 Photos of leaves and flowers, illustrating various types of symmetry and organ arrangements and interactions between flowers and pollinators. Leaves (**a**) *Sempervirens* sp. with a spiral phyllotaxis. (**b**) *Celtis occidentalis* (Cannabaceae), conventional bifacial bilaterally symmetrical leaf with an alternate distichous phyllotaxis. (**c**) *Iris pseudacorus* (Iridaceae), unifacial bilaterally symmetrical leaf, with an alternate distichous phyllotaxis. For flowers, radial (**d**–**e**) and bilateral symmetry (**g**–**i**) are illustrated in the context of 3-merism, which is the basic organization in monocots (**a**, **f**), and 5-merism which is the basic organization in core eudicots (**b**, **g**–**i**). (**d**) *Aphyllanthes monspeliensis* (Asparagaceae). (**e**) *Geranium maderense* (Geraniaceae). (**f**) *Ophrys apifera* (Orchidaceae), zygomorphic flower with a highly modified ventral petal (labellum). (**g**) *Stachys sylvatica* (Lamiaceae), zygomorphic lip flower (lower lip = 3; upper lip = 2 petals). (**h**) *Pisum sativum* subsp. *elatius*

be a major player in the establishment of phyllotactic patterns in *Arabidopsis*, with local maxima at the shoot apical meristem triggering organ formation. The formation of these maxima depends on the asymmetrical relocalization on the cell membrane of efflux carrier proteins of the PIN-formed family. Recent experiments in *Arabidopsis* show that the interplay between auxin distribution and local biomechanical constraints is a major determinant of phyllotactic patterns (Sassi and Traas 2015). Even though genes whose mutations disrupt phyllotaxis have been characterized in *Arabidopsis*, the genetic networks downstream of auxin signaling are still poorly known, and even less is known regarding the bases of phyllotactic pattern diversity.

Leaf Symmetry

Leaves are determinate lateral organs that are generally differentiated at maturity along three axes: proximo-distal, abaxial-adaxial (dorsoventral), and mediolateral (Figs. 1a and 2a–c). Leaves are usually asymmetrical along the proximo-distal and abaxial-adaxial axes and bilaterally symmetrical along the mediolateral axis. In most monocots, leaves typically consist of a proximal sheath wrapped around the stem and a distal flat blade; in other angiosperms, classical proximo-distal differentiation consists of a proximal petiole and a distal flat blade. Flattening generally occurs along the mediolateral axis and results in a high surface-to-volume ratio. It probably evolved very early in the evolution of vascular plants as an adaptive trait for optimizing photosynthesis. Flattening is tightly linked with specialization of the two faces of the blade, creating bifacial leaves with differentiated adaxial (i.e., closest to the SAM) and abaxial sides (Fig. 1a). The adaxial side is the upper side where light capture is optimized, whereas the abaxial side has a higher stomata density enabling gas exchange and transpiration. Internal leaf anatomy is differentiated with palisade mesophyll cells and xylem tissue on the adaxial side and spongy mesophyll cells and phloem on the abaxial side. Microdissection experiments carried out in the 1950s by Ian Sussex showed that a signal from the SAM is required for the establishment of abaxial-adaxial leaf polarity. When communication between the SAM and the young leaf primordium is disrupted, the leaf becomes abaxialized, meaning that abaxial identity is the default identity.

Fig. 2 (continued) (Fabaceae), zygomorphic flag flower (flag = upper petal). (**i**) *Tropaeolum majus* (Tropaeolaceae), zygomorphic flower with a spur borne on the dorsal sepal. Interactions between flowers and insects: (**j**) *Trichius fasciatus* (Diptera) feeding on pollen from *Angelica sylvestris* (Apiaceae), with the umbel forming a landing platform. (**k**) *Bombus* sp. (Hymenoptera) feeding nectar and/or pollen from a zygomorphic flower of *Salvia* sp. (**l**) *Pieris rapae* (Lepidoptera) feeding on nectar from a zygomorphic flower of *Lavandula angustifolia* (Lamiaceae) (Photographs S. Nadot, C. Damerval and A. Decourcelle)

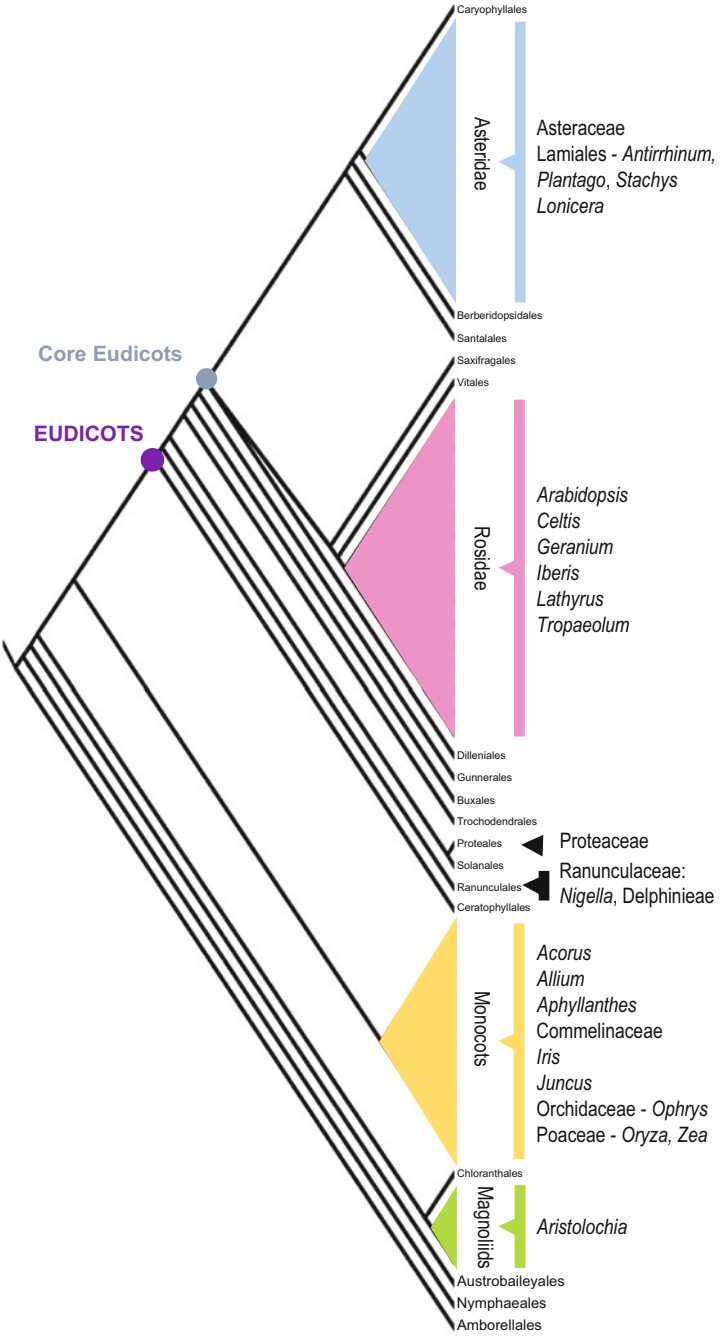


Fig. 3 Simplified angiosperm phylogeny, indicating all genera discussed in the text

Genetic Bases of Asymmetry Along the Abaxial-Adaxial Axis

The genetic network involved in abaxial-adaxial leaf polarity has been unraveled in model species from the core eudicots and the monocots, in *Arabidopsis* and maize in particular (Fig. 3; Yamaguchi et al. 2012; Fukushima and Hasebe 2014). Adaxial determinants include genes from two families of transcription factors: ARP (*ASYMMETRIC LEAVES1 (AS1), ROUGH SHEATH2 (RS2), PHANTASTICA (PHAN)*) and HD-ZIPIII (class III of homeodomain-leucine zipper). The action of the ARP genes is probably mediated by interacting partners. The role of *PHAN* in adaxial identity has been demonstrated in snapdragon (*Antirrhinum majus*), but loss-of-function mutants in *Arabidopsis* of the *PHAN* orthologue *AS1* do not have a consistently abaxialized phenotype, while mutants in maize of the *PHAN* orthologue *RS2* do not have any clear abaxial-adaxial polarity phenotype. In *Arabidopsis*, *AS1* and its unrelated partner *AS2* (*ASYMMETRIC LEAVES2*) positively regulate HD-ZIPIII factors. HD-ZIPIII genes are expressed in the adaxial region and are targeted by regulatory miRNAs (miR165 and miR166) expressed in the abaxial region. Unlike the ARP genes, the role of HD-ZIPIII genes as necessary and sufficient factors of adaxial identity appears conserved between *Arabidopsis* and Poaceae. Abaxial determinants comprise members of the KANADI family and auxin response factors (ARF). KANADI transcription factor genes are expressed in a domain complementary to the HD-ZIPIII genes. They are also necessary and sufficient to promote abaxial identity and are broadly conserved among angiosperms. Both *ETTIN/ARF3* and *ARF4* in *Arabidopsis* are negatively regulated by a small RNA (tasiR-ARF). Antagonistic interactions involving both transcription factors and noncoding small RNAs are important for determining and stabilizing a sharp boundary between the abaxial and adaxial domains, along which the blade will grow (Fig. 1b).

The precise mechanisms acting downstream of the polarity genes and controlling blade outgrowth are not fully understood yet. It has been shown that YABBY transcription factor genes, previously thought to be abaxial determinants, play instead a primary role in blade outgrowth. However, their patterns of expression can differ between species, suggesting the pathway they control is probably not conserved among angiosperms. Genes belonging to two classes of another transcription factor gene family, the *WOX1* and *WOX3/PRESSED FLOWER* subfamilies, which are expressed in the leaf margin and along the abaxial-adaxial juxtaposition region, also play an essential role, at least in *Arabidopsis*. Auxin also appears to be a signal promoting blade outgrowth (Fig. 1b).

Symmetry Changes Along the Mediolateral Axis

Bifaciality and bilateral symmetry along the mediolateral axis are thought to be the ancestral states for the leaf in angiosperms (Harrison et al. 2002). Unifacial leaves, with no abaxial-adaxial differentiation, have evolved multiple times and are frequently found in monocots (Fig. 3) where they nonetheless retain a bifacial sheath. The shift to unifaciality of the blade can lead to a change in leaf symmetry from

bilateral to radial, as found in species of *Allium* or *Juncus*. In other cases, the blade is flattened resulting in bilateral symmetry (e.g., in *Acorus*, *Iris* (Fig. 2c), and some species of *Juncus*). Flattening in unifacial leaves is different from flattening in bifacial leaves in that it involves important cell proliferation toward the SAM side, generating a symmetry plane that is perpendicular to the symmetry plane of bifacial leaves (Fig. 1c). Anatomical studies suggest that unifacial leaves have abaxial characteristics without any differentiation of the epidermal and mesophyll cells and with vascular bundles arranged in a circle with an inner-outer orientation of xylem poles and phloem tissues. A comparative study of two species of *Juncus* with unifacial leaves and with either radial (*J. wallichianus*) or bilateral (*J. prismatocarpus*) symmetry showed that in both species gene expression in the blade tissue was typical of the abaxial domain of the bifacial leaf of rice (Yamaguchi et al. 2010). However, a YABBY gene (*DROOPING LEAF*) was found to be specifically expressed in the proliferative zone close to the SAM only in *J. prismatocarpus* with bilaterally symmetrical leaves. In rice, this gene is expressed in the medial part of leaf primordia where it promotes midrib thickening. Later on in *J. prismatocarpus*, proliferative activity at the leaf margin appears to be correlated with the expression of a *WOX1* gene. These results suggest that part of the genetic circuitry involved in blade outgrowth has been conserved and reused for leaf flattening in *Juncus*. Whether similar processes are involved in the independent evolution of flattened unifacial leaves in other groups remains to be determined.

Floral Symmetry

Flowers form a reproductive unit composed of a series of lateral organs that are considered to be highly modified leaves. Sterile organs surround the fertile organs on a short axis, following an almost invariable order: stamens, producing the male gametophytes (pollen grains), surround the carpels, producing the ovules (each containing a female gametophyte). The sterile organs (frequently a greenish outer calyx (sepals) and a showy inner corolla (petals)) collectively form the perianth and provide protection for the developing fertile organs and attractiveness in the case of animal pollination. The basic number of each type of organ is known as "merism," which is one component of the floral ground plan. Symmetry is another component of floral descriptions and has been used since Theophrastus (371–287 BC). It was recognized very early on that flowers present an overall symmetry that can be radial (actinomorphy – Fig. 2d–e) or bilateral (zygomorphy – Fig. 2f–i) whatever the merism, and that this character could be used, along with other characters, to produce a classification system of flowering plants. Strictly speaking the terms actinomorphy and zygomorphy should only apply to flowers with organs inserted in whorls, but in practice they are extended to flowers with spirally inserted organs. Indeed, as in whorled flowers, it is possible to identify discrete sets of organ types in spiral flowers, even though such flowers are more prone to display organs of intermediate identity. Although symmetry can be defined for each organ whorl (or pseudo-whorl), in practice it is generally applied to the perianth, especially the corolla, and to the

androecium, which often undergoes a reduction in the number of functional stamens in zygomorphic flowers. Zygomorphy can be more or less elaborate, involving organ displacement around the receptacle or strong differentiation along the dorsoventral axis generating different morphologies within a single whorl (e.g., Fig. 2f). Zygomorphic flowers can also vary in the placement of their fertile organs, toward the upper part of the flower (in lip flowers, for example, Fig. 2g) or the lower part of the flower (in flag flowers, for example, Fig. 2h) (Endress 1994). Protruding structures such as spurs or pouches storing nectar can also contribute to zygomorphy (Fig. 2i). In actinomorphic and zygomorphic flowers, individual perianth organs are usually bilaterally symmetrical along their mediolateral axis but asymmetry also exists, for example, in the dorsal and lateral petals of snapdragon or lateral and ventral petals of zygomorphic Fabaceae (Fig. 2h). As lateral organs, floral organs also exhibit proximo-distal and abaxial-adaxial asymmetries, an illustration of which are the conical cells typically found on the adaxial surface of petals that play a major role in pollinator attraction (Glover and Martin 2002).

Plant-Pollinator Interactions Have Fueled the Evolution of Floral Symmetry

Pollinators are believed to be major agents in the evolution of zygomorphy. The fossil record suggests that zygomorphy evolved from actinomorphy around 50 million years after the emergence of angiosperms, in coincidence with the diversification of specialized insect pollinators (Crepet and Niklas 2009). The theory goes that zygomorphic flowers coevolved with specialized insects toward a better placement of their reproductive organs with respect to pollinator body shape, maximizing pollen transfer and resulting in efficient cross-pollination (Fig. 2j–l). Actinomorphic flowers are accessible from all sides (in front view) and are typically pollinated by a wide range of pollinators. By contrast, zygomorphic flowers are more specialized, even though they can be visited by a range of species depending on the environmental context. The shape and size of the flower have been hypothesized to be under stabilizing selective pressure within species with zygomorphic flowers, in order to ensure the best success rate of cross-pollination by specific pollinators and subsequent seed set. Consistent with this hypothesis, several studies have shown that zygomorphic flowers are less variable in size than actinomorphic ones (reviewed in Citerne et al. 2010). A drawback of narrow specialization in zygomorphic flowers is that it can increase species vulnerability if environmental factors lead to a mismatch between blooming period and pollinator activity.

Evolution of Floral Symmetry Across Angiosperms

Multiple lines of evidence suggest that perianth actinomorphy is the ancestral state of the flower. This view is supported by the fossil record, in which actinomorphic flowers predate zygomorphic flowers (Crepet and Niklas 2009), as well as by

phylogeny. Increasingly complete and robust molecular phylogenies of angiosperms (http://www.mobot.org/MOBOT/research/APweb/) now provide a backbone of the tree, which is almost completely resolved at the family level. This means that phylogenetic relationships among the 64 orders and 416 families are almost completely known (Fig. 3) and that the main events of diversification within angiosperms have been dated. The most comprehensive estimate to date found nearly 200 transitions in floral symmetry across the whole of the angiosperms (Reyes et al. 2016), almost three times the previous estimate of 70 (Citerne et al. 2010). For instance, in Proteaceae alone, a basal eudicot family of ca. 1700 species, zygomorphy has been found to have evolved up to 18 times independently from an actinomorphic ancestor, with four reversals to actinomorphy.

The relative importance of historical constraints and selection pressures on the evolution of symmetry can be evaluated statistically by analyzing the co-occurrence, or lack of co-occurrence, of traits in a phylogenetic context. For example, in the Asteridae clade, there is a negative evolutionary relationship between perianth zygomorphy and stamen number, as revealed by the very rare occurrence of zygomorphic flowers with more than ten stamens (Jabbour et al. 2008). Recently, it has been shown that zygomorphy, reduced stamen number, and the presence of a corolla are three character states that act synergistically as a key innovation (O'Meara et al. 2016).

The concept of morphospace provides a novel way (through a mathematical approach based on the ordination of morphological variables) of apprehending the evolution of floral symmetry in different morphological contexts (Chartier et al. 2014). The representation of all trait combinations observed in nature within a space of possible morphologies gives an idea of the possible constraints limiting the evolution of types of flower symmetry.

Establishment of Floral Symmetry During Development

Studies of flower development describe and analyze the processes of organogenesis (organ formation) and morphogenesis (shape acquisition) during the time required for a floral meristem to develop into a fully formed structure ready to bloom. Flower morphology, including symmetry, is generally described at anthesis, when the flower has just opened and is considered to be adult.

The meristem is a changing structure and the type of symmetry recorded at any given developmental stage may be transitory. As a result, symmetry can potentially change several times during development. The symmetry of the early meristem may differ from that of the bud after all organs are initiated and from that of the flower at anthesis. For example, in snapdragon, the early meristem is bisymmetrical (with two perpendicular symmetry planes); the bud is near actinomorphic at sepal initiation but after that point becomes and remains zygomorphic (Vincent and Coen 2004). At anthesis, androecium and corolla are strongly zygomorphic and dorsal and lateral petals are internally asymmetrical, while the ventral petal is bilaterally symmetrical (Fig. 4a). The description of flower symmetry therefore depends on the organ or set of organs considered and on the developmental stage of the flower.

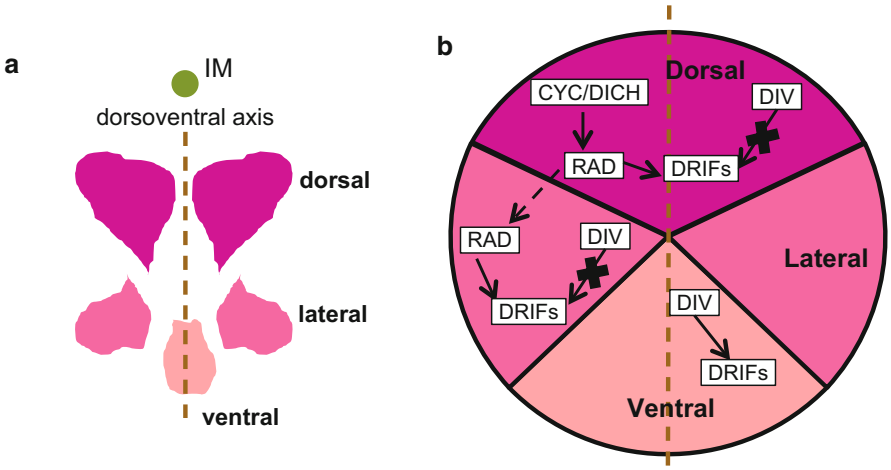


Fig. 4 Illustration of corolla zygomorphy and regulatory gene network specifying petal identity in snapdragon (*Antirrhinum majus*). For details concerning gene names and interactions, see text. (**a**) Diagram of individually dissected petals. The inflorescence meristem (IM) is shown, which determines the dorsoventral axis along which the petals are differentiated. (**b**) The regulatory gene network determining the three types of petal identity: dorsal, lateral, and ventral

In clades where actinomorphy is ancestral and predominant, it is often observed that in zygomorphic species, zygomorphy is established relatively late during development (Endress 1999). For example, in Ranunculaceae, zygomorphy evolved once from actinomorphy in the ancestor of tribe Delphinieae. Flower buds are actinomorphic throughout organogenesis. They become zygomorphic late in development, i.e., after carpel initiation. Dorsal petal primordia develop into spurred petals, whereas the development of ventral petals is arrested shortly after initiation (Fig. 5). In addition, in inflorescences with mostly actinomorphic flowers, it was noted that zygomorphy is established relatively late in the peripheral flowers (e.g., in Asteraceae, Ren and Guo 2015).

Developmental studies provide the basis for formulating evolutionary hypotheses based on comparative analyses. Studying the establishment of symmetry during flower development makes it possible to identify the pivotal stages at which symmetry changes or is constrained (e.g., by the position of the meristem on the inflorescence or by the merism/meristem size ratio). This is necessary for identifying the developmental stages at which molecular investigations should be made to understand how morphological transitions relate to changes in gene expression.

The Genetic Control of Floral Symmetry

The widespread distribution across flowering plants of peloric (radially symmetrical) forms of normally zygomorphic species suggests that floral symmetry is controlled by a few key developmental regulators. Twenty years ago, research groups led by

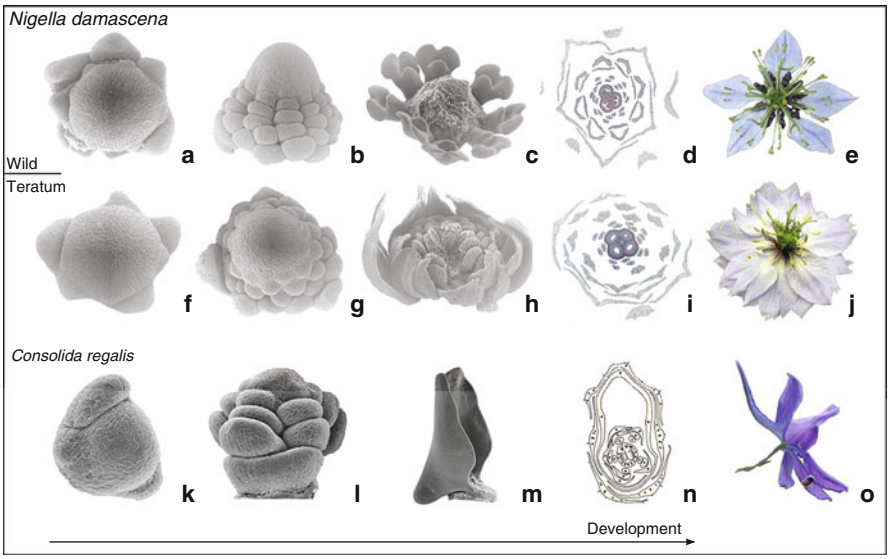


Fig. 5 Developmental series in the flower of *Nigella damascena* and *Consolida regalis* (Ranunculaceae), showing changes in symmetry according to the stage, organ and level of analysis. (**a**–**j**) *Nigella damascena* wild-type (**a**–**e**) and teratological (**f**–**j**) flower morphs. In both morphs, organ primordia initiate on a spiral (**a**–**b**, and **f**–**g**), resulting in a pseudo-actinomorphic meristem. In the wild-type morph, the corolla looks actinomorphic (**c**), although the petals are spirally inserted on the meristem (**c**). In the teratological flower morph without petals (**h**), many sepal-like organs are initiated and the phyllotaxis is also spiral. Flower development as investigated with scanning electron microscopy shows that the pre-anthetic flower is pseudo-actinomorphic. A study of flower anatomy shows that the wild-type morph is pseudo-actinomorphic, the phyllotaxis of the calyx and the androecium being clearly spiral (**d**). The teratological flower morph is internally (from the anatomical aspect) asymmetrical (spiral phyllotaxis, single bract, three sepals) (**i**). Wild-type and teratological anthetic flowers are actinomorphic (**e**, **j**). (**k**–**o**) *Consolida regalis*. At early developmental stages, organogenesis is spiral (**k**–**l**). The bud is zygomorphic due to the corolla reduced to a single petal. The latter has a delayed development compared with the stamens (**l**) and becomes spurred (**m**) when organogenesis is completed. The transverse section (**n**) clearly shows the bilateral symmetry of the floral bud. Note the hollow spur of the dorsal petal nested in the spur of the dorsal sepal. Strictly speaking, the floral bud is pseudo-zygomorphic as the perianth organs and the stamens are spirally initiated. The anthetic flower (**o**) is zygomorphic

Enrico Coen in the UK and Jorge Almeida in Portugal unraveled a network of key developmental genes controlling floral symmetry in snapdragon. In this species, unequal corolla and stamen development along the dorsoventral axis depends on the activity of four genes: *CYCLOIDEA* (*CYC*), *DICHOTOMA* (*DICH*), *RADIALIS* (*RAD*), and *DIVARICATA* (*DIV*). These four genes form two groups that act antagonistically to determine regional identities in the floral meristem. The first group (*CYC-DICH-RAD*) determines dorsal identity. *CYC* and *DICH* are close paralogues belonging to the TCP gene family of plant-specific transcription factors. Both genes are expressed in the dorsal region of the floral meristem prior to organogenesis; their expression becomes restricted to the dorsal petals and staminode (a nonfunctional

and underdeveloped stamen) (*CYC*) and to the dorsal half of the dorsal petals (*DICH*) later in development. In the dorsal staminode, CYC has a negative effect on cell proliferation by repressing cell cycle genes. The activity of CYC and DICH is mediated by *RAD*, a MYB-class transcription factor. CYC and DICH can potentially bind directly to *RAD*, inducing its expression in the dorsal region of the developing flower. Ventral identity is specified by DIV, another MYB transcription factor. DIV activity is controlled posttranscriptionally in the dorsal and lateral floral organs by RAD, which outcompetes DIV for MYB-related DRIFs (DIV and RAD interacting factors) (Fig. 4b).

Whether this network, or some of its components, is conserved in other plant species has been a major focus of research over the past 20 years. It has been shown that some of these key molecular players, and in particular *CYCLOIDEA*-like genes (hereafter *CYC*-like genes), have been recruited repeatedly for the evolution of zygomorphy in diverse lineages. Most studies to date have been conducted in the large core eudicot clade, to which snapdragon belongs (Fig. 3). In core eudicots, a pathway mediated by genes from the *CYC2* clade, a core eudicot-specific *CYC* clade (Howarth and Donoghue 2006), is involved in the control of floral symmetry. In clades where zygomorphy evolved independently (in both Asteridae and Rosidae), asymmetrical *CYC2* expression has been correlated with unequal floral development along the dorsoventral axis, a role that has been corroborated by functional studies in a few model core eudicot species. Although *CYC2* genes have been recruited repeatedly in core eudicots for the control of floral bilateral symmetry, these genes have distinct evolutionary histories in these different lineages, since they have undergone independent duplications that can potentially be the raw material for functional diversification. Independently derived lineage-specific paralogues can differ in the way their roles are partitioned, in their effect on growth and in their region of activity. For instance, asymmetrical *CYC2* expression in core eudicots is primarily on the dorsal side of the flower (sometimes expanding into the lateral region), but there are exceptions where asymmetrical expression is on the ventral side. The effect on growth is also variable: for instance, in *Iberis amara*, a close relative of *Arabidopsis*, persistent asymmetrical dorsal expression of *CYC* in developing flowers reduces petal growth (Busch and Zachgo 2007), unlike in snapdragon where it promotes growth in the later stages of floral development. Ectopic expression in *Arabidopsis* flowers of *CYC* from *I. amara* and snapdragon has opposite effects: petal growth reduction for the former and growth enhancement for the latter, indicating that protein function has diverged between the two (Busch and Zachgo 2007). It is not clear what the ancestral expression pattern of *CYC2* may be, because radial species not derived from zygomorphic ancestors exhibit either persistent radial *CYC2* expression, or transient early expression that can be asymmetric, as in *Arabidopsis* (Cubas et al. 2001). Nevertheless, it appears that repeated spatiotemporal shifts in *CYC2* expression underlie the evolution of asymmetrical floral development along the symmetry plane in core eudicot lineages.

The conservation and co-option of RAD and DIV functions in lineages outside of the core Lamiales (the order to which *A. majus* belongs) are still unclear even though asymmetrical expression of one or the other, or both, has been observed in species from other clades within Asteridae. In core Lamiales, asymmetrical co-expression of *RAD* and *CYC* in the dorsal/lateral regions of the flower may have evolved from a persistent radial expression of both genes, as found in actinomorphic early-diverging Lamiales (Zhong and Kellogg 2015). However, in *Arabidopsis*, endogenous *RAD*-like genes could not be activated by CYC (Baxter et al. 2007), suggesting that *RAD* genes may not be co-opted in the floral symmetry pathway of Rosidae.

Secondary loss of zygomorphy within diverse lineages of core eudicots is associated with spatiotemporal changes in *CYC2* expression, but not generally with gene degeneration and loss, suggesting that these genes may have other functions. Two main types of changes leading to a loss of asymmetrical expression are observed: absent or downregulated expression (often with residual expression early in development) or expansion of the expression domain to all regions of the floral whorl. However, in core Lamiales, secondary loss of zygomorphy is associated not only with changes in *CYC* expression but also with a breakdown of the *CYC-RAD-DIV* regulatory pathway. This is found in the wind-pollinated flowers of *Plantago* where one *CYC* paralogue and *RAD* are lost and where changes in expression of the remaining single *CYC* copy (across the flower bud) (Preston et al. 2011) and *DIV* (regulating stamen rather than petal development) (Reardon et al. 2014) are correlated with its derived radial symmetry.

Outside of the core eudicots, asymmetrical dorsoventral expression of *CYC*-like genes in developing flowers has been described in certain zygomorphic basal eudicots, monocots, and in the magnoliid genus *Aristolochia* (Fig. 3). In the latter, asymmetrical dorsoventral expression is evident only in late floral development, suggesting that the early establishment of zygomorphy may be under the control of other genetic factors (Horn et al. 2015). By contrast in orchids, where floral structure and bilateral symmetry are particularly elaborate (Fig. 2f), *CYC*-like genes do not appear to play an important role in the floral symmetry pathway. The development of distinct organs within the perianth (outer and inner tepals and lip) is linked to differential expression of B-class MADS-box *DEFICIENS*-like (*DEF*-like) genes. The perianth organs are specified by different combinations, known as the "orchid code," and relative expression levels of four *DEF*-like paralogues (Mondragón-Palomino and Theissen 2011). The involvement of MADS-box genes in the floral symmetry pathway may be conserved among monocots, since asymmetrical expression patterns have been recorded in Commelinaceae and Poaceae. Their interactions with *CYC*-like genes, if any, are currently unknown.

CYC-like genes, and in certain cases MADS-box genes, appear to be the central players for asymmetrical floral development in angiosperms, subdividing organ identity within a whorl (Fig. 4a). In independent acquisitions of zygomorphy, novel interactions of these key genes with different targets may have evolved, providing scope for the morphological variation displayed by zygomorphic flowers. The factors regulating *CYC*-like genes and the role of *CYC*-like genes

in actinomorphic species are still poorly known. Such knowledge would help us understand what make these genes prone to repeated recruitment for the control of zygomorphy.

Future Prospects

Angiosperm leaves evolved and diversified from leaves of the ancestral lineage of seed plants emerging in the Late Devonian-Early Carboniferous. Bilateral symmetry along the mediolateral axis is ancestral in leaves. Physical factors impacting photosynthesis (e.g., light, atmospheric CO_2 concentration, heat dissipation) have probably been instrumental in the evolution of flattening and abaxial-adaxial differentiation promoting bilateral symmetry. By contrast, bilateral symmetry in flowers is a derived state within angiosperms, resulting from an ongoing process of coadaptation between pollinators and flowers and postdated by 50 million years the origin of angiosperms in the Early Cretaceous.

It is not surprising that the control of symmetry within individual lateral organs, be it leaves or floral organs, seems to share certain features (auxin fluxes, expression of similar transcription factors, establishment of boundaries by antagonistic molecular interactions), which coexist with particular determinants of organ identity. While asymmetry of individual flower organs along the proximo-distal and abaxial-adaxial axes might be controlled by genetic circuitries similar to those of leaves, symmetry along the mediolateral axis in petals seems to result from different processes, involving *CYC*-like genes but also other factors. The interplay of several actors, including the activity of key transcription factors, even though they differ in the two structures, is a common feature of the control of bilateral symmetry in leaves and flowers. The parallel recruitment of key regulators from the same developmental program is a feature of biological evolution, and in plants, it has been described for the independent evolution of leaves, roots, and C4 photosynthesis, for example. Indeed, present knowledge points to the repeated co-option of *CYC*-like genes for bilateral symmetry in flowers. Studies investigating independent shifts to either radial or bilateral symmetry in unifacial leaves are still too scarce to know if a similar developmental program has been repeatedly co-opted.

Deciphering gene regulatory networks in a comparative framework has become the next stage for understanding the origin of morphological diversity. New technologies will help us progress toward this goal and renew our approach of evolutionary developmental questions in the near future. Developmental studies are fundamental for identifying the crucial stages where the underlying molecular changes are predicted to take place. New nondestructive imaging methods such as the recently developed X-ray micro-computed tomography give access to the three-dimensional organization of developing flowers, enabling the observation of rare or difficult material. Additionally, live-imaging techniques such as light sheet fluorescence microscopy can be used in some species and organs. With new generation sequencing techniques, genome and/or transcriptome sequencing has become feasible for virtually any species, challenging the candidate gene approach and giving

access to potentially novel genes affecting the trait of interest. Techniques such as virus-induced gene silencing are feasible in a large panel of species, enabling functional validation. In addition, better resolved phylogenies and new theoretical and analytical approaches for reconstructing micro- and macroevolutionary patterns will help uncover the driving forces and constraints in phenotype evolution.

Cross-References

► Evolution of Floral Organ Identity

Acknowledgments We acknowledge Thierry Deroin for helpful discussions on flower anatomy and development and careful reading of the manuscript.

References

Baxter CE, Costa MM, Coen ES (2007) Diversification and co-option of *RAD*-like genes in the evolution of floral asymmetry. Plant J 52:105–113

Busch A, Zachgo S (2007) Control of corolla monosymmetry in the Brassicaceae *Iberis amara*. Proc Natl Acad Sci USA 104:16714–16719

Chartier M, Jabbour F, Gerber S, Mitteroecker P, Sauquet H, von Balthazar M, Staedler Y, Crane PR, Schönenberger J (2014) The floral morphospace – a modern comparative approach to study angiosperm evolution. New Phytol 204:841–853

Citerne H, Jabbour F, Nadot S, Damerval C (2010) The evolution of floral symmetry. In: Kader JC, Delseny M (eds) Advances in botanical research, vol 54. Elsevier, London, pp 85–137

Crepet WL, Niklas KJ (2009) Darwin's second "abominable mystery": why are there so many angiosperm species? Am J Bot 96:366–381

Cubas P, Coen E, Zapater JM (2001) Ancient asymmetries in the evolution of flowers. Curr Biol 11:1050–1052

Endress PK (1994) Diversity and evolutionary biology of tropical flowers. Cambridge University Press, Cambridge, UK

Endress PK (1999) Symmetry in flowers: diversity and evolution. Int J Plant Sci 160:S3–S23

Fukushima K, Hasebe M (2014) Adaxial-abaxial polarity: the developmental basis of leaf shape diversity. Genesis 52:1–18

Glover BJ, Martin C (2002) Evolution of adaptive petal cell morphology. In: Cronk QCB, Bateman RM, Hawkins JA (eds) Developmental genetics and plant evolution. Taylor & Francis, London, pp 160–172

Harrison CJ, Cronk QCB, Hudson A (2002) An overview of seed plant leaf evolution. In: Cronk QCB, Bateman RM, Hawkins JA (eds) Developmental genetics and plant evolution. Taylor & Francis, London, pp 395–403

Horn S, Pabón-Mora N, Theuß VS, Busch A, Zachgo S (2015) Analysis of the CYC/TB1 class of transcription factors in basal angiosperms and magnoliids. Plant J 81:559–571

Howarth DG, Donoghue MJ (2006) Phylogenetic analysis of the "ECE" (CYC/TB1) clade reveals duplications predating the core eudicots. Proc Natl Acad Sci USA 103:9101–9106

Jabbour F, Damerval C, Nadot S (2008) Evolutionary trends in the flowers of Asteridae: is polyandry an alternative to zygomorphy? Ann Bot 102:153–165

Mondragón-Palomino M, Theißen G (2011) Conserved differential expression of paralogous *DEFICIENS*- and *GLOBOSA*-like MADS-box genes in the flowers of Orchidaceae: refining the 'orchid code'. Plant J 66:1008–1019

O'Meara B, Smith SD, Armbruster WS, Harder LD, Hardy CR, Hileman LC, Hufford L, Litt A, Magallón S, Smith SA, Stevens PF, Fenster CB, Diggle PK (2016) Non-equilibrium dynamics and floral trait interactions shape extant angiosperm diversity. Proc R Soc B 283:2015304

Preston JC, Martinez CC, Hileman LC (2011) Gradual disintegration of the floral symmetry gene network is implicated in the evolution of a wind-pollination syndrome. Proc Natl Acad Sci USA 108:2343–2348

Reardon W, Gallagher P, Nolan KM, Wright H, Cardeñosa-Rubio MC, Bragalini C, Lee C, Fitzpatrick DA, Corcoran K, Wolff K, Nugent JM (2014) Different outcomes of the MYB floral symmetry genes *DIVARICATA* and *RADIALIS* during the evolution of derived actinomorphy in *Plantago*. New Phytol 202:716–725

Reyes E, Sauquet H, Nadot S (2016) Floral symmetry changed at least 199 times in angiosperms. Taxon 65:945–964

Ren JB, Guo YP (2015) Behind the diversity: ontogenies of radiate, disciform, and discoid capitula of *Chrysanthemum* and its allies. J Syst Evol 53:520–528

Sassi M, Traas J (2015) When biochemistry meets mechanics: a systems view of growth control in plants. Curr Op Plant Biol 28:137–143

Vincent CA, Coen ES (2004) A temporal and morphological framework for flower development in *Antirrhinum majus*. Can J Bot 82:681–690

Yamaguchi T, Yano S, Tsukaya H (2010) Genetic framework for flattened leaf blade formation in unifacial leaves of *Juncus prismatocarpus*. Plant Cell 22:2141–2155

Yamaguchi T, Nukazuka A, Tsukaya H (2012) Leaf adaxial-abaxial polarity specification and lamina outgrowth: evolution and development. Plant Cell Physiol 53:1180–1194

Zhong J, Kellogg EA (2015) Stepwise evolution of corolla symmetry in *CYCLOIDEA2*-like and *RADIALIS*-like genes expression patterns in Lamiales. Am J Bot 102:1260–1267

Part VII

Invertebrate Evo-Devo

Evo-Devo of Butterfly Wing Patterns

Jeffrey M. Marcus

Contents

Abstract

The evolution and development of lepidopteran wing patterns are a valuable system for understanding cellular development and differentiation of phenotypes with clear ecological functions. Butterfly wings are only two cells thick, with single epithelia making up the dorsal and ventral wing surfaces. The color patterns found on lepidopteran wings are formed from a mosaic of scale cells containing pigments or with structural coloration caused by the reflection of particular light wavelengths by ridges found on scale surfaces. Butterfly color patterns are laid down on top of a preexisting developmental genetic architecture that directs wing development and which determines the shape of each wing, allows for the functional specialization of forewings and hindwings and of dorsal and ventral wing surfaces, and which specifies the positions of the longitudinal veins on the wing. This architecture also provides a mechanism by which different regions on the wing can be regulated independently of one another. The Nymphalid ground plan is an archetype that can be used to compare and homologize color patterns from different Lepidoptera species. Many genes related to the determination of many color pattern elements within

J. M. Marcus (✉)
Department of Biological Sciences, University of Manitoba, Winnipeg, MB, Canada
e-mail: marcus@cc.umanitoba.ca

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_174

the Nymphalid ground plan have been identified, and for border ocelli (or eyespot) patterns in particular, a large genetic regulatory network for pattern formation and differentiation has been assembled. Many Lepidoptera model species have contributed to our understanding of butterfly color pattern development and evolution, but recent comparative genomic work examining of intraspecific and interspecific variation in *Heliconius* has identified new genes with important roles in pattern development.

Keywords

Color patterns · Insect wings · Developmental patterning · Cellular differentiation · Nymphalid ground plan

Introduction

The color patterns on the wings of butterflies and moths (order Lepidoptera) are among the most striking patterns in nature, with demonstrated roles in mate choice, thermoregulation, and predator avoidance (including the substrategies of camouflage, disruptive coloration, attack deflection, aposematism, and mimicry). Lepidopteran wing color patterns are among the most complex of any insect and are made up of overlapping wing scales that project above the surface of the wing membrane like tiles or shingles on a roof as seen in Fig. 1. Other insect wings lack scales and primarily feature melanin pigments, while butterfly and moth wings are colored by combinations of pigments produced by the ommochrome (yellow, orange, red, and brown), melanin (grey, black, brown, tan, and yellow), pteridine (white), and urate granule (white) biosynthetic pathways in the scales, as well as by structural colors (including iridescent blue, green, and violet). Structural colors are produced by microscopic ridges composed of layered lamellae on the surfaces of the scales that appear to be produced by the regulation of the polymerization of actin filaments during scale development (Dinwiddie et al. 2014; Parnell et al. 2018). On the whole, Lepidopteran color patterns are particularly suitable for study because they are complex, yet consist of clearly defined subunits, exist primarily in two dimensions, and are structurally simple; features that have helped

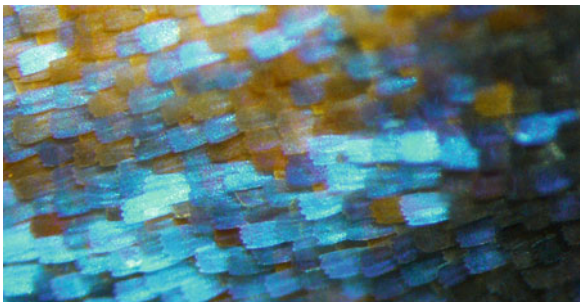


Fig. 1 Tiled scales on the forewing of the nymphalid butterfly *Precis octavia* containing ommochrome (orange) and melanin (black) pigments or showing iridescent blue structural coloration

make them an important model system in the field of evolution and development (Marcus 2005).

Lepidopteran wing scales differentiate during metamorphosis in the latter part of pupal stage of development and measurable quantities of pigments are produced in scale cells during this period. However, the groups of adjacent cells in the developing wing tissues (also known as wing imaginal discs) that are regulated in a coordinated fashion and express the same pigments to form color pattern elements are developmentally determined in the last (often the 5th) larval instar of the caterpillar and in the early pupal stage (Fig. 2). The wing imaginal discs are established much earlier in development and are usually discernable under the

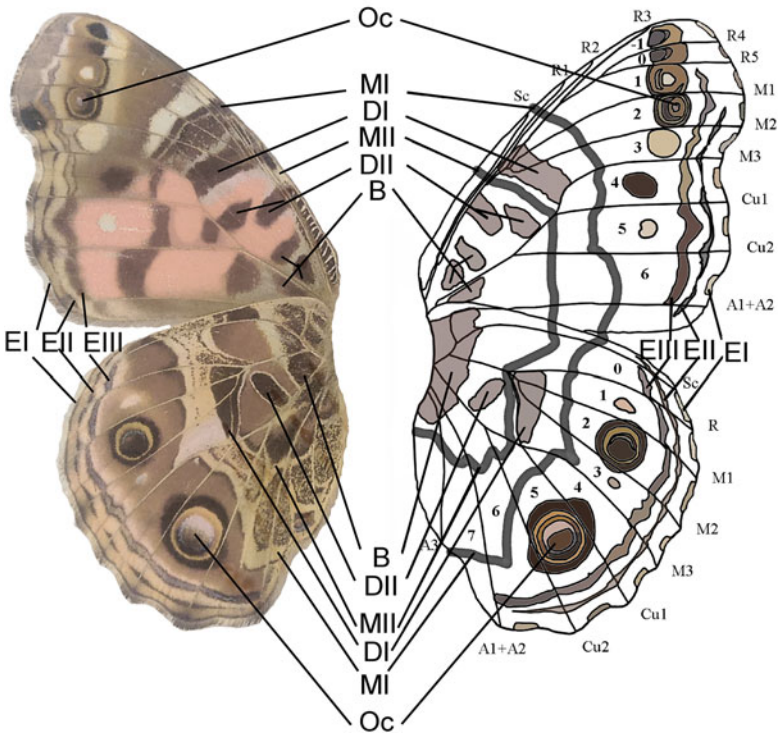


Fig. 2 The Nymphalid ground plan for Lepidopteran wing color pattern organization as expressed on the ventral wing surfaces of the butterfly, *Vanessa braziliensis* (family Nymphalidae), after Abbasi and Marcus (2015, 2017) and labeled using the nomenclature of Schwanwitsch (1924). The ground plan color pattern elements are sometimes divided into three major divisions: the basal symmetry system [consisting of the Basalis (B) pattern element], the central symmetry system [centered on Discalis I (DI) and including Discalis II (DII) and two Medial bands (MII and MI)], and the border symmetry system at the wing margin [containing the Border ocelli (OC, often referred to as eyespots) and the Externa patterns (E): including parafocal (EIII), submarginal (EII), and marginal (EI) elements]. The wing veins in *Vanessa* are also labeled including the Subcosta (Sc); Radius (R); Media (M); Cubitus (Cu); and Anal (A). The wing sectors are numbered in the forewing and hindwing after Abbasi and Marcus (2015, 2017)

microscope by the third larval instar. They rest just inside of the larval body wall in a dorso-lateral position in the second and third thoracic segments. This position makes them surgically accessible and they can easily be manipulated in vivo or removed for in vitro experiments during the larval and pupal stages of development. Adult insect wings, including those of Lepidoptera, consist primarily of dead tissue, and so all developmental processes cease almost immediately after adult emergence from the pupal case as the wings harden and the cells that make up the dorsal and ventral wing epithelia die.

Nymphalid Ground Plan and Wing Patterning in Three Dimensions

The typical color pattern elements on the wings of most butterfly species and also many moths can be understood as derivatives of the Nymphalid ground plan (Fig. 2). The Nymphalid ground plan was originally proposed by Schwanwitsch (1924) and reintroduced into the recent scientific discourse by Nijhout (1991) as a paradigm for understanding the development and determining homologies among the patterns found in different species of Lepidoptera. Individual color pattern elements are sometimes grouped into three "symmetry systems," within which color pattern elements often show correlated phenotypes and may have shared developmental characteristics (Otaki 2012). Yet, discrete color pattern elements within each symmetry system can vary in size, color, and position between species and also between sexes or morphs in polymorphic species (Nijhout 1991). The complete set of the color pattern elements that constitute the Nymphalid ground plan may never have all co-occurred in any individual lepidopteran species, so it should be thought of as an archetype (a conceptual framework) rather than as an ancestral state for color patterns.

The location of each color pattern element within the Nymphalid ground plan is determined by the preexisting developmental architecture of the wings: the cellular signals and patterns of gene expression that establish wing identity (forewing vs. hindwing) and the dorsal-ventral, anterior-posterior, and proximal-distal axes of the wing (Fig. 3). Hindwings become differentiated from the forewings through the expression of the hox gene *Ultrabithorax,* which is otherwise involved in patterning the third thoracic segment of the insect anterior-posterior body axis (Weatherbee et al. 1999). Insect wings are also divided into dorsal and ventral developmental compartments by expression of Apterous, a transcription factor that is expressed in the dorsal wing epithelium (Prakash and Monteiro 2018). The actions and interactions of the two transcription factors Ultrabithorax and Apterous permit the independent upregulation or downregulation of downstream targets on any combination of the four wing surfaces, establishing the inherent capacity for each wing surface to take on different color pattern phenotypes and visual signaling functions.

Within each wing surface, the placement of color pattern elements is specified by signals associated with patterning with the proximal-distal and anterior-posterior

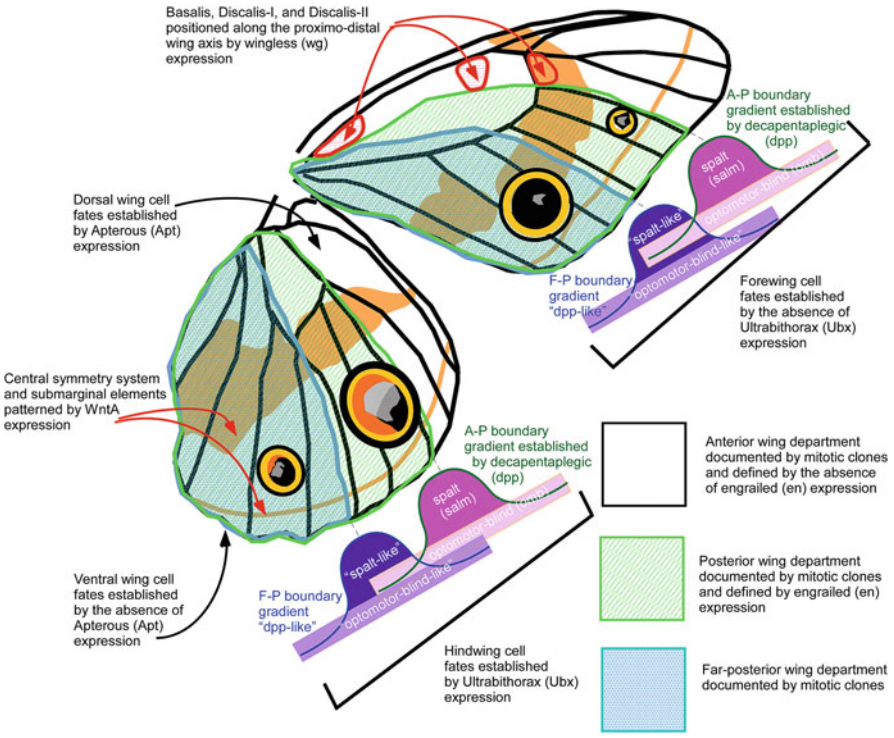


Fig. 3 Establishing the Nymphalid ground plan in three dimensions. Color patterns in species corresponding to the typical Nymphalid ground plan are regulated by transcription factors Apterous (which is expressed in dorsal wing surfaces and specifies dorsal versus ventral cell fates, establishing the dorsal-ventral wing axis (Prakash and Monteiro 2018)), Ultrabithorax (which is expressed in the hindwing and specifies hindwing versus forewing cell fates (Weatherbee et al. 1999)), and Engrailed (which is expressed in the posterior wing compartment and specified anterior versus posterior wing cell fates, establishing the anterior-posterior wing axis (Keys et al. 1999)), as well as the signaling proteins decapentaplegic and the not-yet characterized "decapentaplegic-like" (which also contribute to anterior-posterior axis specification), and wingless and Wnt-A (which specify the color pattern elements along the proximal-distal wing axis) (Carroll et al. 1994; Martin and Reed 2014). Though the expression of these proteins, as well as downstream genes upregulated in response to their expression (including *Spalt, optomotor blind,* and genes with analogous functions (Zhang and Reed 2016)), it is possible for the genetic regulatory apparatus of the wing to place color patterns at precise locations on particular wing surfaces (Abbasi and Marcus 2017)

axes (Fig. 3). The placement of color patterns along the proximal-distal axis is patterned primarily by Wnt ligands, especially wingless (wg) and WntA (Carroll et al. 1994; Martin et al. 2012; Martin and Reed 2014). The size and location of Basalis, Discalis-I, and Discalis-II pattern elements appear to be determined by wingless expression, while the Medial-I and Medial-II of the central symmetry system and the submarginal band (EII) of the border symmetry system are associated with WntA signaling (Fig. 3). Often the final colors of the patterns in the adult butterfly wing produced by wg and WntA signaling are different, suggesting that

these very similar ligands differentially interact with their receptors or have differential effects on downstream components of the signal transduction pathways to produce alternative outcomes for adult wing pigmentation (Martin and Reed 2014). Wnt ligands not only activate signal transduction through the canonical pathway (involving β-catenin), but sometimes also through the c-Jun N-terminal kinase (JNK), and Ca2+ alternative signaling pathways, suggesting one possible mechanism by which similar ligands could produce different color pattern outcomes. However, it is not yet known which signaling pathways are triggered by the Wnt signals on the wings of butterflies (Özsu and Monteiro 2017).

Anterior-posterior axis patterning in insect wings is initiated by the expression of Engrailed transcription factor in the posterior portion of each body segment early in embryogenesis (Abbasi and Marcus 2017). Engrailed expression is maintained throughout development, defining the far posterior compartment of the insect wing (Fig. 3). Engrailed upregulates the expression of hedgehog (hh), a short-range signaling molecule that binds to receptors in a band of cells immediately anterior to the posterior compartment, which respond by producing a longer-range signaling molecule decapentaplegic (dpp) (Keys et al. 1999) at the A-P boundary. High concentrations of dpp result in expression of the transcription factor spalt (sal), while lower concentrations of dpp are sufficient to drive expression of a second transcription factor optomotor-blind (omb).

It has recently been proposed (Abbasi and Marcus 2017) that there is an additional developmental compartment in the far posterior of the wing in Lepidoptera, which further subdivides the wing. This additional compartment boundary may establish a second gradient of a dpp-like signaling molecule and a second set of nested domains of gene expression (sal-like at high concentrations of ligand, omb-like at lower concentrations) similar to those that have been described more anteriorly (Fig. 3). Collectively, these domains of expression in the developing wing are used to define the locations of the incipient wing veins (which serve as channels for blood flow to provide nutrients to wing cells and through which trachea grow permitting cellular gas exchange), and subdividing the wing into a series of sectors. It has also been pointed out that the combination of transcription factors expressed within each wing sector according to this scheme is unique, and might serve as a combinatorial code or "address" by which each wing sector can be targeted individually by the machinery of wing development and consequently express unique color pattern phenotypes. This is most easily observed in the variation among border ocelli (eyespot) phenotypes between wings, wing surfaces, and wing sectors shown by individual butterflies (Figs. 2 and 3).

The Development of Eyespots/Border Ocelli

Of all lepidopteran color patterns that make up the Nymphalid ground plan, our mechanistic understanding of the details of development is greatest for the border ocelli or eyespots. There are more candidate genes with known expression patterns in border ocelli and more gene products with demonstrated effects on border ocelli

phenotypes than are known for any other lepidopteran color pattern (Özsu et al. 2017; Özsu and Monteiro 2017), which has allowed for the construction of computational models for the determination of these phenotypes (Evans and Marcus 2006; Marcus and Evans 2008).

Among the first gene products to be studied extensively with respect to border ocelli was the transcription factor Distal-less, which functions to define the wing margin in many insects, but is also involved in specifying the focus (center) of border ocelli in butterflies and moths (Fig. 4) (Brakefield et al. 1996; Monteiro et al. 2006). The cells that make up the eyespot focus are located in an indentation relative to the rest of the wing epithelium and appear to have an accelerated cell cycle relative to other wing cells. This makes the locations of foci visually identifiable long before pattern differentiation even without staining for genetic markers (Iwasaki et al.

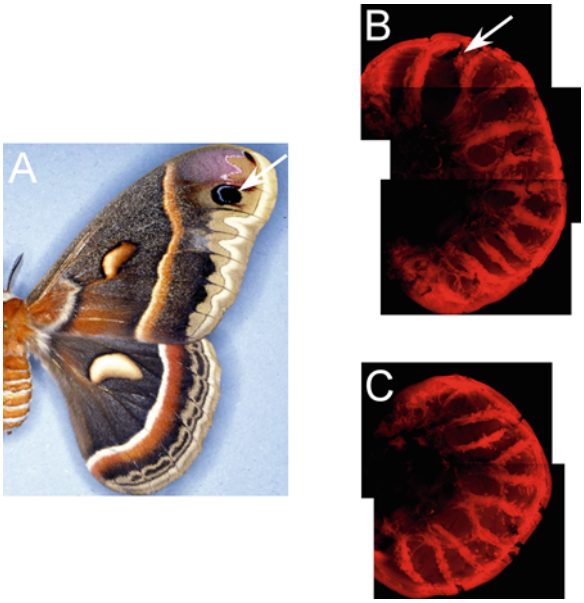


Fig. 4 (**a**) The cecropia moth, *Hyalophora cecropia* (family Saturniidae), has a small border ocellus (white arrow) in the anterior forewing but lacks border ocelli in the hindwing. (**b**) *Hyalophora* late fifth larval instar forewing disc showing expression of Distal-less transcription factor protein in the incipient eyespot focus (white arrow) as visualized with an anti-Distal-less polyclonal primary antibody and a fluorescent TRITC-conjugated mouse anti-rabbit monoclonal secondary antibody, as in butterfly eyespots (Brakefield et al. 1996). (**c**) *Hyalophora* moths lack border ocelli in the adult hindwing and lack foci of Distal-less expression in late fifth larval instar hindwing discs treated in the same way as described above. The tracheae that define the position of the wing veins in the imaginal discs and which are present along the wing imaginal discs margins are autofluorescent. Thus, the fluorescence of the tracheae in panels **b** and **c** is independent of the presence of the primary antibody and is not an indication of Distal-less expression. As in many other Saturniid moths (Monteiro et al. 2006), late fifth larval wing imaginal discs in *H. cecropia* are large (over 1 cm^2 each), so each figure panel is a mosaic of two or three fluorescent photo micrographs taken at 5× magnification

2017). The coincidence of eyespot foci with physical indentations in the wing epithelium facilitated classic experiments involving focus ablation or transplantation that established the focus as the developmental organizer for border ocelli (Nijhout 1991).

In its role as a developmental organizer, the cells of the eyespot focus release a signal that contributes to pattering the surrounding wing tissue into concentric rings (Marcus 2005). The molecular identity of this signal is not yet known, but a large number of gene products involved in specifying the size and location of the focus have been identified (Fig. 5) (Evans and Marcus 2006; Marcus and Evans 2008). Early models for the creation of the concentric rings of the eyespot were based on different focal signal concentration threshold responses associated with each color ring (Brakefield et al. 1996). More recent models suggest that the incipient rings may also be producing ligands in response to the signals from the eyespot focus (Otaki 2011), generating feedback loops that further define and consolidate the ring-like patterns. In the African bush brown butterfly, *Bicyclus anynana* (Nymphalidae: Satyrinae), there is evidence during the early pupal stage (but not during late larval development when the primary focal signal is produced) that wingless ligand is secreted transiently by the focal cells to interact with the surrounding tissue, and in response, a transient signal from the TGF-β family (which includes dpp) is being received by the cells of the eyespot focus from the surrounding tissue (Fig. 5) (Monteiro et al. 2006). This may correspond to the phenomenon of developmental pattern reinforcement in border ocelli identified by Otaki (2011). Once established, each ring of the border ocellus expresses a different combination (or level of expression) of transcription factors (Brunetti et al. 2001), which are suspected to directly or indirectly upregulate components of the biosynthetic pathways required for the production of pigments.

In *Bicyclus anynana,* the border ocellus in the adult butterfly is composed of a white focus, surrounded by an inner black ring and an outer yellow ring. It is thought that the focus is colored by white, ultra-violet reflective pteridine pigments, while both the black and yellow rings are made up of different forms of melanin (Fig. 5) (Matsuoka and Monteiro 2018). The color of butterfly scale cells containing different forms of melanin may be determined by which components of the pathway have been upregulated and/or by the availability of different melanin precursors in the hemolymph relative to the timing of scale cell differentiation (a process regulated by Notch (N) signaling) during the pupal stage (Marcus 2005). Finally, it should be noted that in some other butterfly species, red ommochrome pigments are made in eyespot rings (Brunetti et al. 2001; Marcus 2005) suggesting that other biosynthetic pathways can also be recruited and expressed as part of this genetic regulatory network.

Heliconius and the Importance of Extreme Phenotypes

While the wing color patterns found in most groups of butterflies (and many moths) can be mapped on to the Nymphalid ground plan with relative ease, there are some notable exceptions (Nijhout 1991; Schachat and Brown 2016).

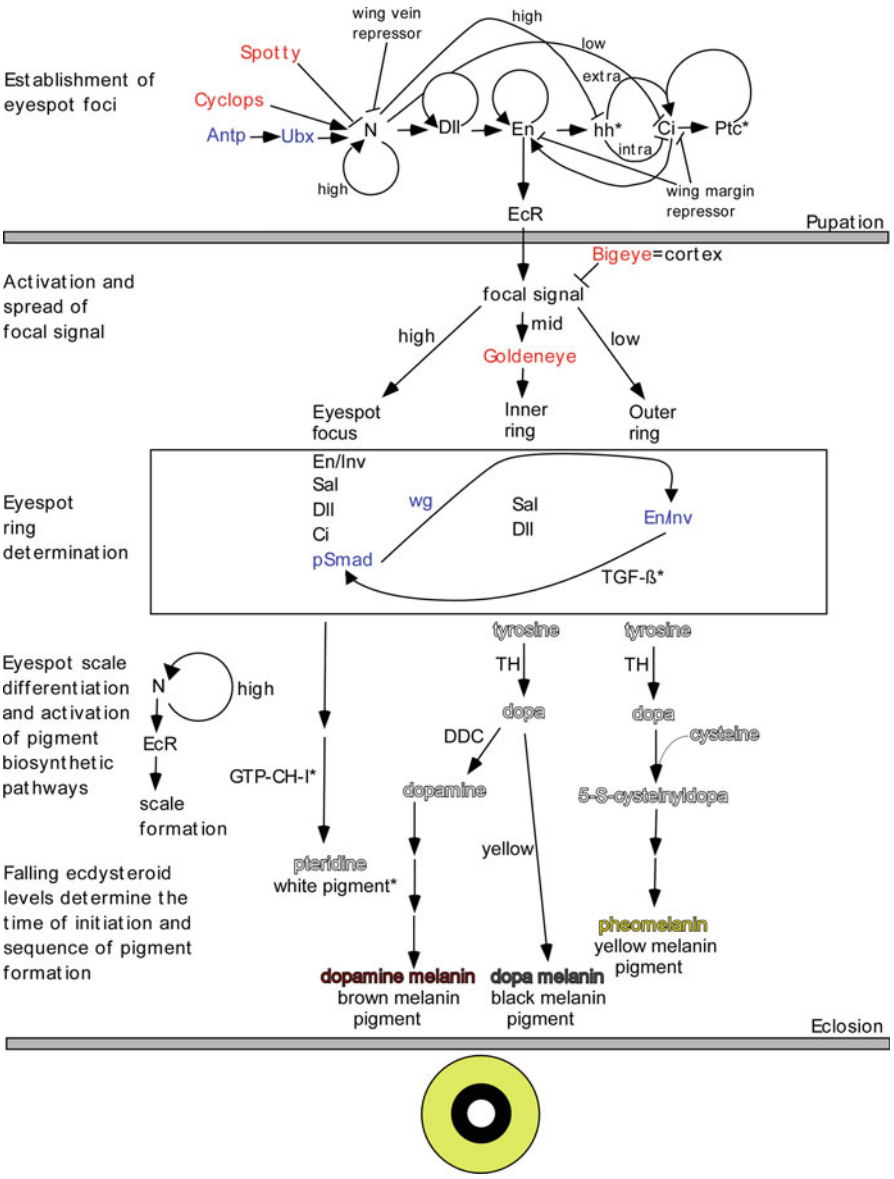


Fig. 5 Developmental model for the formation of border oscelli (also known as eyespots) in *Bicyclus anynana* (Nymphalidae: Satyrinae). Genes labeled in red are spontaneous mutants that appear to function in color pattern development, but with one exception (*Bigeye* appears to be a mutation in the *cortex* locus) have not been characterized at the molecular level. The inferred role of these mutations is based on the assumption that these are loss of function mutations. Genes labeled in blue are components of the hierarchy that are known from *B. anynana*, but for which similar patterns of expression do not appear to be present in two other commonly used model species for eyespot development: *Junonia coenia* and *Vanessa cardui* (both Nymphalidae: Nymphalinae). Pigments and pigment precursors are indicated by hollow letters. Gene products and pigments known from these other models and suspected to be present in *B. anynana* are indicated by asterisks.

Among these exceptions, perhaps best studied are the *Heliconius* butterflies, which for many decades were among the most difficult constellations of color patterns (used for aposematic coloration and involved in Müllerian mimicry rings) to homologize with patterns found elsewhere in the Lepidoptera (Nijhout and Wray 1988). Yet, in spite of these early difficulties (now mostly resolved), the intraspecific and interspecific color pattern variation found in *Heliconius* in combination with advances in genome sequencing have proven to be a powerful tool for uncovering the developmental genetic architecture underlying many color patterns across the Lepidoptera (Heliconius Genome Consortium 2012).

Using a combination of controlled crosses and genome sequencing, it has been determined that most of the major color pattern variation found in *Heliconius* can be attributed to genetic variation (mostly in the regulatory regions) of four genes (Fig. 6). Two of these genes regulate the width of the black melanized bands on the wing. As mentioned above, the gene encoding the secreted ligand *WntA* regulates the proximal-distal width of Discalis I. This gene has shown to correspond to *Heliconius* locus *Sd* (Martin et al. 2012). A second *Heliconius* locus *Yb* regulates the proximal-distal width of several wing bands and has been mapped to the gene that encodes the cell cycle regulator *cortex* (Nadeau et al. 2016). *Cortex* has also been implicated in altering the timing of scale cell development relative to the availability of melanin precursors with effects on the number of highly melanized cells on the wings of *Biston betularia* moths (van't Hof et al. 2016). More generally, *cortex* may be modulating the timing of the maturation of blocks of scale cells relative to other patterning events taking place on the wing as a means of establishing color pattern elements with similar phenotypic characteristics.

Fig. 5 (continued) The presence of TGF-β signaling being received by the cells of pupal eyespot foci is inferred from the localization of the activated downstream protein pSMAD in those cells (Monteiro et al. 2006). Attempts to detect upregulated expression of Ptc and hh in the vicinity of the eyespot foci of *B. anynana* have not been successful (Tong et al. 2012), but both of these gene products are found in association with *Junonia* eyespot foci (Marcus 2005; Evans and Marcus 2006) and mRNAs from both of these genes have been detected in appropriately staged wing tissue transcriptomes from *Bicyclus* (Özsu and Monteiro 2017). Eyespot development can be broken down into four stages (Brakefield et al. 1996; Nijhout 1996): (1) the number and position of eyespot foci are established, (2) a focal signal is secreted from the eyespot focus and that patterns the surrounding tissue (note: the molecular identity of the focal signal is undetermined), (3) the rings of the eyespot are then determined in response to different concentrations of the focal signal. Finally, (4) the wing scales that contain the pigment differentiate and the pigment molecules are synthesized in response to falling ecdysteroid levels. White pteridine pigments are produced first, followed by yellow melanin pigments, with black and brown melanin pigments being produced last. (Abbreviations: *Antp* Antennapedia, *Ubx* Ultrabiothorax, *N* Notch, *Dll* Distalless, *En* Engrailed, *Inv* Invected, *hh* hedgehog, *Ptc* Patched, *Ci* Cubitus interruptus, *EcR* Ecdysone receptor, *Sal* Spalt, *wg* wingless, *TGF-β* transforming growth factor beta, *pSMAD* phospho-SMAD, *GTP-CH I* GTP cyclohydrolase I, *TH* tyrosine hydroxylase, *DDC* DOPA decarboxylase.) Genetic interactions shown here have been previously reported in (Marcus 2005; Evans and Marcus 2006; Marcus and Evans 2008), supplemented by data from more recent literature (Monteiro et al. 2006; Tong et al. 2014; Nadeau et al. 2016; Özsu et al. 2017; Özsu and Monteiro 2017; Matsuoka and Monteiro 2018)

Two other *Heliconius* genes are responsible for determining the color of specific color pattern elements (Fig. 6). Locus *D* in *H. erato* and *H. melpomene* and locus *G* in *H. cydno* have all been shown to correspond to the gene *optix,* a transcription factor that upregulates the ommochrome biosynthetic pathway responsible for the bright red wing pigments xanthommatin and dihydroxyxanthommatin (Reed et al. 2011). In most insects, the expression of *optix* occurs primarily in the cells giving rise to the eye, and the subsequent deployment of these pigments is to insulate the photoreceptors in each ommatidium from stray photons of light escaping from adjacent ommatidia in the adult compound eye, enhancing visual acuity. Expression of these pigments in the insect wing appears to have occurred after the divergence of the Lepidoptera from their sister taxon, the caddisflies (Order Trichoptera), which display only a very limited repertoire of patterning on their wings (Marcus 2018). Finally, *Heliconius* locus *K* has been shown to correspond to the gene *aristaless1,* a

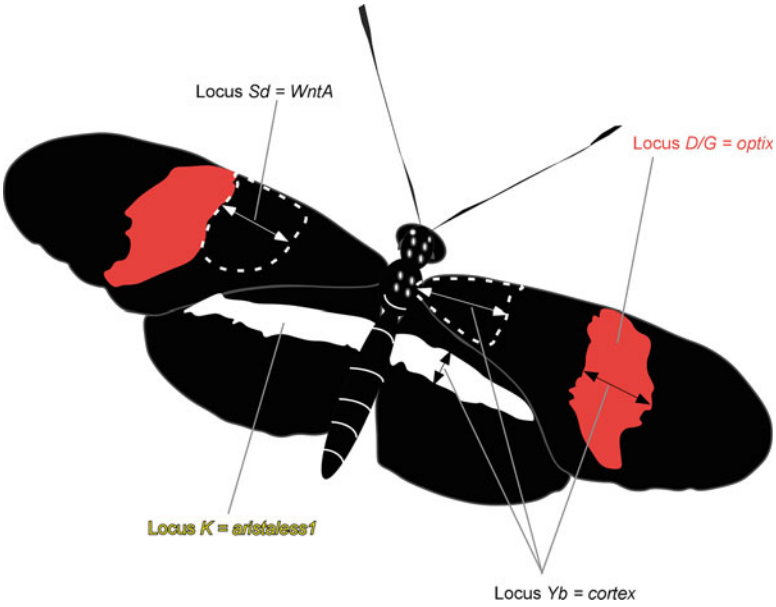


Fig. 6 Butterflies in the genus *Heliconius* have derived color patterns that represent extensive modification of the Nymphalid ground plan. These patterns vary substantially within and between species and are a classic example of Müllerian mimicry where multiple species have converged on similar aposematic coloration to deter predation. Depicted is *H. erato peterivina* showing the color patterns affected by the four loci with the greatest phenotypic effects on *Heliconius* aposematism: locus *Sd* (mapped to *WntA,* which encodes a secreted ligand that regulates the proximal-distal width of Discalis I (Martin et al. 2012)), locus *Yb* (*cortex,* a cell-cycle regulator that regulates the proximal-distal width of several wing bands (Nadeau et al. 2016)), locus *D/G* (*optix,* a transcription factor that upregulates the ommochrome biosynthetic pathway responsible for the red wing pigments xanthommatin and dihydroxyxanthommatin, known as locus *D* in *H. erato* and *H. melpomene* and known as locus *G* in *H. cydno* (Reed et al. 2011)), and locus *K* (*aristaless1,* a transcription factor that suppresses the portion of the ommochrome pathway responsible for synthesizing the yellow pigment 3-hydroxy-L-kynurenine (Westerman et al. 2018))

transcription factor that suppresses the portion of the ommochrome pathway responsible for synthesizing the yellow pigment 3-hydroxy-L-kynurenine (Westerman et al. 2018).

Conclusions

Experimental approaches involving classical genetics, comparative genomics, developmental biology, computational modeling, insect endocrinology, evolutionary comparisons of morphology, gene editing, and gene expression studies have each contributed to advancing our knowledge of the evolution and development of butterfly color patterns. This wide array of approaches, in combination with a diversity of model lepidopteran species under study, has provided us with an increasingly nuanced mechanistic understanding, as well as an increasing appreciation for what portions of the developmental machinery responsible for butterfly color patterns that have been most responsive to evolutionary pressures. Integration of mechanistic and evolutionary approaches with ecological studies that measure the effects of phenotypic change on trait function in the natural environment are expected to launch the field of butterfly evo-devo into additional new and exciting directions in the future.

Cross-References

- ▶ Developmental Plasticity and Evolution
- ▶ Evo-Devo and Niche Construction
- ▶ Evo-Devo and Phylogenetics
- ▶ Mechanisms of Pattern Formation, Morphogenesis, and Evolution
- ▶ Modeling Evolution of Developmental Gene Regulatory Networks

References

Abbasi R, Marcus JM (2015) Colour pattern homology and evolution in Vanessa butterflies (Nymphalidae: Nymphalini): eyespot characters. J Evol Biol 28:2009–2026

Abbasi R, Marcus JM (2017) A new A-P compartment boundary and organizer in holometabolous insect wings. Sci Rep 7:16337. https://doi.org/10.1038/s41598-017-16553-5

Brakefield PM, Gates J, Keys D, Kesbeke F, Wijngaarden PJ, Monteiro A, French V, Carroll SB (1996) Development, plasticity and evolution of butterfly eyespot patterns. Nature 384:236–242

Brunetti CR, Selegue JE, Monteiro A, French V, Brakefield PM, Carroll SB (2001) The generation and diversification of butterfly eyespot colour patterns. Curr Biol 11:1578–1585

Carroll SB, Gates J, Keys DN, Paddock SW, Panganiban GEF, Selegue JE, Williams JA (1994) Pattern formation and eyespot determination in butterfly wings. Science 265:109–114

Dinwiddie A, Null R, Pizzano M, Chuong L, Leigh Krup A, Ee Tan H, Patel NH (2014) Dynamics of F-actin prefigure the structure of butterfly wing scales. Dev Biol 392:404–418

Evans TM, Marcus JM (2006) A simulation study of the genetic regulatory hierarchy for butterfly eyespot focus determination. Evol Dev 8(3):273–283

Heliconius Genome Consortium (2012) Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. Nature 487:94–98. https://doi.org/10.1038/nature11041
Iwasaki M, Ohno Y, Otaki JM (2017) Butterfly eyespot organiser: in vivo imaging of the prospective focal cells in pupal wing tissues. Sci Rep 7:40705
Keys DN, Lewis DL, Selegue JE, Pearson BJ, Goodrich LV, Johnson RJ, Gates J, Scott MP, Carroll SB (1999) Recruitment of a *hedgehog* regulatory circuit in butterfly eyespot evolution. Science 283:532–534
Marcus JM (2005) Jumping genes and AFLP maps: transforming lepidopteran color pattern genetics. Evol Dev 7(2):108–114
Marcus JM (2018) Our love-hate relationship with DNA barcodes, the Y2K problem, and the search for next generation barcodes. AIMS Genetics 5(1):1–23. https://doi.org/10.3934/genet.2018.1.1
Marcus JM, Evans TM (2008) A simulation study of mutations in the genetic regulatory hierarchy for butterfly eyespot focus determination. Biosystems 93(3):250–255
Martin A, Reed RD (2014) Wnt signaling underlies evolution and development of the butterfly wing pattern symmetry systems. Dev Biol 395(2):367–378
Martin A, Papa R, Nadeau JH et al (2012) Diversification of complex butterfly wing patterns by repeated regulatory evolution of a *Wnt* ligand. Proc Natl Acad Sci USA 109:12632–12637
Matsuoka Y, Monteiro A (2018) Melanin pathway genes regulate color and morphology of butterfly wing scales. Cell Rep 24(1):56–65
Monteiro A, Glaser G, Stockslager S, Glansdorp N, Ramos D (2006) Comparative insights into questions of lepidopetran wing pattern homology. BMC Dev Biol 6:52
Nadeau NJ, Pardo-Diaz C, Whibley A et al (2016) The gene cortex controls mimicry and crypsis in butterflies and moths. Nature 534:106
Nijhout HF (1991) The development and evolution of butterfly wing patterns. Smithsonian Institution Press, Washington
Nijhout HF (1996) Focus on butterfly eyespot development. Nature 384:209–210
Nijhout HF, Wray GA (1988) Homologies in the colour patterns of the genus *Heliconius* (lepidopteran: Nymphalidae). Biol J Linn Soc 33:345–365
Otaki JM (2011) Color-pattern analysis of eyespots in butterfly wings: a critical examination of morphogen gradient models. Zool Sci 28:403–413
Otaki JM (2012) Color pattern analysis of nymphalid butterfly wings: revision of the nymphalid groundplan. Zool Sci 29(9):568–576
Özsu N, Monteiro A (2017) Wound healing, calcium signaling, and other novel pathways are associated with the formation of butterfly eyespots. BMC Genomics 18:788
Özsu N, Chan QY, Chen B, Das Gupta M, Monteiro A (2017) *Wingless* is a positive regulator of eyespot color patterns in *Bicyclus anynana* butterflies. Dev Biol 429(1):177–185
Parnell AJ, Bradford JE, Curran EV et al (2018) Wing scale ultrastructure underlying convergent and divergent iridescent colours in mimetic *Heliconius* butterflies. J R Soc Interface 15(141):20170948
Prakash A, Monteiro A (2018) *apterous A* specifies dorsal wing patterns and sexual traits in butterflies. Proc R Soc Lond B 285:20172685. https://doi.org/10.1098/rspb.2017.2685
Reed RD, Papa R, Martin A et al (2011) *Optix* drives the repeated convergent evolution of butterfly wing pattern mimicry. Science 333(6046):1137–1141
Schachat SR, Brown RL (2016) Forewing color pattern in Micropterigidae (Insecta: Lepidoptera): homologies between contrast boundaries, and a revised hypothesis for the origin of symmetry systems. BMC Evol Biol 16:116. https://doi.org/10.1186/s12862-016-0687-z
Schwanwitsch BN (1924) On the groundplan of wing-pattern in nymphalids and certain other families of rhopalocerous Lepidoptera. Proc R Soc Lond B 34:509–528
Tong X, Lindemann A, Monteiro A (2012) Differential involvement of hedgehog signaling in butterfly wing and eyespot development. PLoS One 7(12):e51087. https://doi.org/10.1371/journal.pone.0051087
Tong X, Hrycaj S, Podlaha O, Popadic A, Monteiro A (2014) Over-expression of *Ultrabithorax* alters embryonic body plan and wing patterns in the butterfly *Bicyclus anynana*. Dev Biol 394(2):357–366

van't Hof AE, Campagne P, Rigden DJ, Yung CJ, Lingley J, Quail MA, Hall N, Darby AC, Saccheri IJ (2016) The industrial melanism mutation in British peppered moths is a transposable element. Nature 534:102–105

Weatherbee SD, Nijhout HF, Grunert LW, Halder G, Galant R, Selegue J, Carroll S (1999) Ultrabithorax function in butterfly wings and the evolution of insect wing patterns. Curr Biol 9(3):109–115

Westerman EL, VanKuren NW, Massardo D et al (2018) *Aristaless* controls butterfly wing color variation used in mimicry and mate choice. Curr Biol. https://doi.org/10.1016/j.cub.2018.08.051

Zhang L, Reed RD (2016) Genome editing in butterflies reveals that Spalt promotes and distal-less represses eyespot colour patterns. Nat Commun 7:11769. https://doi.org/10.1038/ncomms11769

Twisted Shells, Spiral Cells, and Asymmetries: Evo-Devo Lessons Learned from Gastropods

Maryna P. Lesoway and Jonathan Q. Henry

Contents

Abstract

Gastropods (snails) are members of the phylum Mollusca and have been studied in a range of evolutionary developmental biology contexts. As members of the third major branch of the bilaterians, Spiralia (Lophotrochozoa) and the most speciose group outside of the arthropods, gastropods present a microcosm of body plan diversity. Historically, gastropods served as major players in comparative embryology research, and the cellular homologies of the spiral cleavage pattern continue to be important in understanding how changes in early development lead to diverse larval and adult phenotypes. The hallmarks of the gastropod body plan (torsion, asymmetry, external coiled shell) and various aspects of their development are being explored in the light of new understanding of gastropod phylogeny and conserved molecular developmental mechanisms. This chapter explores some of the latest evo-devo lessons that snails have taught us and suggests areas for renewed attention.

M. P. Lesoway (✉) · J. Q. Henry
Department of Cell and Developmental Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA
e-mail: mlesoway@illinois.edu; j-henry4@illinois.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_179

Keywords

Spiral cleavage · Biomineralization · Left-right asymmetry · Torsion · mRNA localization

Introduction

> "Ontogeny does not recapitulate phylogeny: it creates it."
> Walter Garstang (1922)

At the end of the nineteenth century, gastropods were among the leading models used in developmental biology. Comparative embryology used spiral-cleaving embryos, including the gastropods *Crepidula* (the slipper snail) and *Tritia* (the mud snail, formerly *Ilyanassa*), to explore early development, patterns of spiral cleavage, and cellular homologies, explicitly linking development and evolution. Classical examinations of cell lineages by Edwin Grant Conklin, Frank Rattray Lillie, and Edmund Beecher Wilson showed that despite their highly divergent adult body plans, the spiral cleavage pattern is conserved across molluscs, annelids, and some other groups, demonstrating that early development could be used to understand animal relationships and evolution. Gastropods were instrumental in Garstang's challenge to Haeckel's biogenetic law. The biogenetic law stated that ontogeny recapitulates phylogeny and required that embryos pass through a series of ancestral stages during development, adding novelties only at the end of development. Garstang used examples from gastropod larvae to illustrate that changes could take place at any point in a developmental series. However, gastropods and spiralians were neglected as developmental biologists began to embrace experimental embryology and developmental genetics took off, prompting a shift to more genetically tractable systems. It was not until the latter part of the twentieth century when molecular phylogenies forced a rethinking of bilaterian relationships and molecular developmental techniques became more widespread that interest in these animals was reinvigorated. This has been accelerated by the development of techniques including high-throughput sequencing and genome editing that can be used in a range of species, providing powerful tools for non-model organisms.

As the most speciose group of animals outside of the arthropods, gastropods exhibit a wide range of adult phenotypes, with largely conserved early development, presenting a microcosm of spiralian diversity. The combination of spiral cleavage, varied adult morphology, and a well-represented fossil record has made snails and their relatives a useful group for exploration of the evolutionary links between early development and adult morphology. In this chapter, we take a broad view of gastropod evolutionary developmental biology. We highlight key examples, discuss some of the most intriguing puzzles, and contemplate the future of the field. Due to

space limitations, we are unable to cover all aspects of gastropod evolutionary developmental biology; however, we aim to provide a diverse sampling of the lessons offered by snail evo-devo, past and present.

What Is a Gastropod?

Gastropod molluscs are spiralians (lophotrochozoans), characterized by an external coiled univalved shell (Fig. 1a) and a torted or twisted body plan, demonstrated by crossing of the pleurovisceral nerve cords and a twisted gut (Fig. 1b, c). The gastropod body plan has undergone a great deal of diversification and varies widely within this basic theme. This includes shelled, shell-less, and even bivalved forms, coiled and non-coiled shells, burrowing wormlike forms, and pelagic forms. Most species dwell in the sea but are also found in freshwater and terrestrial habitats. Diversity extends to reproductive styles (including brooding or free-spawning) and developmental modes (indirect or direct development, with feeding or nonfeeding larvae). Development begins with spiral cleavage (see below), leading to development of a ciliated trochophore larva (Fig. 1d), and/or a free-swimming veliger larva (Fig. 1e, f). The trochophore (Fig. 1d) is roughly ovoid in shape, with an anterior, apical ciliary tuft and a circumferential ciliated band, consisting of a prototroch and metatroch. The compound cilia of these bands produce the force necessary for swimming and feeding. In the veliger, the ciliated band is expanded to form the velum (Fig. 1e, f), also used for swimming and feeding. The velum and larval body can be retracted fully into the shell and protected by the operculum, which acts as a trapdoor. The larval velum and the apical organ are lost at metamorphosis.

Spiralians are one of three major groupings of bilaterian animals, apart from the ecdysozoans and deuterostomes (Fig. 2a). Determining phylogenetic relationships among the spiralians (Fig. 2a) and molluscs (Fig. 2b) has proven challenging, although phylogenomic studies confirm the monophyly of molluscs within the Spiralia. Gastropod relationships have long been the source of much speculation and controversy. Recent efforts using phylogenomic approaches to tackle gastropod relationships (Zapata et al. 2014) have confirmed the monophyly of the gastropods and the distinction of five major sub-groupings: vetigastropods, patellogastropods, neritomorphs, caenogastropods, and heterobranchs (Fig. 2b–e). The latter two are considered by most analyses to form a monophyletic grouping, the Apogastropoda (Fig. 2b–e). However, the more common placement of the patellogastropods as basal to other gastropods (Eogastropoda) is not supported by Zapata et al. (2014). Understanding of deep relationships among gastropod groups remains fluid, and rooting of the tree is not clear (Fig. 2c–e). Understanding these relationships is of major importance for evolutionary hypotheses of development, including understanding the origins of novel characters and the expected polarity of change of various developmental features.

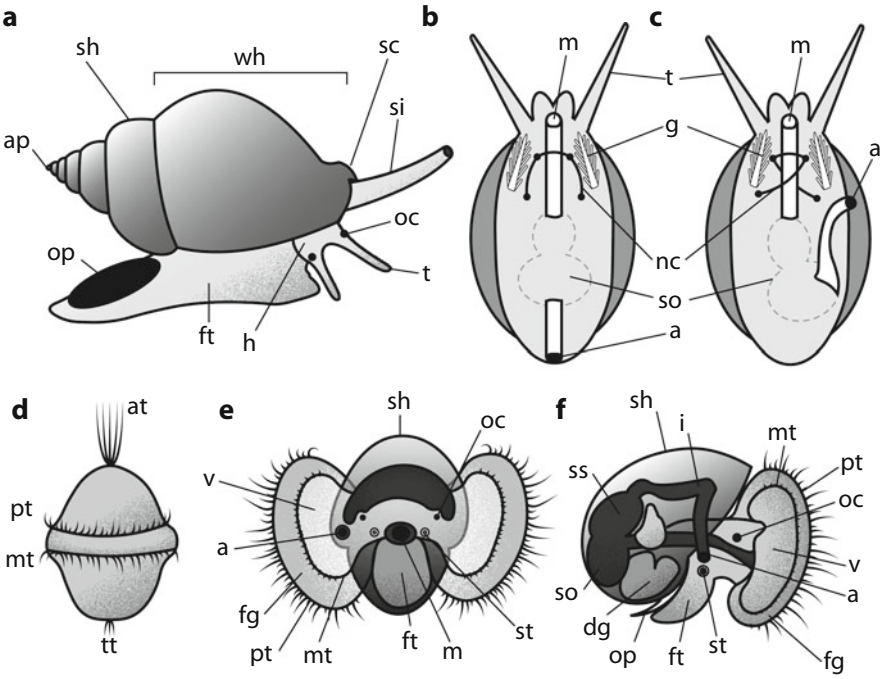


Fig. 1 The general gastropod body plan. (**a**) Snails are characterized by a dorsal coiled univalved shell and ventral head and foot. Shelled forms typically have a hardened operculum that serves to close the shell opening when the animal is retracted into the shell. (**b**, **c**) Gastropods have a torted body plan, typified by an anteriorly deflected anus and crossed nerve cords (**c**). This is often contrasted with the hypothetical ancestral mollusc (HAM) (**b**), which hypothesizes that the torted body plan emerged from a non-torted ancestor with a posterior anus and uncrossed nerve cords. (**d**–**f**) Typical larval forms of gastropods include the trochophore, (**d**), and the veliger, illustrated here in frontal, (**e**), and side views, (**f**). The trochophore is encircled by ciliary bands (e.g., prototroch) and topped with a ciliated apical organ (**d**). The veliger has a shell, and the ciliary bands are elaborated into a large velum, which is lost at metamorphosis (**e**, **f**). a, anus; ap, apex; at, apical tuft; dg, digestive gland; fg, food groove; ft, foot; g, gill; h, head; i, intestine; m, mouth; mt, metatroch; nc, nerve cord; oc, ocellus; op, operculum; pt., prototroch; sc, siphonal canal; sh, shell; si, siphon; ss, style sac; st, statocyst; tt, telotroch; v, velum; wh, whorl

Spiral Forms Most Beautiful: Spiral Cleavage, Embryonic Organization, and Pattern Formation

The early development of gastropods and most other molluscs (excepting the cephalopods), as well as other spiralian phyla (including the Annelida, Nemertea, Platyhelminthes, etc.), is characterized by spiral cleavage (Fig. 3). Much of our knowledge of the spiral cleavage pattern comes from the study of gastropods, though admittedly, relatively few species have been characterized in depth. Spiral cleavage is characterized by the twisting pattern of cleavages that is first apparent at the eight-

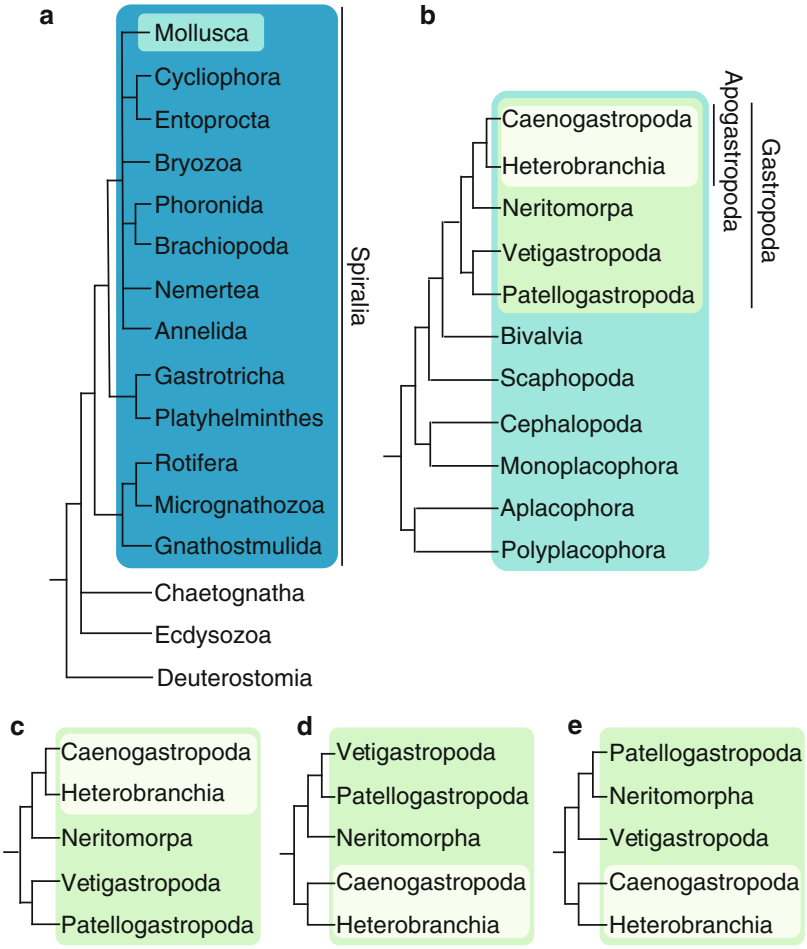


Fig. 2 Current phylogenetic hypotheses of spiralians, molluscs, and gastropods. (**a**) Spiralians (lophotrochozoans) represent one of the major clades of bilaterian metazoans, distinct from ecdysozoans and deuterostomes. Spiralian relationships remain difficult to determine but include more than a third of known phyla. (**b**) Within the Mollusca, gastropods are monophyletic and likely sister to bivalves. (**c**–**e**) Phylogenomic data confirms five major gastropod groups, but rooting of the tree is not clear (based on Zapata et al. 2014)

cell stage (Fig. 3a, b). Conklin's (1897) landmark description of the development of *Crepidula fornicata* included a nomenclature for cell lineages, which continues to be used with minor changes to this day (e.g., Lyons et al. 2012, 2015). The first two cleavages, which can be either equal (Fig. 3a, b) or unequal (Fig. 3c, d), are perpendicular to the fertilized egg's animal-vegetal axis and divide the embryo roughly into quadrants, termed A, B, C, and D, which map loosely to future parts of the embryo, left, ventral, right, and dorsal, respectively, in forms with dextral cleavage. At the third cleavage, the cleavage planes shift to lie somewhat oblique to

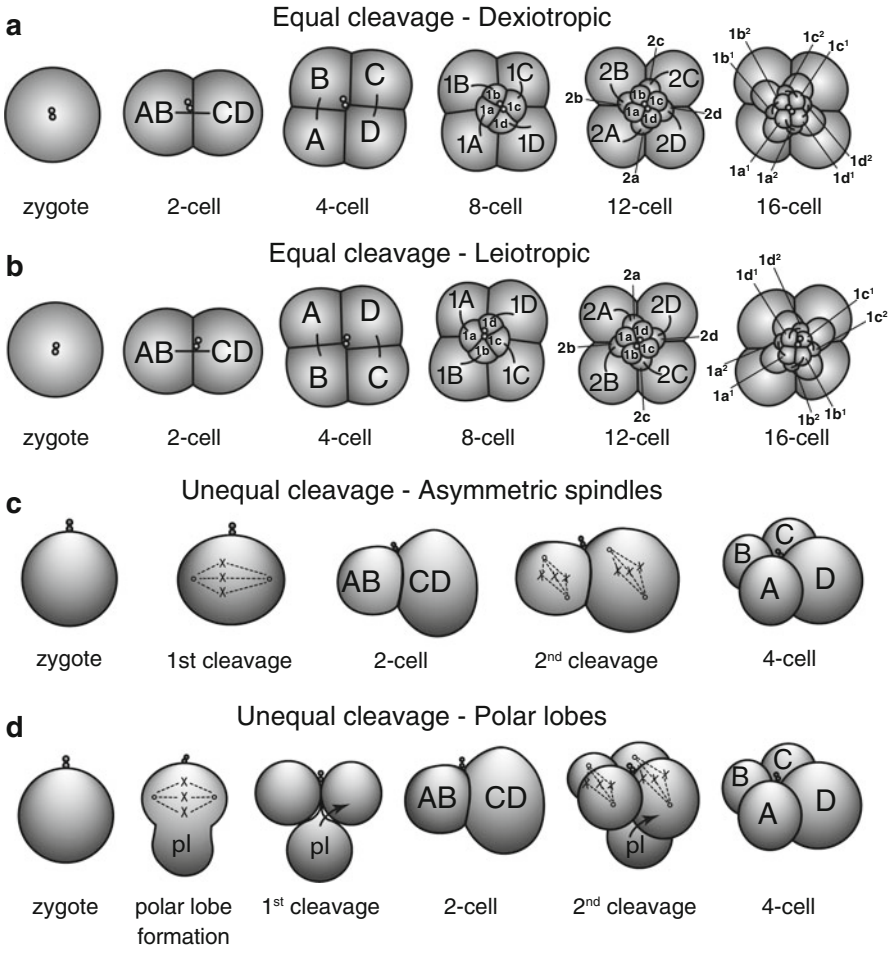


Fig. 3 Spiral cleavage in the gastropods. (**a**–**d**) Cleavage can be equal (**a**, **b**) or unequal (**c**, **d**). The direction of the spiral at third cleavage (resulting in the eight-cell stage) can be either clockwise (**a**, dexiotropic) or counterclockwise (**b**, leiotropic). Unequal cleavage can be due to asymmetric divisions resulting from asymmetric positioning of the cleavage spindle (**c**), the formation of a vegetal cytoplasmic protrusion called the polar lobe (**d**), or a combination of these mechanisms (not shown). See text for full description. Lines show direction of cleavages and relationships of sister cells. Cells are labelled with presumptive identities following the nomenclature of Conklin (1897). Two small round polar bodies are located at the animal pole. Cleavage spindles formed during the two main forms of unequal divisions are shown in **c** and **d**. Embryos in (**a**) and (**b**) are viewed from the animal pole, embryos in (**c**) and (**d**) are seen in lateral views. pl, polar lobe

the animal-vegetal axis, generating a series of (typically) smaller daughter cells, called "micromeres," located at the animal pole (Fig. 3a, b). These daughter cells come to sit in the furrows located between the opposing vegetal sister cells, which are generally larger and termed "macromeres." As cleavages proceed, the axes of

division (the cleavage spindles) tilt in opposite directions with each cycle, resulting in a pattern of alternating micromeres. The direction of the third cleavage can be clockwise (dexiotropic) or counterclockwise (leiotropic), which has implications for later development (Fig. 3a vs. b, see below). The first quartet of micromeres, termed 1a, 1b, 1c, and 1d (Fig. 3a, b), and the corresponding macromeres, 1A, 1B, 1C, and 1D (Fig. 3a, b), are formed at the third division. The second quartet is formed at the fourth division and so on. Usually, four quartets of micromeres are produced. Spiral cleavage is conserved such that individual cell lineages can be compared across different species and even different phyla. Each lineage contributes to specific parts of the embryo. For example, the fourth quartet includes the 4d (mesentoblast) lineage, one key source of mesoderm (endomesoderm). This lineage is also significant as subsequent divisions of the 4d cell are bilateral, breaking the alternating spiral cleavage pattern (Conklin 1897; Lyons et al. 2012). These cleavages produce bilateral germinal bands, giving rise to various mesodermally and endodermally derived structures in the embryo.

The specification of the different cell quadrants depends on the initial cleavage divisions, which can be characterized as equal or unequal. This is of particular significance for the D quadrant, the source of the dorsal organizer that directs the development of adjacent cells, as well as the source of much of the mesoderm in gastropods and other spiralians (i.e., 4d). Initial specification of the D quadrant is either via induction by progeny of the first quartet of micromeres, in the case of equal cleavage (Fig. 3a, b), or by differential apportioning of cytoplasmic determinants, in the case of unequal cleavage (Fig. 3c, d) (Freeman and Lundelius 1992). Unequal cleavage is accompanied by shifting of the cleavage spindles (Fig. 3c), the formation of a polar lobe (Fig. 3d), or some combination of these mechanisms. For example, in *Tritia obsoleta*, a large polar lobe is allocated to one of the daughter cells during the first two cleavages, producing an early asymmetry and determining the identity of the larger D quadrant blastomere at the four-cell stage (Fig. 3d). Removal of the polar lobe of *T. obsoleta* before the first cleavage produces a radialized embryo, demonstrating the role of the polar lobe and D quadrant in inducing the dorsoventral axis (Clement 1952). In equally cleaving embryos, such as the equally cleaving limpet *Patella vulgata*, induction of the D quadrant occurs after the formation of the third quartet of micromeres (van den Biggelaar and Guerrier 1979). Prior to this, all macromeres are capable of becoming the D quadrant, and removal of the first quartet cells produces a radialized embryo (van den Biggelaar and Guerrier 1979; Henry et al. 2017a).

Detailed lineages of the D quadrant have only recently been produced. For example, Lyons et al. (2012) described the lineage of the 4d mesentoblast in *Crepidula*, providing evidence for the origins of the primordial germ cells and other tissues. Detailed knowledge of this important and highly conserved cell lineage is valuable for understanding the embryological basis of the origins of novel structures within a stereotyped cleavage pattern. Similar detailed lineage studies have revealed differences in the origins of mesoderm and endoderm and identity of the organizer cell and specific cell fates in other gastropods (and other spiralians), for example, in *T. obsoleta* (Chan and Lambert 2014), demonstrating the flexibility of development within the conserved spiral cleavage pattern.

Understanding cellular origins is only the first step to understanding the molecular processes governing the development of spiralian animals. In gastropods, the embryonic organizer has been identified as the 3D and/or the 4d cell where this has been investigated (Henry et al. 2017a). The Erk-1/2 MAPK pathway has been implicated in the establishment of the organizer in many gastropods. For example, MAPK is activated in the 3D and 4d cells followed by the first quartet micromeres in *T. obsoleta* (Lambert and Nagy 2001). Disruption of MAPK activity disrupts D quadrant specification and organizer activity, producing radialized embryos (Lambert and Nagy 2001). Activation of MAPK in equally cleaving gastropods is generally restricted to the 3D cell. However, the identity of upstream members of the signaling cascade and the role of MAPK in D quadrant specification, developmental mode, and other species-specific differences remain open questions.

Spiral cleavage was previously described as mosaic or determinate, with each cell having a specific developmental fate. However, cell fates are specified by a combination of cell autonomous and conditional mechanisms. During spiral cleavage, developmentally significant mRNAs localize to specific quartets of micromeres (Lambert and Nagy 2002; Rabinowitz and Lambert 2010). This process has been examined in detail in *T. obsoleta*. During prophase, mRNAs are located diffusely throughout the cytoplasm or localized to the cell cortex. At interphase, specific mRNAs concentrate in the pericentriolar matrix adjacent to the nucleus localizing to the centrosome. As division proceeds, these mRNAs decouple from the centrosome, shifting to the cytoplasm or cell cortex, and may be transferred asymmetrically to specific daughter cells, resulting in a particular pattern of distribution associated with the birth of various quartets of cells (Lambert and Nagy 2002). The patterns of localization are complex and distinguish each tier of micromeres with their own specific complement of mRNAs. These mRNAs have subsequently been shown to play a role in specifying the fates of these cells. Coupled with the asymmetric signals from the D quadrant organizer, specific cell fates are distinguished both along the animal-vegetal and the dorsoventral axes.

The timing and specification of the D quadrant and changes in the pattern of spiral cleavage may be tied to evolutionary patterns in development of gastropods. Equal cleavage is thought to be the ancestral cleavage type (Fig. 3a, b, Freeman and Lundelius 1992; van den Biggelaar and Haszprunar 1996). Shifts in the timing of D quadrant specification have been used to understand gastropod phylogenetic relationships, and acceleration of the timing of organizer determination (via asymmetric cleavage divisions, Fig. 3c, d) correlates to more derived gastropod groups (Freeman and Lundelius 1992; van den Biggelaar and Haszprunar 1996). This acceleration coincides with the presence of feeding larvae and is thought by many to have been a requirement for the accelerated development of juvenile structures in pre-metamorphic stages. For example, the production of a polar lobe allocating cytoplasmic determinants allows cell fates (including those of the D quadrant) to be specified at an earlier stage in development (Freeman and Lundelius 1992). This in turn may have been important in accelerating development of mesodermally derived structures and possibly the evolution of feeding larvae.

Asymmetries Left and Right

The spiral nature of gastropod cleavage is echoed in the spiral nature of the adult shell. Embryos with clockwise (dexiotropic) cleavage (Fig. 4a) grow into right-handed snails (Fig. 4c), while embryos with counterclockwise (leiotropic) cleavage (Fig. 4b) become left-handed snails (Fig. 4d). In most living species, the shell coils to the right (dextral), with left-coiling (sinistral) or planispiral (bilaterally symmetrical) shells being less common (Vermeij 1975). The preponderance of right-handedness in gastropod shells is not well understood but has implications for reproduction in species with internal fertilization – individuals that do not share the same handedness may have difficulty mating (Asami et al. 1998). Handedness in the shell also produces corresponding selective pressures on animals that prey on right-coiled gastropods (e.g., snakes, Hoso et al. 2007).

Handedness is largely genetically determined. In the pond snail, *Lymnaea stagnalis*, genetic crosses and the manipulation of cellular contents demonstrate that dextral coiling is dominant to sinistral coiling (Freeman and Lundelius 1982), and a single maternally expressed locus confers handedness. More recently, the locus of shell coiling in *L. stagnalis* was identified as the *diaphanous-related formin* gene, *Ldia2* (Davison et al. 2016). *Formin* is a protein affecting actin nucleation and elongation. *Ldia2* is asymmetrically localized at first cleavage in *L. stagnalis*, demonstrating asymmetry at the expression level well before being morphologically apparent in the embryo (Fig. 4e–g). Pharmacological inhibition disrupts chiral twisting at the third cleavage, supporting the identification of formin as a molecular cause of early asymmetry in *L. stagnalis* (Davison et al. 2016).

Other well-characterized molecular markers of asymmetry, *nodal* and *pitx*, are also expressed asymmetrically. In embryos of *Lottia gigantea* and *Biomphalaria glabrata*, left-right asymmetric embryonic expression corresponds to the dextral (*L. gigantea*) or sinistral (*B. glabrata*) pattern of cleavage and shell coiling, respectively (Fig. 4a–d, h, i) (Grande and Patel 2009). Asymmetrical *nodal* expression is established in early cleavage and is upstream of *pitx* expression (Grande and Patel 2009). Blocking *nodal* activity by pharmacological means leads to the loss of shell coiling and symmetrical *pitx* expression in *Biomphalaria* embryos (Grande and Patel 2009). The relationship between the direction of cleavage and shell coiling has been demonstrated in *L. stagnalis*. Physically altering the position of the micromeres at third embryonic cleavage, upstream of initial *nodal* expression in the embryo, changes the location of *nodal* expression and subsequent adult handedness (Kuroda et al. 2009). Right-handed (dexiotropic) embryos that are mechanically shifted to have left-handed (leiotropic) cleavage grow up to have left-handed (sinistral) shell coiling, while right-shifted embryos of sinistrally cleaving embryos show right-handed (dextral) shell coiling as adults. It remains to be seen how *nodal* and *pitx* expression is regulated and what upstream mechanisms, including physical interactions between cells, mediate handedness in cleavage and shell coiling.

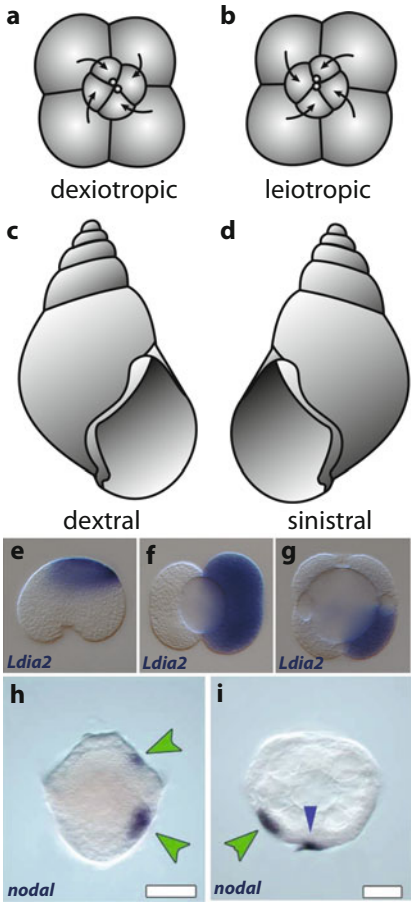


Fig. 4 Asymmetry and shell coiling in gastropods. (**a**–**d**) Direction of the cleavages at the eight-cell stage corresponds to the direction of shell coiling in the adult. Dexiotropic cleavage (**a**) leads to dextral (right-handed) shell coiling (**c**), while leiotropic cleavage (**b**) results in sinistral shell (left-handed) coiling (**d**). Most gastropods have a dextral coiling shell. Sinistral coiling is found in some species or populations of snails. (**e**–**g**) The formin gene *Ldia2* in *Lymnaea stagnalis* is asymmetrically expressed during first cleavage (**e**) and second cleavage (**f**) and expressed in one blastomere at the four-cell stage (**g**). (**h**, **i**) *Nodal* is expressed asymmetrically during cleavage (not shown) and during later stages of development. At the early trochophore stage, *nodal* is expressed in the right cephalic region (upper green arrowhead) and in the right lateral post-trochal ectoderm (lower green arrowhead) in the dextral snail *Lottia gigantea* (**h**) and in the left lateral post-trochal ectoderm (green arrowhead) in the sinistral snail, *Biomphalaria glabrata* (**i**). The blue arrowhead indicates the developing shell gland. Embryos are shown in animal view (**a**, **b**, **f**, **g**), side view (**e**), and dorsal view (**h**, **i**). Scale bars represent 50 μm. Panels (**e**–**g**) adapted from Davison et al. (2016) under a CC-BY license. Panels (**h**, **i**) adapted from Grande and Patel (2009), by permission from Nature Publishing

Larval Biology and Life History Evolution

Life history and larval development, particularly the origins of larval feeding, have been the subject of interest to evolutionary and developmental biologists alike. Differences in dispersal ability due to the presence or absence of a planktonic larval form may have implications for speciation and extinction events, and the study of larval morphology has formed the basis for various evolutionary scenarios. The ancestral gastropod larva is argued to be represented by a swimming, nonfeeding larva. Although phylogenetic hypotheses are equivocal on the subject (e.g., Zapata et al. 2014), indirect development with larval planktotrophy likely emerged at least once in the apogastropoda and may have evolved multiple times in gastropods. While rare in other groups (e.g., echinoderms), molecular phylogenetic and morphological evidence shows that it is possible for planktotrophy to re-evolve following a loss in a number of gastropod groups. For example, detailed phylogenetic analyses and larval morphology show at least one instance where larval planktotrophy re-emerged after a loss in the calyptraeid family of gastropods (Collin 2004), which includes the slipper snails (*Crepidula* spp.), the cup and saucer snails (*Crucibulum* spp.), and the hat shells (*Calyptraea* spp.). While all calyptraeids brood their offspring, developmental mode is highly diverse, including feeding (planktotrophic) larvae, nonfeeding (lecithotrophic) larvae, direct development with crawl-away juveniles, and direct development via consumption of nutritive embryos (Lesoway et al. 2014). The loss of feeding larvae had been thought to be irreversible owing to the low likelihood of regaining complex feeding and swimming structures. For example, echinoderm larvae show dramatic reduction of larval characters in lecithotrophic forms (Strathmann 1978). However, encapsulated development has the potential to allow for retention of larval structures in the calyptraeids and other groups where feeding larvae are thought to have re-emerged. This includes the retention of features such as the ciliary bands required for larval swimming and feeding (i.e., prototroch, metatroch, and food groove) in direct developers, including species that feed on nutritive embryos during encapsulated development (Collin 2004; Lesoway et al. 2014). Further, gastropod larvae do not undergo the same catastrophic metamorphosis seen in sea urchins and other echinoderms, and metamorphosis is often reduced externally to shedding of the larval velum. In gastropods, many juvenile structures begin their development precociously during larval stages; hence the re-emergence of planktotrophy following a loss appears to be more widespread in the gastropods than thought possible in other groups.

Hard Parts and Body Plan Puzzles

The forms and diversity of snail shells capture the imagination of anyone who has picked one up to listen to the echoes of the ocean, or as a souvenir of a beach holiday (Fig. 5). The process of shell biomineralization is a long-standing area of inquiry,

Fig. 5 Gastropod shell diversity. Color, patterning, shape, and sculpturing are highly variable, among (and often within) species. (**a**) Conch-type shell with apical spines. (**b**) Ventral (apertural) view of the hat shell *Calyptraea lichen* showing the interior shelf. (**c**) Vermicularid-type shell, with an open coil. (**d**) Shell color may be complex and variable, as exemplified by the strawberry top shell, *Clanculus puniceus*. (**e**) Aperture of a periwinkle, *Littorina* sp. (**f**) Many vetigastropods, such as the keyhole limpet, *Diodora aspera*, have one or more apertures. (**g**) High-spired shell of a turret or tower-type snail. (**h**) A nearly planispiral (coiled in one plane) shell of the tigersnail, *Anguispira alternata*. (**i**) Whelk-type shell. (**j**) Cowry-type shell with a narrowed aperture. The mantle edge typically extends to cover most of the shell in the live animal. (**k**) Spindle-type shell with an elongate siphonal canal. (**l**) Limpet-type shell with ridges. (**m**) Cone-type shell. (**n**) Olive-type shell. (**o**) The globose shell of *Hexaplex radix* features many spiny varices. Images not to scale

and there is great interest in understanding the mechanisms leading to the diversity of shell shape, coloration, and structure in gastropods (Fig. 5). The gastropod shell consists of layers of calcium carbonate crystals in the form of calcite or aragonite, embedded in an organic extracellular matrix. The layers can include an outer organic layer, the periostracum, and inner mineralized layers. These may include prismatic and nacreous layers of calcium carbonate crystals, organized in columns or sheets, respectively. Embryological studies have shown that despite the variety of adult shell morphology, shell development in gastropods typically begins with the formation of a thickened dorsal shell field, followed by an invagination to form the shell gland, a

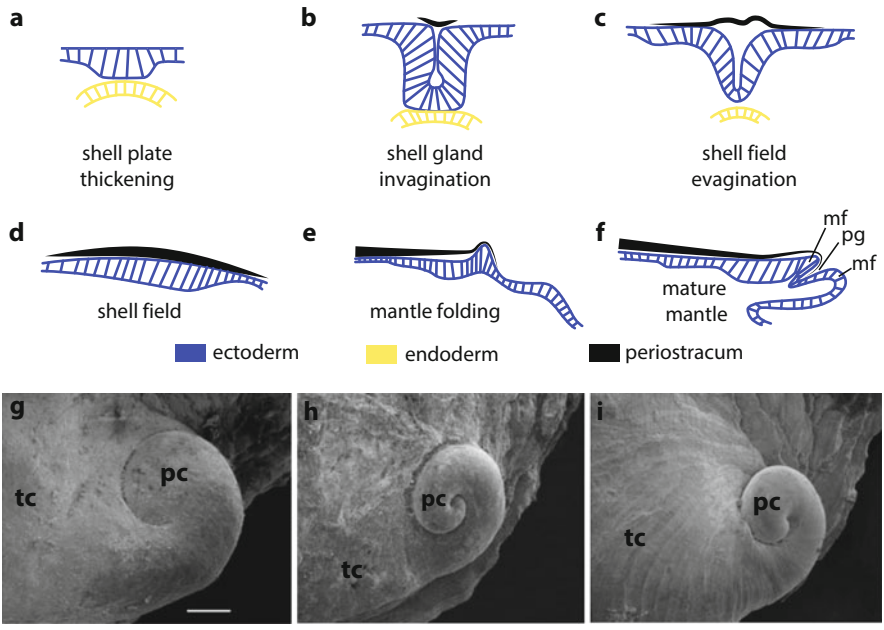


Fig. 6 Embryonic/larval shell development and shell forms. Despite the diversity of gastropod shells, early development is largely conserved. (**a**) Inductive interactions between the ectoderm and endoderm initiate thickening of the shell plate at the dorsal side of the embryo. (**b**) Invagination of the shell plate forms the shell gland. (**c**) The shell gland evaginates to form the shell field, which begins to produce the embryonic protoconch. (**d**–**f**) As development progresses, the shell field folds to form the mantle fold which continues to secrete the larval and adult shell. (**g**–**i**) Signs of early development are retained at the shell apex. Direct-developing snails with large eggs have a globose shell apex (**g**). Indirect development from small eggs produces a small, tightly coiled shell apex (**h**). Sculpturing often differs between the embryonic protoconch and the teleoconch (**i**), and there may also be a distinct demarcation between the embryonic and the larval shell. mf, mantle fold; pc, protoconch; pg, periostracal groove; tc, teleoconch. Scale bar represents 500 μm. Panels (**a**–**f**) after Kniprath (1981). Panels (**g**–**i**) adapted from Collin (2005) by permission from Oxford University Press

highly conserved structure in gastropod embryonic and larval development (Fig. 6a–f, see Kniprath 1981 for review). In the heterobranch, *Lymnaea stagnalis*, interactions between ectodermal and endodermal tissues lead to the production of a thickened shell field, followed by invagination of the shell gland. Later in development, the shell field evaginates, producing the shell-forming mantle edge (Kniprath 1981). Significant interest in the mechanisms underlying biomineralization in biological systems and the potential impacts of ocean acidification are bringing some understanding of the molecular mechanisms of shell secretion in gastropods to light. For example, *engrailed* is expressed in the shell field of gastropod embryos including *Patella* and *Lymnaea* and is thought to set up the boundary delimiting the shell field in conjunction with *dpp* expression (Nederbragt et al. 2002). Asymmetric expression of *dpp* is associated with shell coiling and driven by differential

regulation of cell proliferation and growth (Shimizu et al. 2013). In *L. stagnalis*, *dpp* expression is higher on either the left or the right side, depending on the direction of shell coiling (Shimizu et al. 2013). Blocking *dpp* expression in *L. stagnalis* produces straight shells, supporting a role for *dpp* in shell coiling. Further, *dpp* expression in the limpets *P. vulgata* and *Nipponacmea fuscoviridis* is the same on both sides, correlating with their conical, more symmetrical shell shapes (e.g., Fig. 5f, l).

Comparative proteomics and transcriptomic screens have found that shell secretion is highly variable within gastropods (and molluscs more generally). Transcriptomic comparisons of the nacreous secretome of the vetigastropod *Haliotis asinina* and the owl limpet *L. gigantea* and three species of the bivalve *Pinctada* show relatively low levels of conservation of secreted components, suggesting that molluscan shell secretion genes have evolved very rapidly (Jackson et al. 2010). Of the genes that are shared among these molluscs, several are known from other biomineralizing animals, suggesting some level of conservation. Indeed, the level of evolutionary conservation of a "biomineralization GRN" is a tantalizing question for further research.

The gastropod shell is readily fossilized and abundant in the fossil record, leaving a historical record of their evolution. In some cases, the shell also retains evidence of early developmental mode, making gastropods excellent for understanding the evolution of development in deep time. The shell first develops as a pre-metamorphic protoconch (Fig. 6g–i) secreted by the shell field (Fig. 6c, d) during the early embryonic phase and by the mantle tissue at later stages. Post-metamorphosis, the teleoconch (Fig. 6g–i) is secreted by the mantle tissue (Fig. 6f). In snails with a planktonic feeding larva, the protoconch can sometimes be distinguished into earlier embryonic and later larval protoconch by differences in shell sculpturing. The size and shape of these parts of the shell differ with developmental mode (Fig. 6g–i). Indirect-developing snails with a swimming, feeding veliger larva often come from smaller eggs and have a tightly coiled protoconch (Fig. 6h). There may be distinct changes in shell sculpturing between the embryonic and larval protoconch as well as between the protoconch and teleoconch. Direct developers or planktonic nonfeeding larvae (lecithotrophs) frequently have a large, globose protoconch and typically lack protoconchal ornamentation (Fig. 6g). Because remnants of the embryonic and larval shell are retained at the apex of the adult shell, these characters suggest the early development of long dead, fossilized gastropods and have been used to speculate about the evolution of developmental modes. However, these fine structures can often be degraded by weathering, even in living gastropods. Internal casts (steinkern) of fossil gastropod shells, which retain the size and overall shape of the shell, have also been used (more controversially as the external patterning of the shell is not retained) to date the origins of larval planktotrophy in the gastropods to the Ordovician (Nützel et al. 2006). However, evidence for the ancestral mode of development in the gastropods remains equivocal and continues to be a source of debate and disagreement.

Another hallmark of the gastropod body plan is the torted organization of the body (Fig. 1b, c). The dorsal visceropallium of gastropods appears twisted 180° relative to the ventral head and foot, and the anus is deflected anteriorly. The nerve

cords are also twisted, although in many species (typically shell-less forms), this arrangement may be absent. Torsion is distinct from shell coiling, and gastropods which have a shell that lacks coiling (i.e., limpet-like, Fig. 5f, l) may still show internal signs of torsion such as crossed nerve cords (Fig. 1b, c). The evolutionary origins of this aspect of the gastropod body plan remain the subject of much debate and speculation. Based on early observations of the process of torsion during the development of basal gastropods, torsion was hypothesized to have originated as a larval adaptation to planktonic life, resulting in the twisted adult body plan (Garstang 1929). Garstang's torsion hypothesis has proven difficult to test, and no evidence supports the idea that post-torsional veligers are less susceptible to predation than pre-torsional veligers (Pennington and Chia 1985). While appealing as an explanation, Garstang's hypothesis also conflates the process of developmental torsion during embryogenesis with the evolutionary pattern of a torted adult body plan. Page (2006) reviews the controversy and literature surrounding torsion and notes that this confusion is a source of much of the debate surrounding the origins of the gastropod body plan. Evolutionary scenarios of the gastropod body plan have often relied on a hypothetical ancestral mollusc (the "HAM") with a posterior mantle cavity and anus (Fig. 1b), with torsion resulting in the modern body plan with an anterior mantle cavity and anus (Fig. 1c). However, this hypothetical ancestor is not based on fossil evidence, and the HAM scenario is tautological (Page 2006). Comparative developmental evidence suggests an alternative hypothesis, with early asymmetry in growth of the mantle producing ontogenetic torsion (Kurita and Wada 2011) and providing a mechanism for the torsion pattern (Page 2006).

The mechanisms of torsion during development are not clear-cut either. Contractile activity of larval retractor muscles has been suggested to be responsible for ontogenetic torsion, based on the position of larval muscle attachments and contractile activity in the early veliger. However, several studies have raised doubt about the ability of the larval retractor muscles to produce torsion during development. Challenges to this hypothesis include a lack of shell stiffness for muscles to work against in embryos undergoing torsion (Hickman and Hadfield 2001), and pharmacological disruption of muscle attachments in embryos undergoing torsion failed to disrupt the process of torsion (Page 2002). Furthermore, pharmacological disruption of *nodal* signaling in early development blocks both asymmetric growth of the mantle epithelium and torsion, but not the growth of the larval retractor muscles (Kurita and Wada 2011). While torsion is considered by some to be "solved" as an evolutionary question, the relative lack of detailed comparative developmental data and the evidence challenging the traditional torsion hypothesis makes this classical question in evo-devo in need of re-evaluation.

Everything Old Is New Again: Future Directions

Many of the organisms that E.G. Conklin and colleagues explored at the beginning of the last century have re-emerged and are thriving as spiralian developmental models. For example, *Tritia* and *Crepidula* have been used to produce modern cell

lineage analyses, and the use of artificial mRNA constructs and time-lapse imaging and other experimental manipulations has shed light on complex morphogenetic processes in early development, such as gastrulation (e.g., Lyons et al. 2015). Increasing availability of sequencing data and the advent of accessible gene-editing techniques (e.g., CRISPR/Cas9) has meant that many systems traditionally lacking in genomic resources, including the gastropods, are now flourishing. The first demonstration of successful gene editing using CRISPR/Cas9 in a spiralian was done in *C. fornicata* (Perry and Henry 2015). Recent years have seen the publication of a flurry of transcriptomic databases, and a handful of gastropod genomes (e.g., *Lottia*, *Aplysia*, and *Biomphalaria*) have become available with more on the horizon. The pond snail *Lymnaea* and more basal gastropods like *Patella* and *Haliotis* continue to provide insight into shell secretion and biomineralization. More recently, the direct-developing congener of *C. fornicata*, *C. atrasolea*, has been developed as a laboratory-based model of spiralian development (Henry et al. 2017b). Tractability as an experimental system with readily manipulated embryos, year-round embryo production, and direct development make *C. atrasolea* ideal for developmental study. Comparisons between *Crepidula* species with different modes of development (e.g., planktotrophy, *C. fornicata*; direct development, *C. atrasolea*; direct development with nutritive embryos, *C. navicella*, Lesoway et al. 2014) will shed light on the mechanisms that have allowed diversity of developmental mode within this group and may elucidate the origins of larval forms more broadly.

Conclusion

The gastropod body plan continues to hold evolutionary mysteries – the contrast of the highly conserved spiral pattern of development with the diversity of adult phenotypes presents an excellent case study for the evolution of novel forms from conserved developmental origins. In addition to the morphological diversity of gastropods, torsion, the evolution of larval forms and transitions among modes of development, variation in shell shape and the mechanisms underlying biomineralization, the segmentation status of early molluscs, and more are all ready for deeper examination under the evo-devo paradigm. Revived and new animal models, new techniques, and comparative genetic and morphological approaches provide the tools to address ongoing controversies and long-standing evolutionary questions making the gastropods an exciting nexus for continued lessons in evo-devo.

Cross-References

- ▶ Eco-Evo-Devo
- ▶ Evolution of Floral Organ Identity
- ▶ Heterochrony
- ▶ Mechanisms of Pattern Formation, Morphogenesis, and Evolution
- ▶ Methods and Practices in Paleo-Evo-Devo

Acknowledgments The authors acknowledge the invaluable support of the National Science Foundation (NSF) and were supported by NSF grant IOS-1558061 to JQH (JJH). MPL was supported by a postdoctoral fellowship from the Fonds de recherche du Québec – Nature et technologies (FRQ-NT).

References

Asami T, Cowie RH, Ohbayashi K (1998) Evolution of mirror images by sexually asymmetric mating behavior in hermaphroditic snails. Am Nat 152(2):225–236. https://doi.org/10.1086/286163

Chan XY, Lambert JD (2014) Development of blastomere clones in the *Ilyanassa* embryo: transformation of the spiralian blastula into the larval body plan. Dev Genes Evol 224(3):159–174. https://doi.org/10.1007/s00427-014-0474-z

Clement AC (1952) Experimental studies on germinal localization in *Ilyanassa*. I. The role of the polar lobe in determination of the cleavage pattern and its influence in later development. J Exp Zool 121(3):593–625. https://doi.org/10.1002/jez.1401210310

Collin R (2004) Phylogenetic effects, the loss of complex characters, and the evolution of development in calyptraeid gastropods. Evolution 58(7):1488–1502

Collin R (2005) Development, phylogeny, and taxonomy of Bostrycapulus (Caenogastropoda : Calyptraeidae), an ancient cryptic radiation. Zool J Linn Soc-Lond 144(1):75–101

Conklin EG (1897) The embryology of *Crepidula*: a contribution to the cell lineage and early development of some marine gasteropods. J Morphol 13(1):1–226

Davison A, McDowell Gary S, Holden Jennifer M, Johnson Harriet F, Koutsovoulos Georgios D, Liu MM, Hulpiau P, Van Roy F, Wade Christopher M, Banerjee R, Yang F, Chiba S, Davey John W, Jackson Daniel J, Levin M, Blaxter Mark L (2016) *Formin* is associated with left–right asymmetry in the pond snail and the frog. Curr Biol 26(5):654–660. https://doi.org/10.1016/j.cub.2015.12.071

Freeman G, Lundelius JW (1982) The developmental genetics of dextrality and sinistrality in the gastropod *Lymnaea peregra*. Rouxs Arch Dev Biol 191(2):69–83. https://doi.org/10.1007/bf00848443

Freeman G, Lundelius JW (1992) Evolutionary implications of the mode of D quadrant specification in coelomates with spiral cleavage. J Evol Biol 5(2):205–247. https://doi.org/10.1046/j.1420-9101.1992.5020205.x

Garstang W (1922) The theory of recapitulation: A critical re-statement of the Biogenetic Law. Proc Linn Soc Lond Zool 35:81–101

Garstang W (1929) The origin and evolution of larval forms. Rep Br Assoc Adv Sci Sect D 96:77–98

Grande C, Patel NH (2009) *Nodal* signalling is involved in left–right asymmetry in snails. Nature 457(7232):1007–1011. https://doi.org/10.1038/nature07603

Henry JQ, Lyons DC, Perry KJ, Osborne CC (2017a) Establishment and activity of the D quadrant organizer in the marine gastropod *Crepidula fornicata*. Dev Biol 431(2):282–296. https://doi.org/10.1016/j.ydbio.2017.09.003

Henry JQ, Lesoway MP, Perry KJ, Osborne CC, Shankland M, Lyons DC (2017b) Beyond the sea: *Crepidula atrasolea* as a spiralian model system. Int J Dev Biol 61(8–9):479–493. https://doi.org/10.1387/ijdb.170110jh

Hickman CS, Hadfield MG (2001) Larval muscle contraction fails to produce torsion in a trochoidean gastropod. Biol Bull 200(3):257–260. https://doi.org/10.2307/1543507

Hoso M, Asami T, Hori M (2007) Right-handed snakes: convergent evolution of asymmetry for functional specialization. Biol Lett 3(2):169–173. https://doi.org/10.1098/rsbl.2006.0600

Jackson DJ, McDougall C, Woodcroft B, Moase P, Rose RA, Kube M, Reinhardt R, Rokhsar DS, Montagnani C, Joubert C, Piquemal D, Degnan BM (2010) Parallel evolution of nacre building gene sets in molluscs. Mol Biol Evol 27(3):591–608. https://doi.org/10.1093/molbev/msp278

Kniprath E (1981) Ontogeny of the molluscan shell field: a review. Zool Scr 10(1):61–79. https://doi.org/10.1111/j.1463-6409.1981.tb00485.x

Kurita Y, Wada H (2011) Evidence that gastropod torsion is driven by asymmetric cell proliferation activated by TGF-β signalling. Biol Lett. https://doi.org/10.1098/rsbl.2011.0263

Kuroda R, Endo B, Abe M, Shimizu M (2009) Chiral blastomere arrangement dictates zygotic left–right asymmetry pathway in snails. Nature 462(7274):790–794. https://doi.org/10.1038/nature08597

Lambert JD, Nagy LM (2001) MAPK signaling by the D quadrant embryonic organizer of the mollusc *Ilyanassa obsoleta*. Development 128(1):45–56

Lambert JD, Nagy LM (2002) Asymmetric inheritance of centrosomally localized mRNAs during embryonic cleavages. Nature 420(6916):682–686. https://doi.org/10.1038/nature01241

Lesoway MP, Abouheif E, Collin R (2014) The development of viable and nutritive embryos in the direct developing gastropod *Crepidula navicella*. Int J Dev Biol 58:601–611. https://doi.org/10.1387/ijdb.140136rc

Lyons DC, Perry KJ, Lesoway MP, Henry JQ (2012) Cleavage pattern and fate map of the mesentoblast, 4d, in the gastropod *Crepidula*: a hallmark of spiralian development. EvoDevo 3(1):1–26

Lyons DC, Perry KJ, Henry JQ (2015) Spiralian gastrulation: germ layer formation, morphogenesis, and fate of the blastopore in the slipper snail *Crepidula fornicata*. EvoDevo 6(1):24. https://doi.org/10.1186/s13227-015-0019-1

Nederbragt AJ, van Loon AE, Dictus W (2002) Expression of *Patella vulgata* orthologs of *engrailed* and *dpp-BMP2/4* in adjacent domains during molluscan shell development suggests a conserved compartment boundary mechanism. Dev Biol 246(2):341–355. https://doi.org/10.1006/dbio.2002.0653

Nützel A, Lehnert O, Frýda J (2006) Origin of planktotrophy – evidence from early molluscs. Evol Dev 8(4):325–330. https://doi.org/10.1111/j.1525-142X.2006.00105.x

Page LR (2002) Ontogenetic torsion in two basal gastropods occurs without shell attachments for larval retractor muscles. Evol Dev 4(3):212–222. https://doi.org/10.1046/j.1525-142X.2002.02005.x

Page LR (2006) Modern insights on gastropod development: reevaluation of the evolution of a novel body plan. Integr Comp Biol 46(2):134–143. https://doi.org/10.1093/icb/icj018

Pennington JT, Chia F-S (1985) Gastropod torsion: a test of Garstang's hypothesis. Biol Bull 169(2):391–396. https://doi.org/10.2307/1541490

Perry KJ, Henry JQ (2015) CRISPR/Cas9-mediated genome modification in the mollusc, *Crepidula fornicata*. Genesis 53(2):237–244. https://doi.org/10.1002/dvg.22843

Rabinowitz JS, Lambert JD (2010) Spiralian quartet developmental potential is regulated by specific localization elements that mediate asymmetric RNA segregation. Development 137(23):4039–4049. https://doi.org/10.1242/dev.055269

Shimizu K, Iijima M, Setiamarga DH, Sarashina I, Kudoh T, Asami T, Gittenberger E, Endo K (2013) Left–right asymmetric expression of *dpp* in the mantle of gastropods correlates with asymmetric shell coiling. EvoDevo 4(1):15. https://doi.org/10.1186/2041-9139-4-15

Strathmann RR (1978) Evolution and loss of feeding larval stages of marine invertebrates. Evolution 32(4):894–906

van den Biggelaar JAM, Guerrier P (1979) Dorsoventral polarity and mesentoblast determination as concomitant results of cellular interactions in the mollusk *Patella vulgata*. Dev Biol 68(2):462–471. https://doi.org/10.1016/0012-1606(79)90218-5

van den Biggelaar JAM, Haszprunar G (1996) Cleavage patterns and mesentoblast formation in the gastropoda: an evolutionary perspective. Evolution 50(4):1520–1540

Vermeij GJ (1975) Evolution and distribution of left-handed and planispiral coiling in snails. Nature 254:419. https://doi.org/10.1038/254419a0

Zapata F, Wilson NG, Howison M, Andrade SCS, Joerger KM, Schroedl M, Goetz FE, Giribet G, Dunn CW (2014) Phylogenomic analyses of deep gastropod relationships reject *Orthogastropoda*. Proc Biol Sci 281(1794):20141739. https://doi.org/10.1098/rspb.2014.1739

Evo-Devo Lessons Learned from Hemichordates

Kuni Tagawa

Contents

Abstract

Hemichordates are exclusively marine animals related to vertebrate chordates, like ourselves, and to non-vertebrate chordates, such as lancelets and ascidians; therefore, they are useful to understand our ancestral state. Hemichordates are also associated with the radially symmetrical echinoderms, organisms such as sea stars and sea urchins, because of similarities in embryogenesis and larval form. Hemichordate larvae are believed to resemble the hypothetical dipleurula larva, which is thought to have been a crucial stage in metazoan evolution. Hemichordates include about 130 described species and are divided into two classes: the free-living Enteropneusta and the sessile Pterobranchia. Enteropneusts are commonly called *giboshi mushi* in Japanese and acorn

K. Tagawa (✉)
Marine Biological Laboratory, Graduate School of Integrated Sciences for Life, Hiroshima University, Onomichi, Hiroshima, Japan
e-mail: kuni@hiroshima-u.ac.jp

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_183

worms in English, since the enteropneust proboscis resembles a traditional ornament on the tops of posts or balustrades of bridges, shrines or temples, and acorns. Pterobranchs are small animals that form colonies of clones connected via stalks. This chapter reviews recent studies on hemichordates, focusing on phylogeny, paleontology, molecular developmental biology, and genomics, to review what we have learned about animal evolution and development from these obscure organisms.

Keywords

Hemichordates · Acorn worm · Animal evolution · Deuterostome · Direct and indirect development

Introduction

Hemichordates were seldom mentioned in biology textbooks and were rarely found in nature when the author started to work on them more than 20 years ago; therefore, they were unfamiliar, even to most biologists. However, the history of hemichordate studies is actually lengthy, and many studies since the late twentieth century are related to the origin and evolution of chordates (Tagawa et al. 2001). Molecular biological techniques have progressed rapidly in recent decades, changing conventional experimental embryology into molecular developmental biology and reviving the view of evolution from a developmental perspective. In the late twentieth century, as the new field "evo-devo" re-emerged, researchers attempted to better understand animal evolution through comparative developmental biology (Lowe 2008). This new perspective caused the obscure hemichordates to assume greater importance in the field of developmental biology.

The author has been studying the Hawaiian hemichordate, *Ptychodera flava*, to understand the origin and evolution of chordates, by analyzing genes important in the development of major chordate features (Tagawa et al. 2001). During this time, genome projects involving various model organisms have expanded to include non-model organisms, such as hemichordates (Simakov et al. 2015). Now extensive genomic information about many organisms is available to conduct comparative analyses (Tagawa 2016). This treatise reviews that which has been learned from recent advances in hemichordate biology about evolution and development in the past quarter century.

Brief History of Hemichordate Research

The history of hemichordate research is shown chronologically in Table 1 for ease of reference. In 1825, Johann Friedrich Eschscholtz, a German naturalist, described the first hemichordate, *Ptychodera flava*, although he thought it was a holothurian, a class of echinoderms. A German zoologist, Johann Wilhelm

Table 1 The history of hemichordates research

Year	Scientists	What is done	Species	Class[a]
1825	Johann Friedrich Eschscholtz	Named and described the first hemichordate as a sea cucumber	*Ptcyhodera flava*	E
1850	Johanness Peter Müller	Developed the term "tornaria" for enteropneust larvae		
1866	Alexander Onufriewitsch Kowalevsky	Found gill slits in enteropneusts	*Balanoglossus clavigerus*	E
1866	Georg Ossian Sars	Found the first pterobranch	*Halilophus mirabilis*	P
1869, 1870	Elie Metchnikoff	Observed the transformation from tornaria into an adult worm	*Balanoglossus*	E
1869	Michael Sars	Named the first pterobranch	*Halilophus mirabilis*	P
1869	George James Allman	Described and named a species of bryozoan	*Rhabdopleura normani*	P
1870	Carl Gegenbaur	Coined the name "Enteropneusta"		E
1872, 1873	Alexander Emanuel Agassiz	Confirmed Metchnikoff's observation	*Balanoglossus*	E
1872, 1874	Georg Ossian Sars	Described pterobranchs	*Rhabdopleura mirabilis*	P
1877	Edwin Ray Lankester	Named the "Pterobranchia"		P
1882, 1887	William Carmichael McIntosh	Named and described pterobranchs	*Cephalodiscus dodecalophus*	P
1885	William Bateson	Proposed the name "Hemichordata"	*Balanoglossus kowalevskii*	E
1887	Sidney Frederic Harmer	Showed that pterobranchs are closely related to enteropneusts	*Cephalodiscus dodecalophus*	H
1893	Johann Wilhelm Spengel	Reclassified species as enteropneusts, instead of sea cucumbers	*Ptcyhodera flava*	E
1899	Arthur Willey	Assigned the Pterobranchia and Enteropnuesta to the rank of class		H

[a]E represents the class Enteropneusta or acorn worms, and P represents the class Pterobranchia. H represents both E and P, the phylum Hemichordata
Blanks in the species and class columns indicate "Not applicable"

Spengel (1893), later reclassified it as a hemichordate. Alexander Onufriewitsch Kowalevsky (1866), a Russian embryologist and zoologist, analyzed the anatomy of a second described species, *Balanoglossus clavigerus*, and found that it has gill slits. A German anatomist Karl Gegenbaur (1870) coined the name Enteropneusta for animals with these characters. Spengel and van der Horst developed modern hemichordate systematics. A German physiologist, Johanness Peter Müller (1850), termed the pelagic enteropneust larvae "tornaria," but he thought they were starfish larvae. A Russian zoologist, Élie Metchnikoff (1869, 1870), observed the

development of these larvae and found that they transformed into enteropneusts, and this observation was later confirmed by an American scientist, Alexander Emanuel Agassiz (1872, 1873). An English geneticist, William Bateson (1884–1886), studied the development of *Saccoglossus kowalevskii* and some other species and concluded that enteropneusts are closely related to vertebrates. He proposed that the name "Hemichordata" be substituted for "Enteropneusta," designating it as a fourth subphylum of the Chordata (1885).

Concurrently, a Norwegian marine biologist, Georg Ossian Sars (1872), found the first specimen of another class of hemichordates, the Pterobranchia, and his father, Michael Sars (1874), named it *Halilophus mirabilis* (later *Rhabdopleura mirabilis*), although they thought that it is closely related to the Hydrozoa and Bryozoa. An Irish naturalist, George James Allman (1869), classified a specimen from the Shetland Islands as a bryozoan and named it *Rhabdopleura normani*. Today, both are regarded as the same species, and the latter scientific name was officially adopted. British zoologist, Sir Edwin Ray Lankester (1877), gave the name Pterobranchia to this clade, but he also thought these animals were bryozoans. A Scottish marine zoologist, William Carmichael McIntosh (1882), named and described a species collected in the Magellan Straits, *Cephalodiscus dodecalophus.* British zoologist Sir Sidney Frederic Harmer (1887) showed that it is closely related to the Enteropneusta and included it in the Hemichordata. Arthur Willey (1899), a British-Canadian zoologist, incorporated the genera *Cephalodiscus* and *Rhabdopleura* into the Pterobranchia and assigned the rank of class to both the Pterobranchia and the Enteropneusta.

Molecular Phylogeny

Phylogenetically, hemichordates are located in the clade Deuterostomia of the Bilateria. The Deuterostomia, inferred by the Austrian biologist Karl Grobben (1908), consist of only three phyla: Chordata, Echinodermata, and Hemichordata. Among deuterostomes, hemichordates were traditionally allied with chordates since they exhibit similar adult morphology to that of chordates, such as a stomochord or buccal diverticulum with homology to the notochord, a hollow, dorsal nerve cord or collar cord, corresponding to the neural tube, and pharyngeal gill slits (Fig. 1). The advent of molecular phylogeny, comparing 18S ribosomal DNA sequences in the late twentieth century, showed that they are less closely related to chordates than to echinoderms and revived the clade Ambulacraria, a name coined by Metchnikoff (Fig. 2). However, the question of whether colonial tube-dwelling pterobranchs are sister to vermiform enteropneusts or whether they should be nested within them has been much debated. Also, enteropneust phylogeny requires revision in light of recent investigations of deep-sea acorn worms (Holland et al. 2005). Phylogenetic analysis based on morphology splits the solitary enteropneusts into four families: the direct-developing Harrimaniidae, two groups of indirect developers, the Spengelidae and Ptychoderidae, and the deep-sea spaghetti worms of the family Saxipendiidae. More recently, one more family, the deep-sea family Torquaratoridae was added (Cannon et al. 2009).

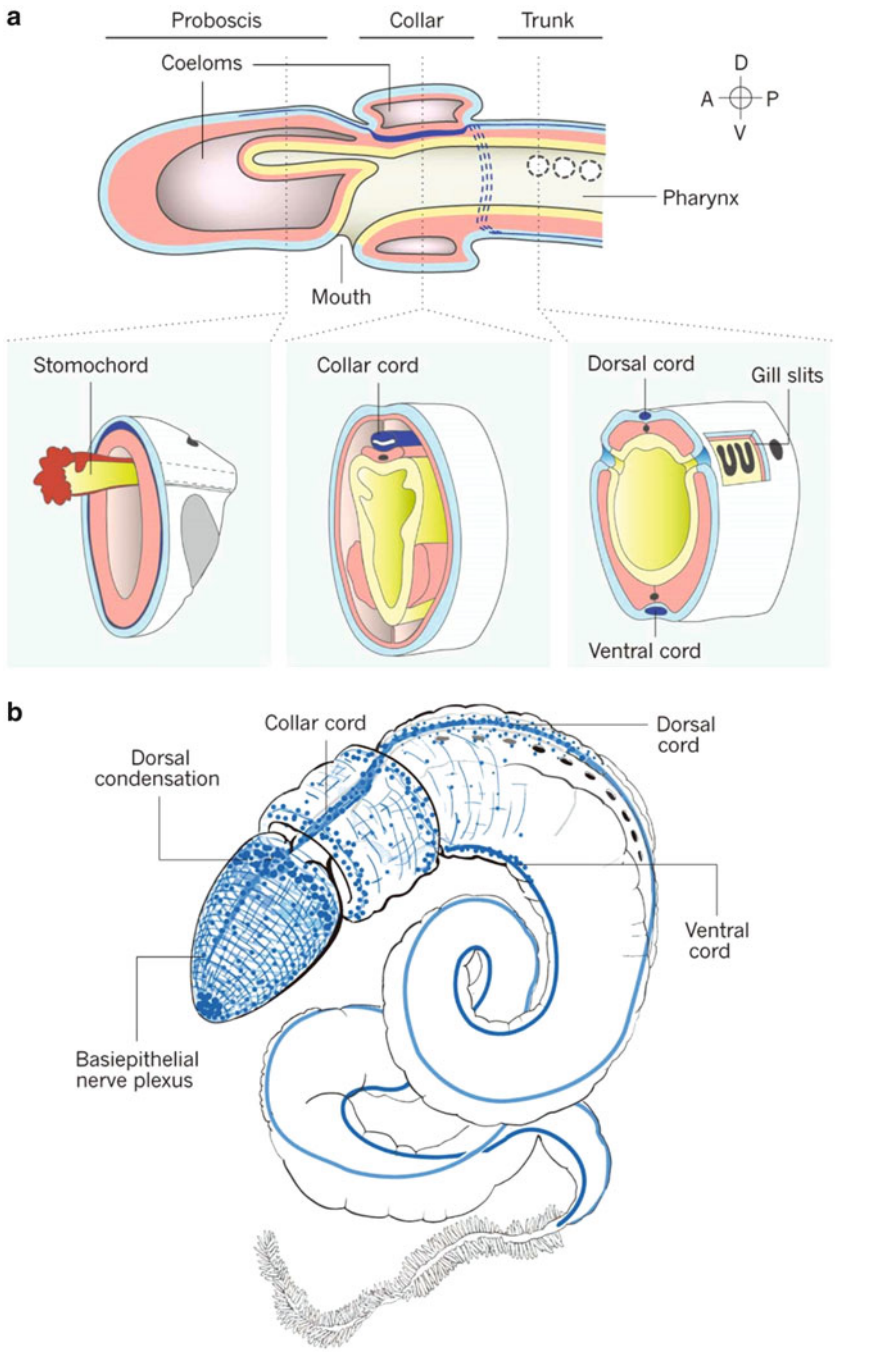


Fig. 1 Key anatomical features of the enteropneust body plan. (**a**) Longitudinal and transverse sections through an adult enteropneust hemichordate, highlighting morphological characters that have figured prominently in classic hypotheses of deuterostome evolution and chordate origins.

Previous phylogenetic studies based on 18S ribosomal DNA sequences showed that enteropneusts are paraphyletic with pterobranchs and sister to harrimaniids. Cannon et al. (2014) updated the phylogeny with expanded taxonomic sampling and mitochondrial DNA sequences and showed that the Saxipendiidae should be included in the family Harrimaniidae, while the Torquaratoridae is allied with the family Ptychoderidae, comprising three families of enteropneusts. They also showed that pterobranchs (Cephalodiscida and Rhabdopleurida) are sister to harrimaniids. Recent phylogenomic comparisons of transcriptomes for all hemichordate families revealed the reciprocal monophyly of the Enteropneusta and Pterobranchia and placed the deep-sea family Torquaratoridae within the Ptychoderida (Cannon et al. 2014). Our current analysis, based on comparative genomics of metazoans, also supports the monophyly of hemichordates and places the pterobranchs as a sister group to enteropneusts rather than within them (Simakov et al. 2015; Fig. 3). This phylogeny is probably more stable than any previous system and is expected to remain so unless new fossil evidence suggests otherwise (Tagawa 2016).

Fossil Evidence (Paleontological Studies)

Modern phylogenetic interpretations rely heavily on analysis of DNA sequences, while fossil evidence sometimes provides critical clues about phylogenetic relationships, although generally soft-bodied organisms, like the vermiform Enteropneusta, are less likely to be preserved in the fossil record. Because their bodies decompose readily, the fossil record of hemichordates is very poor and is largely restricted to the organic housing or tubarium (coenecium or rhabdosome) of colonial pterobranchs. The two major ranks commonly assigned to the Pterobranchia are the Cephalodiscida and the Rhabdopleurida (Fig. 3). Both groups are relatively well known from living representatives but have yielded a rather meager fossil record (Maletz 2014). Graptolites, in comparison, have a relatively complete and rich fossil record with regard to skeletal details, whereas their soft anatomy is almost completely unknown, except for a few poorly preserved remnants (Maletz 2014). The extinct Graptolithina is well known as an index fossil from the Middle Cambrian through the Lower Carboniferous period. Traditionally, it was suggested that the Graptolithina is sister to the class Pterobranchia (Fig. 3a). However, a recent phylogenetic study based on analysis of a 32-character morphological data set (Mitchell et al. 2013) suggested that the Graptolithinae, including the Rhabdopleuridae, is a sister taxon to the Cephalodiscidae (Fig. 3b).

Fig. 1 (continued) A anterior, *P* posterior, *D* dorsal, *V* ventral. (**b**) The nervous system of an adult enteropneust, showing both the broad basiepithelial plexus throughout the ectoderm and nerve chords along the dorsal and ventral midlines. Blue spots represent cell bodies and lines represent neural processes. (Reprinted by permission from Springer Customer Service Centre GmbH: Springer Nature, Nature, The deuterostome context of chordate origins, Lowe et al. 2015)

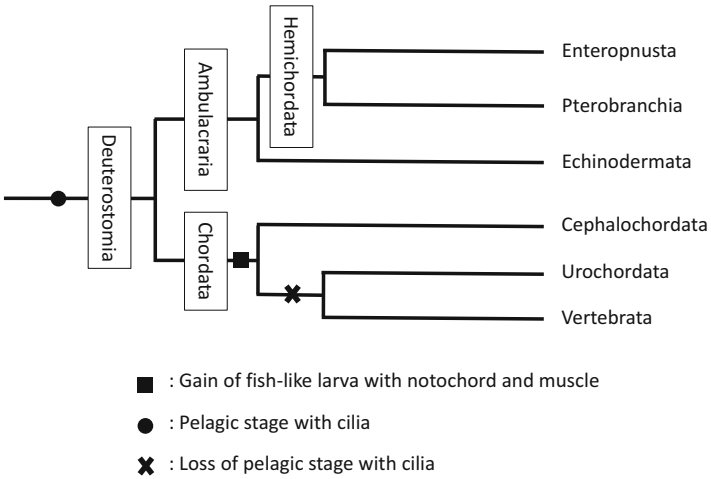


Fig. 2 Deuterostome relationships and lifestyles. Deuterostome groups can be divided into two clades: Chordata and Ambulacraria. The Chordata consists of cephalochordates, such as amphioxus. The Urochordata includes sea squirts. Vertebrata comprises vertebrates. The Ambulacraria is comprised of the bilateral Hemichordata and the radially symmetrical Echinodermata. The Hemichordata is divided into two classes, solitary free-living wormlike enteropneusts and tiny sessile colonial pterobranchs. Irrespective of their origin, deuterostome antecedents had planktotrophic larval stages with ciliary movement, as in acorn worms and amphioxus (shown by ●). During the course leading to tunicates and vertebrates, however, planktonic larvae with ciliary movement were lost (shown by **X**). Fish-like larval stages with muscles and a notochord for active swimming were acquired in the last common chordate ancestor (shown by ■)

Caron et al. (2013) addressed the fundamental issue of whether the ancestor of deuterostomes was a free-living, wormlike creature or a sessile, colonial animal, by describing fossilized marine worms from the Burgess Shale of Western Canada, which dates to 505 million years ago, in the Middle Cambrian period. Before this unusual report, the earliest known fossil enteropneusts were Triassic, between 250 and 200 million years old. The fossils, named *Spartobranchus tenuis*, were originally classified as *Ottoia tenuis*, a priapulan, by the famous American paleontologist, Charles Doolittle Walcott, in 1911. Caron et al. (2013), however, showed that they were not only enteropneusts but that they also lived at least part of their lives in tubes. This particular dwelling habit was unknown among enteropneusts but was common among their relatives, the pterobranchs. Fossils suggest possible intermediate morphology between enteropneusts and pterobranchs and imply that the ancestor of pterobranchs adopted a tube-dwelling habit before becoming colonial and sessile and before evolving elaborate tentacles. This discovery had a significant impact on hemichordate phylogeny, suggesting that mobile wormlike animals, acorn worms, or enteropneusts were the ancestor of vertebrates, not sessile colony dwellers, like pterobranchs.

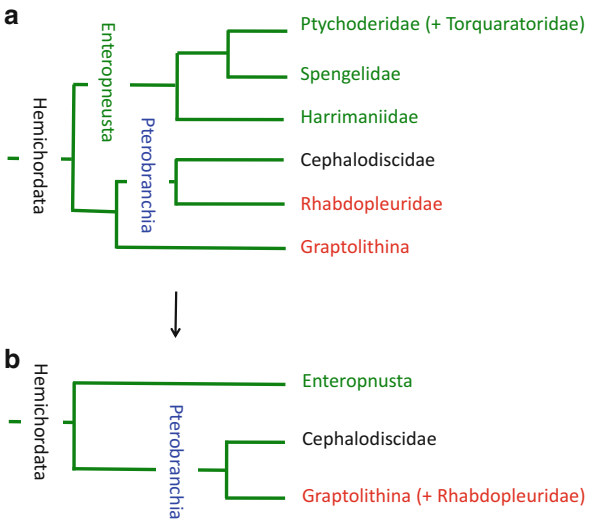


Fig. 3 Hemichordate phylogeny and new pterobranch relationships. (**a**) Enteropneusts are divided into three families: indirect developers, the Ptychoderidae, including the representative of the family, *Ptychodera flava*, and deep-sea acorn worms, the Torquaratoridae; indirect developers, the Spengelidae, including species that swarm like fish; and direct developers, the Harrimaniidae, including the representative of the family *Saccoglossus kowalevskii*. The Pterobranchia is traditionally divided into two extant families, Cephalodiscidae and Rhabdopleuridae. The fossil Graptolithina is sister to the class Pterobranchia in this cladogram. (**b**) Current proposed pterobranch relationships. Enteropneust family names are abbreviated to emphasize pterobranch relationships. Note the fossil Graptolithina is sister to extant Cephalodiscidae and extant Rhabdopleuridae is included within Graptolithina (compare to Fig. 2)

Molecular Developmental Biology

Many hemichordate genes involved in development have been isolated, and their expression patterns have been examined by whole-mount in situ hybridization to compare with those of other animals, especially model organisms, such as vertebrates and *Drosophila*. Functional assays with exogenous drug treatments and microinjection of small interfering RNAs into fertilized eggs of the hemichordate, *S. kowalevskii* have been quite successful (Lowe et al. 2006; Darras et al. 2011, 2018). Such studies are too numerous to detail here, but a significant aspect of axial patterning for body plan evolution is discussed in this section. The three axes, animal-vegetal (AV), anterior-posterior (AP), and dorsal-ventral (DV), are established during bilaterian embryogenesis by surprisingly conserved suites of genes.

Animal-Vegetal Patterning in Hemichordates

The AV axis initiates formation of the three germ layers, ectoderm, endoderm, and mesoderm. Ectoderm derives from the animal pole, and endomesoderm later divides

into endoderm and mesoderm from the vegetal pole. The formation of endomesoderm is triggered by beta-catenin, an intracellular signal transducer in the canonical Wnt signaling pathway. In both hemichordates and echinoderms (Darras et al. 2011), knockdown of the gene results in excess ectoderm and no endomesoderm, an embryonic state called "animalization." On the other hand, stabilization of the gene product throughout the embryo results in excess endomesoderm and no ectoderm, termed "vegetalization" of the embryo (Darras et al. 2011). This mechanism has been demonstrated in protostomes such as nemerteans, and beta-catenin is also involved in endoderm formation in diploblastic cnidarians. Together with these results, it is suggested that this process is ancestral to eumetazoan lineages.

Mesoderm specification from endomesoderm occurs by a signal from neighboring cells or tissues, i.e., "induction" in all deuterostomes except ascidians, in which it occurs by cell-autonomous specification of a sequestered cytoplasmic determinant. The two main signaling pathways, Nodal and FGF, are involved in mesoderm specification in vertebrates. FGF signaling also specifies anterior mesoderm in non-vertebrate chordates, amphioxus, and in hemichordates, suggesting an ancient role of FGF in deuterostome mesoderm formation, although Notch-Delta signaling is important in early mesoderm specification of echinoid echinoderms.

Anteroposterior Patterning in Hemichordates

Many early developmental steps of AP axis formation are highly conserved among deuterostome taxa and probably date to the bilaterian ancestor. Wnt signaling is again important for establishing AP patterning in hemichordates as well as other bilaterians (Darras et al. 2018; Fig. 4). In vertebrates, Wnt protein produced posteriorly acts as a posteriorizing signal in all three germ layers, and Wnt antagonists produced anteriorly originate from the mesoderm of Spemann's organizer. Their interaction sets up a graded Wnt distribution to prefigure the eventual anatomical AP axis. Over-activation of the Wnt pathway by GSK3beta leads to anterior truncation and ectoderm posteriorization in hemichordates, while blocking of the Wnt pathway by overexpression of Wnt antagonists has opposite effects, resulting in a phenotype complementary to that of Wnt activation. Current data from hemichordates provide strong support for the hypothesis that the canonical Wnt signaling pathway as intimately involved in early evolution of the AP axis (Darras et al. 2018). The most conspicuous finding from hemichordates is that canonical Wnt signaling regulates the same region of the transcriptional network that defines ectodermal, neuraxis AP patterning in vertebrates, which is not shared by invertebrate chordates. Co-expression of genes such as *sfrp1/5*, *fgf8/17/18*, *foxG*, *retinal homeobox*, *dlx*, and *nk2-1* defines ectodermal territories that later form proboscis ectoderm in hemichordates and forebrain in vertebrates anteriorly (Fig. 4). Similarly, expression domains of *emx*, *barH*, *dmbx*, and *pax6* define collar ectoderm of hemichordates and midbrain of vertebrates in posterior regions. More posteriorly, domains of *gbx*, *engrailed*, *pax2/5/8*, and the collinearly expressed Hox genes regulate pharynx and trunk patterning of hemichordates and the hindbrain and spinal cord in vertebrates. AP map similarities even extend to the three signaling centers, producing the same signals and occupying equivalent map positions that are

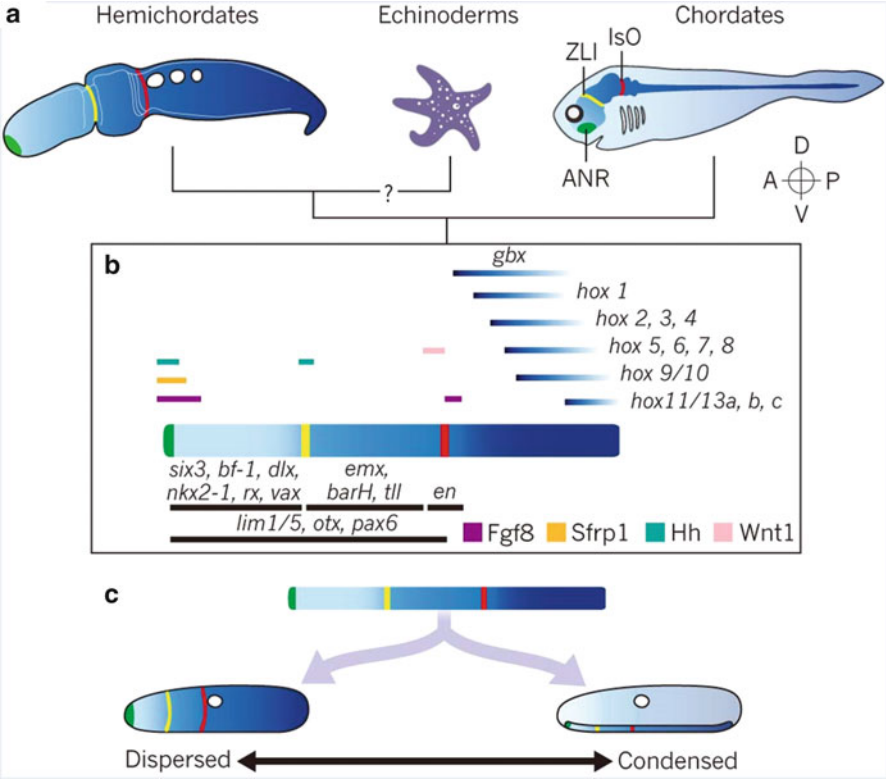


Fig. 4 A conserved molecular network for the deuterostome anteroposterior axis. (**a**) Schematic representation of the distribution of ectodermal expression domains of anteroposterior (AP) transcription factors (blue gradient) and ectodermal signaling centers (green, yellow, and red) in relation to body plans of deuterostome phyla. Chordate neuroectodermal signaling centers depicted are the anterior neural ridge (ANR), zona limitans intrathalamica (ZLI), and isthmic organizer (IsO). Broad conservation of expression domains between hemichordates and chordates allows for reconstruction of an ancestral patterning network, which is shown without any explicit inference of ancestral morphologies (**b**). Insufficient data exist from echinoderms to infer to what extent they share this conserved AP patterning network during adult patterning, although much of the anterior network is conserved in larvae. (**b**) Domain map for conserved transcription factors and signaling ligands in relation to the AP axis. (**c**) Current data allow for reconstruction of a conserved molecular coordinate system for the AP axis of the last common deuterostome ancestor, but not for reconstruction of discrete morphologies of that ancestor, because this AP patterning network is deployed in a variety of morphological contexts, as evidenced by comparative data from hemichordates (dispersed; AP expression domains encircling the body) and chordates (condensed; AP domains largely restricted to regions near the dorsal midline). *A* anterior, *P* posterior, *D* dorsal, *V* ventral. (Reprinted by permission from Springer Customer Service Centre GmbH: Springer Nature, Nature, The deuterostome context of chordate origins, Lowe et al. 2015)

important for vertebrate brain patterning and for hemichordate ectodermal development at the anterior tip, proboscis-collar boundary, and collar-trunk boundary (Fig. 4). In hemichordates, the conserved AP map of ectodermal expression domains covers both neural and epidermal tissue, and domains encircle the body. In chordates, most

comparative studies have focused on the role of this network in patterning the dorsal CNS, but more recent studies demonstrate that expression of many genes extends ventrally into sensory neurons and epidermis, suggesting a more general role in ectodermal patterning.

Dorsoventral Patterning in Hemichordates

The DV axis has been a subject of intensive study in animal evolution since the famous inversion hypothesis proposed by French naturalist, Étienne Geoffroy Saint-Hilaire (1822), that the ventral side of arthropods is homologous to the dorsal side of vertebrates. Recently it was shown that one midline of an embryo produces BMP, a member of the TGF-beta family, and the opposite midline produces the BMP antagonist, Chordin. This antagonistic interaction between BMP and Chordin generates a graded distribution of Bmp across the embryos, resulting in a corresponding graded distribution of activated Smad1/5 transcription factors in embryonic cells. The Smad1/5 gradient stimulates and represses different genes encoding transcription factors and other signaling pathways to generate a long-lasting DV map of expression domains of these genes. These gene expression patterns establish development of different tissues and cell types in different regions along the DV axis.

In hemichordate embryos, BMP and BMP modulators are expressed in ectoderm of the dorsal midline and Chordin, and anti-dorsalizing morphogenetic protein (Admp) is expressed in the ectoderm of the ventral midline, as in protostomes and echinoderms, but opposite to the pattern in chordates (Fig. 5). In this context, DV axis inversion, inferred by the BMP-Chordin gradient, occurred at the split between non-chordate (ambulacrarian) and chordate deuterostomes. Lowe et al. (2006) addressed the functional effects of BMP signaling in the hemichordate, *S. kowalevskii*. When embryos were exposed globally to exogenous BMP, the same overexpression effects as injection of BMP mRNA resulted. Embryos were dorsalized morphologically and molecularly in a dose-dependent manner. However, overexpression of BMP did not result in repression of neural fates, contrary to its effects in chordates and *Drosophila*, raising the possibility that BMP-Chordin gene networks were not deployed in the nervous system of the last common ancestor of bilaterians. On the other hand, endogenous Bmp knockdown by injection of small interfering RNAs into fertilized eggs resulted in a complementary phenotype, ventralized embryos. These findings substantiate general views on DV axis formation, leaving chordates as a unique phylum among bilaterians (Fig. 5).

Larval Evolution

The majority of animals have planktonic life stages, larvae with body plans, ontogenies, and ecologies distinct from those of adults. How did a larval stage evolve and how could it be lost? There are two conceivable scenarios in regard to larval origins (Raff 2008; Fig. 6). One is that the first animals were small, pelagic forms similar

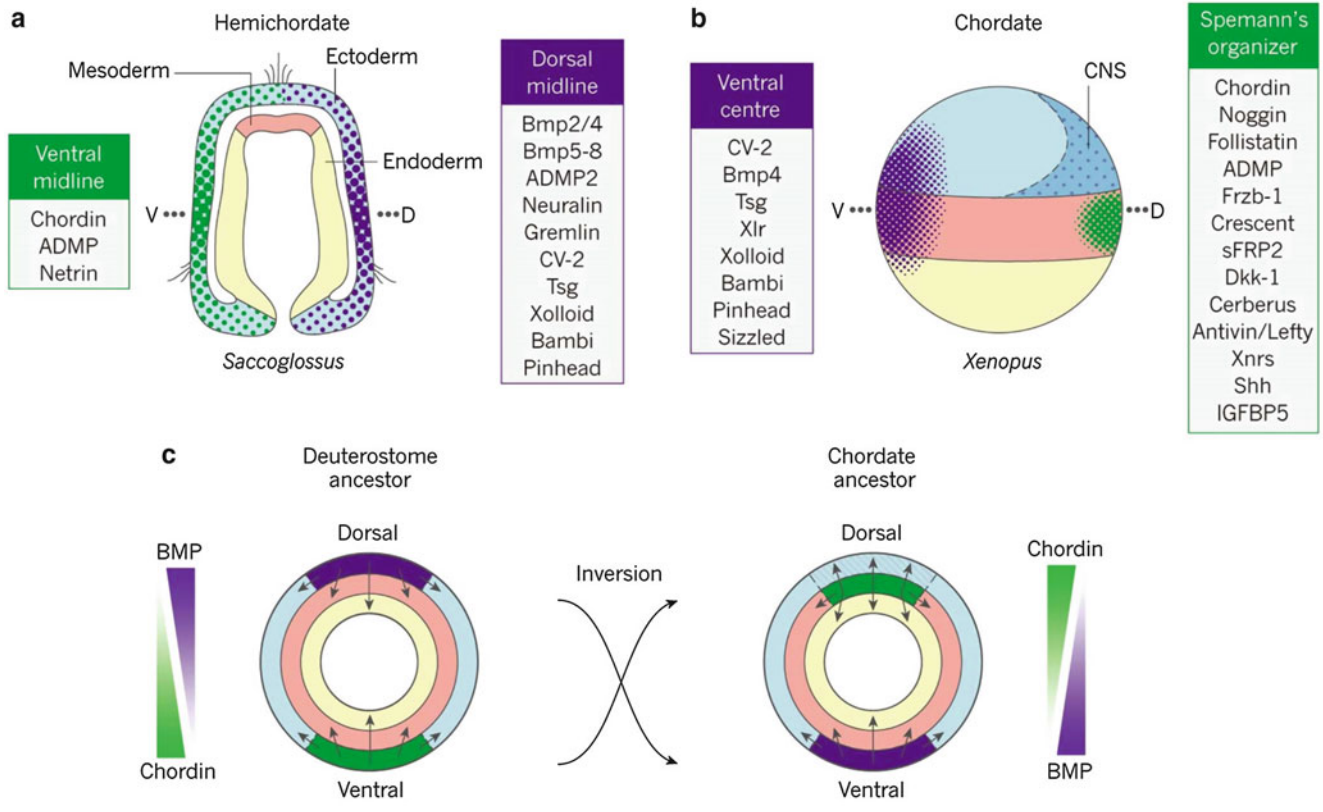


Fig. 5 Comparison of dorsoventral patterning mechanisms of hemichordates and chordates. (**a**) BMP-Chordin signaling components expressed in the dorsal and ventral midline ectoderm (blue) in the late gastrula stage of *Saccoglossus kowalevskii*. (**b**) BMP-Chordin signaling components expressed either on

to extant larvae and that adult bilaterian body plans evolved subsequently (Fig. 6a). The other is that adult bilaterian body plans evolved first, and then larval body plans arose by interpolation into direct-developing ontogenies (Fig. 6b). Enteropneusts or acorn worms, the largest branch of hemichordates, have direct and indirect developing species, as do most bilaterian clades (Fig. 7). Embryogenesis of direct developers occurs inside eggs at the bottom of the sea, and embryos hatch as juveniles. In contrast, indirect developers hatch into lovely, transparent, swimming, *tornaria* larvae. They swim and drift for months in the ocean, capturing food with ciliary bands until they are ready to metamorphose into wormlike juveniles that eventually settle on the sea bottom for adult life (Fig. 7).

Gonzalez et al. (2017) recently compared larval and adult body plans of the indirect developing hemichordate, *Schizocardium californicum*, by describing 27 transcription factors involved in ectodermal AP patterning. Their results show that the gene expression pattern in young juveniles is surprisingly similar to that of the direct-developing hemichordate, *S. kowalevskii*. In contrast, the expression pattern in *tornaria* larvae corresponds only to anterior gene expression territories of juveniles. Early *tornaria* lacks whole sets of genes, such as Hox genes, expressed in the trunk region, but they are progressively expressed in later *tornaria*, in preparation for metamorphosis. Gonzalez et al. (2017) concluded that temporal regulation of the posterior developmental program was a key factor in the evolutionary transition between the two life history strategies in enteropneusts.

How can we interpret this result in regard to evolutionary aspects of metazoan life history? Do we opt for convergence of hemichordate tornaria and echinoderm dipleurula larvae or a common evolutionary origin? Recent data from hemichordates and other bilaterians appear to support the former, suggesting that a larval stage might be a derived condition in bilaterians (Fig. 6). A better understanding of larval and adult body patterning across different bilaterian lineages will be crucial to advance this debate about evolution of animal life histories.

Insights from Comparative Genomics

By comparing genomes of extant organisms, we try to reconstruct genomic features of extinct ancestors. Such endeavors may provide insight into patterns of genome diversity and how organisms evolved through gain, loss, and modification of genomic features. The increasing number of sequenced genomes from less well-studied taxa improves our view of ancestral genomes.

Fig. 5 (continued) the ventral side or dorsally in Spemann's organizer in the early gastrula of *Xenopus*. CNS, central nervous system. (**c**) Inversion of dorsoventral (DV) signaling centers and relocation of the Chordin source from ectoderm (yellow) to mesoderm (red) were innovations in DV patterning at the base of the chordates (ancestral location shown by gray shading). (Reprinted by permission from Springer Customer Service Centre GmbH: Springer Nature, Nature, The deuterostome context of chordate origins, Lowe et al. 2015)

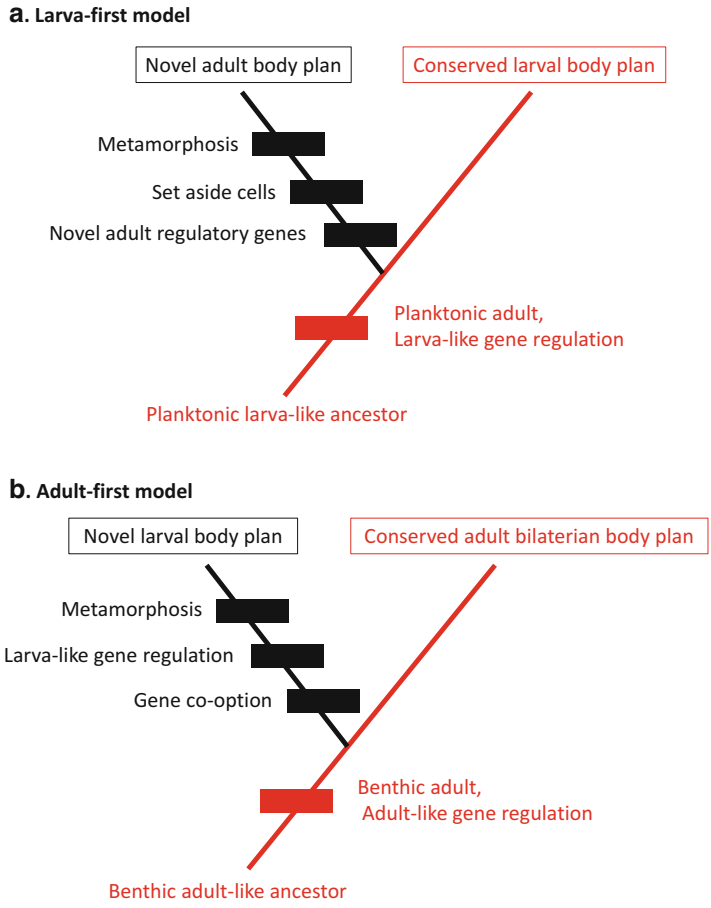


Fig. 6 **Two possible scenarios of bilaterian origins**. Two scenarios posit amounts of evolutionary change along branches leading to more derived developmental changes. (**a**) Larva-first model. Most evolution of developmental characters lies on the branch leading to the benthic adult, with the larva retaining ancestral features. (**b**) Adult-first model. Current data from indirect developing hemichordate now favor this model. Most evolution of developmental characters lies in the line to the planktonic larva, with the adult retaining ancestral features. Both models illustrate single lineages, but in the metazoan radiation, numerous lineages evolved in parallel. A large degree of homoplasy resulted in either case. (Modified after Raff (2008) with permission of the Royal Society)

Simakov et al. (2015) sequenced and analyzed genomes of acorn worms to infer ancestral genomic features of deuterostomes. They used specimens from the two main lineages of enteropneust hemichordates, which diverged more than 370 million years ago: an Indo-Pacific species, *Ptychodera flava*, from Hawaii and an Atlantic species, *Saccoglossus kowalevskii*, from North America. The former develops indirectly via a *tornaria* larva that metamorphoses to a juvenile worm after several months in the planktonic stage, while the latter develops directly into a juvenile worm within days. The haploid lengths of the two genomes are both

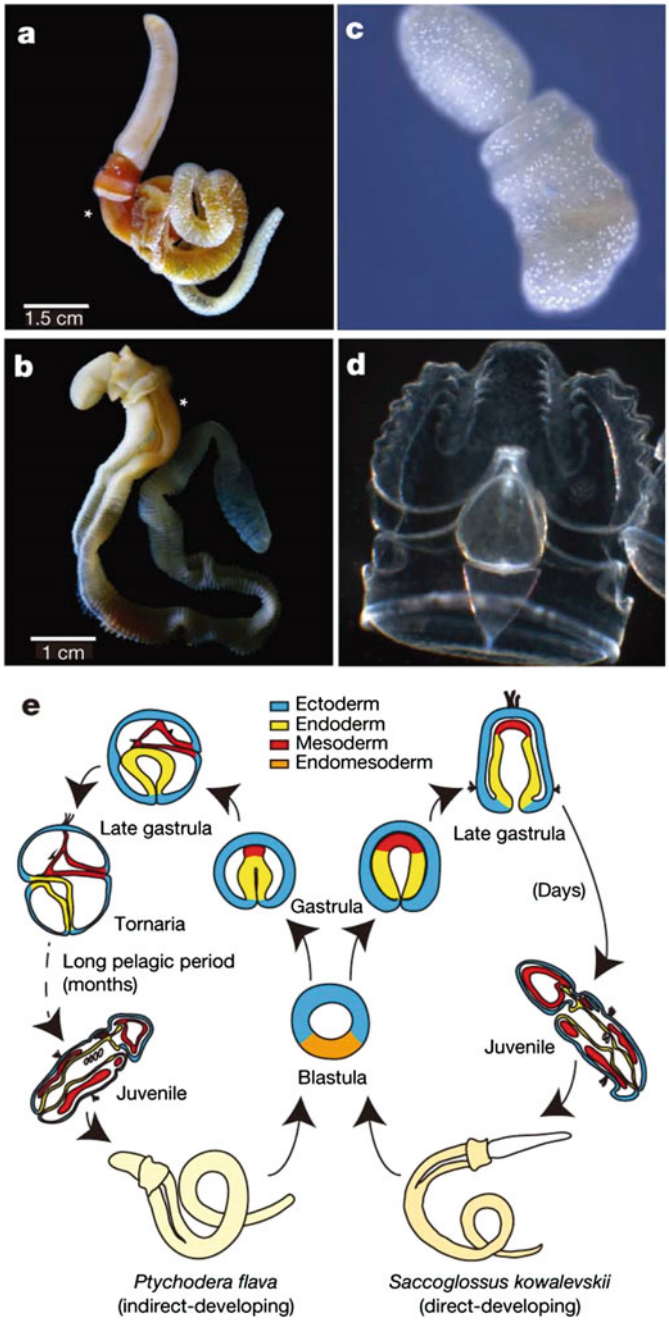


Fig. 7 Hemichordate model systems and their embryonic development. The hemichordate phylum includes the enteropneusts (acorn worms) and pterobranchs (minute, colonial, tube-dwelling; not shown). (**a**, **c**) *Saccoglossus kowalevskii* (harrimaniid (direct-developing) enteropneust) adult (**a**) and juvenile (**c**) with gill slits. (**b**, **d**) *Ptychodera flava* (ptychoderid

about one gigabase pair (1 Gbp) but differ in nucleotide heterozygosity (1.3% in *P. flava* and 0.5% in *S. kowalevskii*). Estimated gene numbers total >18,566 for *P. flava* and 19,270 for *S. kowalevskii*. Both acorn worm genomes show similar bulk gene content and similar repetitive landscapes. Simakov et al. identified 8,716 families of ancestral genes in these genomes, implying their presence in the deuterostome ancestor. Descendants of these ancestral genes account for ~14,000 genes in extant deuterostome genomes, because of gene duplication and other processes.

Exon-intron structures of genes were generally well-conserved among hemichordates and other metazoans, and the authors were able to infer 2,061 ancestral deuterostome splice sites. They found 23 introns and 4 coding sequence indels present only in deuterostomes (shared between at least one ambulacrarian and chordates) among orthologous bilaterian genes. These shared, derived characters may be useful to diagnose clade memberships of new candidate organisms.

Based on whole-genome alignments, Simakov et al. identified 6,533 conserved noncoding elements (CNE) longer than 50 bp that are found in all five deuterostome clades including two hemichordates, amphioxus, sea urchins, and humans. Identified CNEs overlap extensively with human long, noncoding RNAs (3,611 CNE loci). As with vertebrates, those alignments usually do not exceed 250 bp, and they also occur in clusters. Among these conserved sequences is a previously identified vertebrate brain and neural tube specific enhancer, located close to the *sox14/21* ortholog in all five species.

It was difficult to find chromosome-scale organization of gene linkage, macro-synteny, between sea urchin and chordate genomes, since assembly of the sea urchin draft genome was very limited. Hemichordate genomes exhibit extensive conserved synteny with the most basal chordates, the cephalochordates. Owing to the hemichordate sequences, many shared features were discovered among deuterostome genomes, implying that many well-characterized features of chordate genomes are not chordate-specific but arose earlier in animal evolution.

With regard to micro-synteny, hundreds of tightly linked, conserved gene clusters of three or more genes, including Hox and ParaHox clusters, occur in both acorn worms. Hemichordates and amphioxus share more micro-syntenic linkages than either does with sea urchins, vertebrates, or available protostomes. Conservation of micro-syntenic linkages can occur due to low rates of genomic rearrangement or, more interestingly, as a result of selection to retain linkages between genes and their cis-regulatory elements. The most prominent finding from our comparative genomics of hemichordates and other metazoans is that a cluster of six genes, known as the

Fig. 7 (continued) (indirect-developing) enteropneust) adult (**b**) and tornaria stage larva (**d**). Gill slits labelled with an asterisk in **a** and **b**. (**e**) Comparison of the direct and indirect modes of development of the two hemichordates, indicating the long pelagic larval period in *Ptychodera* until the settlement and metamorphosis as a juvenile. (Reprinted by permission from Springer Customer Service Centre GmbH: Springer Nature, Nature, Hemichordate genomes and deuterostome origins, Simakov et al. 2015)

"deuterostome pharyngeal gene cluster," shows deuterostome-specific micro-synteny regarding the order of four transcription factor genes, *nkx2.1*, *nkx2.2*, *pax1/9*, and *foxA*, along with two non-transcription factor genes *slc25A21* (mitochondrial 2-oxodicarboxylate carrier) and *mipol1* (mirror-image polydactyly 1), which are putative "bystander" genes containing regulatory elements for *pax1/9* and *foxA*, respectively (Fig. 8a). What is interesting is that the four transcription factor genes are expressed in pharyngeal endoderm and foregut endoderm of juvenile hemichordates (Fig. 8b). Co-expression of these four clustered genes during pharyngeal development strongly supports the functional importance of their clustering.

The same ordered cluster is also present on a single scaffold in the crown-of-thorns sea star, *Acanthaster planci*, an echinoderm that does not possess gill pores and in amniote vertebrates that lack gill slits (Fig. 8a). The fact that these genes are not clustered in available protostome genomes suggests that clustering of the four ordered transcription factors and bystander genes in the deuterostome stem served a regulatory role in evolution of the pharyngeal apparatus, the signature trait of deuterostomes.

Nearly 30 deuterostome-specific genes that might lead to functional innovations in deuterostomes have been discovered. Over a dozen of those genes have readily identifiable homologs in marine microbes, often cyanobacteria or eukaryotic microalgae, that are not known in other metazoans. Such genes are associated with the sialic acid biosynthetic pathway, and 5 of the 11 steps of this pathway are inferred as deuterostome novelties (Fig. 9a). Sialic acid is a family of nine-carbon acidic monosaccharides and can be found as components of oligosaccharide chains of mucins, high-molecular-weight glycoconjugates mostly secreted as principal components of mucus in animals. Acorn worms are covered with mucus, and the mucus is also used for feeding. In the hemichordate and amphioxus genomes, novel and expanded families of genes encoding polypeptide backbones of glycoproteins are found with von Willebrand type D and/or cysteine-rich domains, including mucin, as large, tandemly duplicated clusters with varied expression patterns. This is strong evidence to support the importance of glycoproteins for mucociliary feeding and other hemichordate activities, since these are not found in the sea urchin genome. Also, the pharynx of hemichordates is heavily ciliated, like that of amphioxus. Cells of the pharyngeal walls in hemichordates secret abundant mucins and glycoproteins, like those of the ventral endostyle in amphioxus. In this manner, these glycoproteins probably enhanced mucociliary filter-feeding capture of food particles from the microbe-rich marine environment and protected the inner and outer tissue surfaces in the deuterostome ancestor.

Another example of gene family expansion in hemichordate genomes is the transforming growth factor-beta (TGF-beta) signaling pathway (Fig. 9b–d). Signaling ligands Lefty, a Nodal antagonist, and Univin/Vg1/GDF1, a Nodal agonist, are deuterostome innovations that modulate Nodal signaling during major developmental events in axial patterning (Fig. 9b and c). *Univin* is tightly linked to the related bilaterian *bmp2/4* in the genomes of hemichordates and amphioxus, as suggested previously in the sea urchin genome, supporting its origin by tandem duplication and divergence from an ancestral *bmp2/4*-type gene.

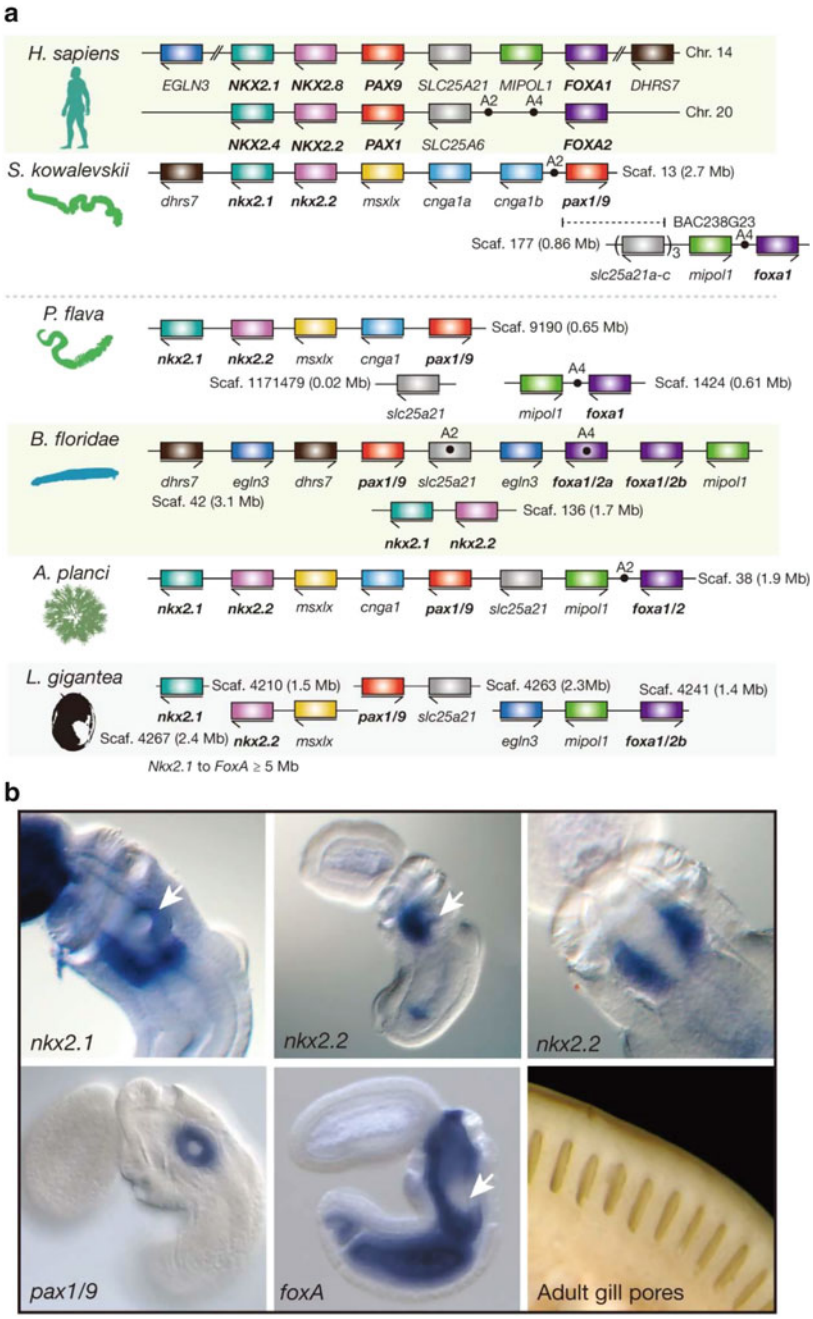


Fig. 8 Conservation of a pharyngeal gene cluster across deuterostomes. (**a**) Linkage and order of six genes including four genes encoding transcription factors Nkx2.1, Nkx2.2, Pax1/9, and FoxA and two genes encoding non-transcription factors Slc25A21 (solute transporter) and Mipol1

TGFβ2 signaling is a deuterostome innovation that controls cell growth, proliferation, differentiation, and apoptosis at later developmental stages. Corresponding to the novel TGFβ2 ligand, the type II receptor has a novel ectodomain. The extracellular matrix protein, thrombospondin 1, which activates TGFβ2 in vertebrates, contains a deuterostome-specific combination of domains, including three thrombospondin type 1 domains that bind the TGFβ2 pro-domain region (Fig. 9d). While these signaling novelties have clear sequence similarities to pan-bilaterian components, they construct long-stem branch clades in phylogenetic trees, indicating extensive sequence divergence in the deuterostome stem. Together, these specific innovations appear to contribute to the increased and complex patterning of Smad2/3-mediated signaling in deuterostomes, compared with protostomes and other metazoans.

Conclusions and Perspectives

Hemichordates occupy a unique and pivotal position in animal evolution, not only of deuterostomes but also of bilaterians, as shown above. Recent studies of hemichordates have uncovered several principles. First, molecular phylogenetic analysis of hemichordates confirms the union of three deuterostome phyla, hemichordates, echinoderms, and chordates, into a single clade, as well as the reciprocal monophyly of enteropneusts and pterobranchs. Second, paleontological analysis provides evidence that acorn worms lived in tubes in the Middle Cambrian period. Third, comparative molecular developmental biology in hemichordates challenges the classic hypothesis of animal body plan evolution, especially in relation to the origin

Fig. 8 (continued) (mirror-image polydactyly 1 protein), which are putative "bystander" genes containing regulatory elements of *pax1/9* and *foxA*, respectively. The pairing of *slc25A21* with *pax1/9* and of *mipol1* with *foxA* also occurs in protostomes, indicating bilaterian ancestry. This cluster is not present in protostomes such as *Lottia* (Lophotrochozoa), *Drosophila melanogaster*, and *Caenorhabditis elegans* (Ecdysozoa) or in the cnidarian, *Nematostella*. *SLC25A6* (the *slc25A21* paralog on human chromosome 20) is a potential pseudogene. The dots marking A2 and A4 indicate two conserved noncoding sequences first recognized in vertebrates and amphioxus but also present in *S. kowalevskii* and partially in *P. flava* and *A. planci*. (**b**) The four transcription factor genes of the cluster are expressed in the pharyngeal/foregut endoderm of the *Saccoglossus* juvenile: *nkx2.1* is expressed in a band of endoderm at the level of the forming gill pore, especially ventrally and posterior to it (arrow), and in a separate ectodermal domain in the proboscis. It is also known as thyroid transcription factor1 due to its expression in the pharyngeal thyroid rudiment in vertebrates. The *nkx2.2* gene is expressed in pharyngeal endoderm just ventral to the forming gill pore, shown in side view (arrow indicates gill pore) and ventral view; and *pax1/9* is expressed in the gill pore rudiment itself. In *S. kowalevskii*, this is its only expression domain, whereas in vertebrates it is also expressed in axial mesoderm. The *foxA* gene is expressed widely in endoderm but is repressed at the site of gill pore formation (arrow). An external view of gill pores is shown; up to 100 bilateral pairs are present in adults, indicative of the large size of the pharynx. (Reprinted by permission from Springer Customer Service Centre GmbH: Springer Nature, Nature, Hemichordate genomes and deuterostome origins, Simakov et al. 2015)

of chordates, and provides a foundation for a new hypothesis about the deuterostome ancestor. Fourth, comparative genomic data from two hemichordate genomes provides important evidence for the existence of deuterostome-specific genes in association with their characteristic ancestral traits, such as pharyngeal gill development and macro-synteny at macro-chromosome scale.

Because of those hemichordate studies, it now appears that the deuterostome and/or our ancestor probably resembled an extant enteropneust, with pharyngeal gill

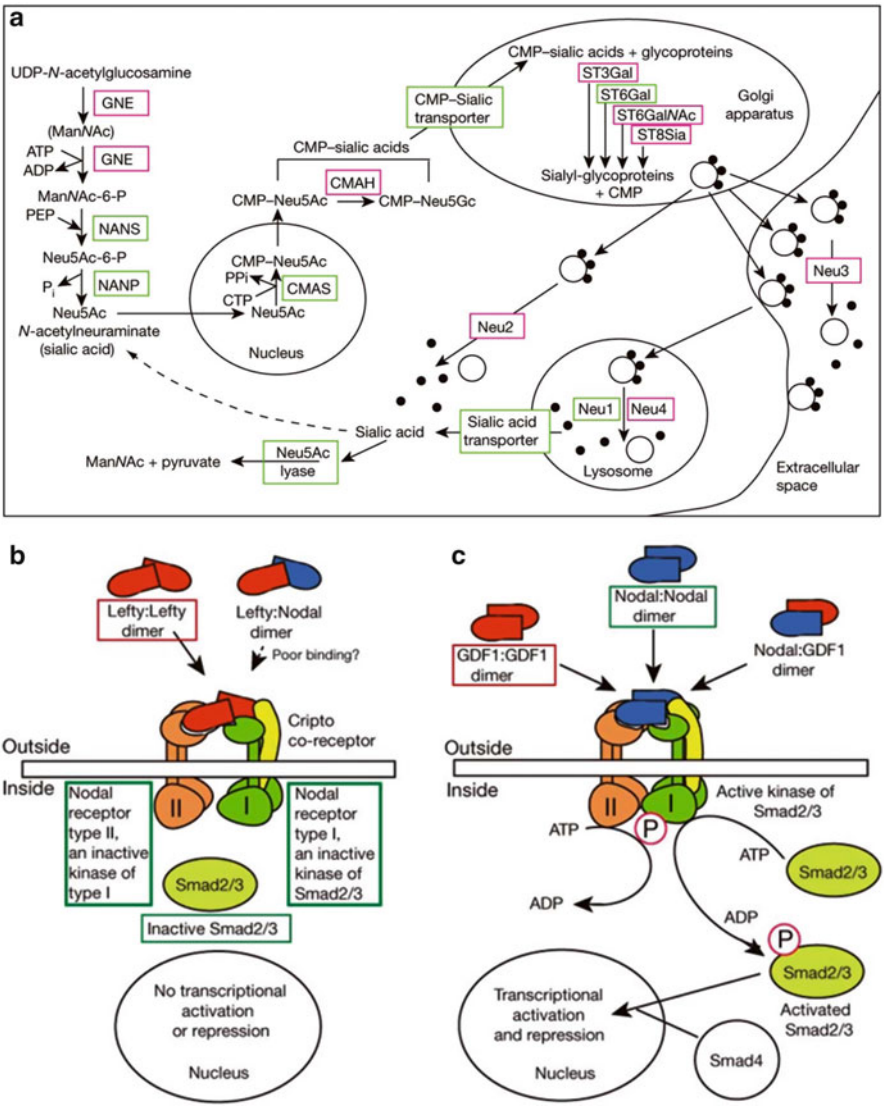


Fig. 9 (continued)

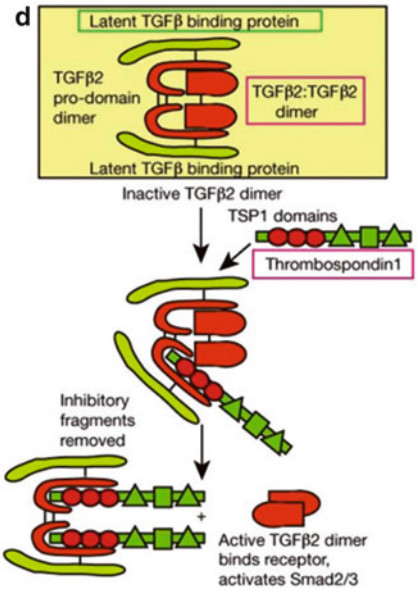


Fig. 9 Examples of deuterostome gene novelties. (**a**) Steps of biosynthesis of sialic acid and its addition to and removal from glycoproteins. (**b**–**d**) Novel genes in TGFβ signaling pathways. The encoded proteins are shown and include Lefty (**b**) an antagonist of Nodal signaling, which activates Smad2/3-dependent transcription when not antagonized; Univin (**c**) an agonist of Nodal signaling, also called Vg1, DVR1, and GDF1; and TGFβ2 (**d**) a ligand that activates Smad2/3-dependent transcription by binding to a deuterostome-specific TGFβ receptor type II, which contains a novel ectodomain (not shown). Also shown in **d** is the novel protein thrombospondin 1 that activates TGFβ2 by releasing it from an inactive complex, by way of its TSP1 domains. Red boxes around protein names indicate their deuterostome novelty. Green boxes around the names indicate genes with pan-metazoan/bilaterian ancestry and without accelerated sequence change in the deuterostome lineage. (Reprinted by permission from Springer Customer Service Centre GmbH: Springer Nature, Nature, Hemichordate genomes and deuterostome origins, Simakov et al. 2015)

slits and mucociliary filter feeding. Deep homologies are also recognized between divergent anatomical morphologies and their developmental origins. At the same time, however, there are still large evolutionary gaps, especially leading from invertebrate deuterostome, stemlike hemichordates through non-vertebrate, chordate cephalochordates to vertebrates, with such chordate innovations as a notochord, somites and a dorsal hollow neural tube (central nervous system). It can be suggested that a motile, free-living, hemichordate-like deuterostome ancestor gave rise to a cephalochordate-like chordate ancestor, but how was the vertebrate ancestor with vertebrate-specific innovations generated from it? Also, how can we integrate larval development and adult development in the genome of the same individual? This chapter cannot cover all research on hemichordates, including regeneration and classical hypotheses related to chordate anatomy (Holland et al. 2015). For further reading, those interested can find the

details in an earlier review (Tagawa 2016) and another current review (Arimoto and Tagawa 2018). More information covering chordate origins and evolution is found in a book by Satoh (2016).

It is noteworthy that the most basal chordates, cephalochordates, have two different swimming stages (Fig. 2). One is pelagic with cilia, like Ambulacraria during embryonic stages, and the other swims actively with muscles during a fish-like larval stage. More derived chordates, such as urochordates and vertebrates, do not have (or may have lost) the former stages but have the latter stages, and this may be a key to understanding deuterostome evolution, since now we have genome sequences to compare details of the development of each at micro and macro levels. Extensive conservation between the acorn worm and amphioxus genomes among deuterostomes, and detailed comparative studies provide clues to generate new ideas about animal evolution.

In any case, to add a missing piece of the puzzle, further efforts in hemichordate research are needed in a variety of areas, including regeneration, in order to understand which evolutionary changes in genomes underlie which evolutionary changes in traits, including development, anatomy, and animal life history. Of course, comprehensive studies across different bilaterian lineages will be crucial. The deeper our analysis goes, the better our understanding of evolution and development will become.

Cross-References

- ▶ Alexander Onufrievich Kowalevsky (1840–1901)
- ▶ A Macroevolutionary Perspective on Developmental Constraints in Animals
- ▶ Convergence
- ▶ Developmental Homology
- ▶ Evo-Devo and Phylogenetics
- ▶ Evolution of Complexity
- ▶ Evolvability
- ▶ Mechanisms of Pattern Formation, Morphogenesis, and Evolution
- ▶ The Developmental Hourglass in the Evolution of Embryogenesis
- ▶ The Evolution of Cleavage in Metazoans

Acknowledgments I would like to thank the editor, Dr. Ehab Abouheif, for inviting me to write this chapter. I also would like to express my gratitude to the outstanding investigators in the hemichordate research field, who have always stimulated me in both positive and negative ways. I wish to express my sincere thanks to Springer Nature and the Royal Society for allowing me to reproduce their data as Figs. 1, 4, 5, 7, 8, 9, and 6, respectively, in this chapter. Special thanks to Professor Nori Satoh and Dr. Steven D. Aird, who checked my poor Japanese English carefully and gave me many useful comments and suggestions. Finally, I would like to acknowledge all members of my lab, past and present, who have always been supportive. I apologize that I could not fully quote sources due to limitations imposed upon the number of citations.

References

Agassiz AE (1872) Tornaria, the young stage of Balanoglossus. Amer Natural 6:636–637

Agassiz AE (1873) The history of Balanoglossus and Tornaria. Mem Amer Acad Arts Sci 9:421–436

Allman GJ (1869) *Rhabdopleura normani*, Allman, nov. gen. et sp. In: Norman AM (ed) Shetland final dredging report. Part II. Crustacea, Tunicata, Polyzoa, Echinodermata, Actinozoa, Hydrozoa and Porifera. Reports of the British Association of the Advancement of Science 1868:311–312

Arimoto A, Tagawa K (2018) Regeneration in the enteropneust hemichordate *Ptychodera flava* and its evolutionary implication. Develop Growth Differ 60:400–408

Bateson W (1885) The later stages in the development of *Balanoglossus kowalevskii*, with a suggestion as to the affinities of the Enteropneusta. Q J Microsc Sci 25(Suppl):81–122

Cannon JT, Rychel AL, Eccleston H, Halanych KM, Swalla BJ (2009) Molecular phylogeny of hemichordata, with updated status of deep-sea enteropneusts. Mol Phylogenet Evol 52:17–24

Cannon JT, Kocot KM, Waits DS, Weese DA, Swalla BJ, Santos SR, Halanych KM (2014) Phylogenomic resolution of the hemichordate and echinoderm clade. Curr Biol 24:2827–2832

Caron JB, Conway Morris S, Cameron CB (2013) Tubicolous enteropneusts from the Cambrian period. Nature 495:503–506

Darras S, Gerhart J, Terasaki M, Kirschner M, Lowe CJ (2011) β-catenin specifies the endomesoderm and defines the posterior organizer of the hemichordate *Saccoglossus kowalevskii*. Development 138:959–970

Darras S, Fritzenwanker JH, Uhlinger KR, Farrelly E, Pani AM, Hurley IA, Norris RP, Osovitz M, Terasaki M, Wu M, Aronowic ZJ, Kirshner M, Gerhart JC, Lowe CJ (2018) Anteroposterior axis patterning by early canonical Wnt signaling during hemichordate development. PLoS Biol 16(1):e2003698. https://doi.org/10.1371/journal.pbio.2003698

Eschscholtz JF (1825) Bericht über die zoologische Ausbeute während der Reise von Kronstadt bis St. Peter und Paul. Oken's Isis for 1825, (6): 733–747.

Geoffroy St-Hilaire E. Considerations generales sur la vertèbre [in French]. *Mém. Mus. Hist. Nat.* 9, 89–119 (1822)

Gegenbaur C (1870) Grundzüge der vergleichenden Anatomie. Zweite. umgearbeitete Auflage. Wilhelm Engelmann, Leipzig, 892 p

Gonzalez P, Uhlinger KR, Lowe CJ (2017) The adult body plan of indirect developing hemichordates develops by adding a Hox-patterned trunk to an anterior larval territory. Curr Biol 27:87–95

Grobben K (1908) Die systematische einteilung des tierreiches. Verh Zool Bot Ges Wien 58:491–511

Harmer SF (1887) Appendix to Report on *Cephalodiscus dodecalophus*. In C. Wyville Thompson and J. Murray (eds.), Rep. scient. results voy. H.M.S. Challenger during the years 1873-76. Zool. 20(52):39–47

Holland ND, Clague DA, Gordon DP, Gebruk A, Pawson DL, Vecchione M (2005) Lophenteropneust' hypothesis refuted by collection and photos of new deep-sea hemichordates. Nature 434:374–376

Holland ND, Holland LZ, Holland PW (2015) Scenarios for the making of vertebrates. Nature 520:450–455

Kowalevsky AO (1866) Anatomie des Balanoglossus delle Chiaje. Mem Acad Imp Sci St Petersbourg 7:1–18

Lankester ER (1877) Notes on the embryology and classification of the animal kingdom: comprising a revision of speculations relative to the origin and significance of the germ-layers. Q J Microsc Sci 17:399–454

Lowe CJ (2008) Molecular genetic insights into deuterostome evolution from the direct-developing hemichordate *Saccoglossus kowalevskii*. Philos Trans R Soc B 363:1569–1578

Lowe CJ, Terasaki M, Wu M, Freeman RM Jr, Runft L, Kwan K, Haigo S, Aronowics J, Lander E, Gruber C, Smith M, Kirshner M, Gerhart J (2006) Dorsoventral patterning in hemichordates: insights into early chordate evolution. PLoS Biol 4(9):e291. https://doi.org/10.1371/journal.pbio.0040291

Lowe CJ, Clarke DN, Medeiros DM, Rokhsar DS, Gerhart J (2015) The deuterostome context of chordate origins. Nature 520:456–465

Maletz J (2014) Hemichordata (Pterobranchia, Enteropneusta) and the fossil record. Palaeogeogr Palaeoclimatol Palaeoecol 398:16–27

McIntosh WC (1882) Preliminary notice of *Cephalodiscus*, a new type allied to Prof. Allman's *Rhabdopleura*, dredged in H M S "Challenger". Ann Mag Nat Hist, (5)10:337–348

Metchnikoff E (1869) Ueber Tornaria. Nachr K Ges. Wiss Univ Göttingen 15:287–292

Metchnikoff E (1870) Untersuchungen über die Metamorphose einiger Seethiere. I. Ueber Tornaria. Z Wiss Zool 20:131–144

Mitchell CE, Melchin MJ, Cameron CB, Maletz J (2013) Phylogenetic analysis reveals that Rhabdopleura is an extant graptolite. Lethaia 46:34–56

Müller JP (1850) Über die Larven und die Metamorphose der Echinodermen. Abh Akad Wiss Berlin, 1848, Physiology, 75–110

Raff RA (2008) Origins of the other metazoan body plans: the evolution of larval forms. Philos Trans R Soc B 363:1473–1479

Sars M (1868) Remarks on the distribution of animal life in the depths of the sea (in Norwegian). *Forh. VidenskSelsk. Krist.*, 1868, pp. 246–75

Sars GO (1872) On some remarkable forms of animal life from the great deep off the Norwegian coast. 1. Christiana Univ Program for the 1st half-year, 1869, Pt 1.

Sars GO (1874) On *Rhabdpleura mirabilis* (M. Sars). Quart J Micr Sci 14:23–44

Satoh N (2016) Chordate origins and evolution. Academic, Boston

Simakov O, Kawashima T, Marlétaz F, Jenkins J, Koyanagi R, Mitros T, Hisata K, Bredeson J, Shoguchi E, Gyoja F, Yue JX, Chen YC, Freeman RM, Sasaki A, Tomoe Hikosaka-Katayama T, Sato A, Fujie M, Baughman KW, Levine J, Gonzalez P, Cameron C, Fritzenwanker JH, Pani AM, Goto H, Kanda M, Arakaki N, Yamasaki S, Qu J, Cree A, Ding Y, Dinh HH, Dugan S, Holder M, Jhangiani SN, Kovar CL, Lee SL, Lewis LR, Morton D, Nazareth LV, Okwuonu G, Santibanez J, Chen R, Richards S, Muzny DM, Gillis A, Peshkin L, Wu M, Tom Humphreys T, Su YH, Putnam NH, Schmutz J, Fujiyama A, Yu JK, Tagawa K, Worley KC, Gibbs RA, Kirschner MW, Lowe CJ, Satoh N, Rokhsar DS, Gerhart J (2015) Hemichordate genomes and deuterostome origins. Nature 527:459–465

Spengel JW (1893) Die Enteropneusten des Golfes von Neapel und der angrenzenden Meeres-Abschnitte. Fauna und Flora des Golfes von Neapel, Monographie, Engelmann, Lipzig, vol 18, pp 1–758

Tagawa K (2016) Hemichordate models. Curr Opin Genet Dev 39:71–78

Tagawa K, Satoh N, Humphreys T (2001) Molecular studies of hemichordate development: a key to understanding the evolution of bilateral animals and chordates. Evol Dev 3:443–454

Willey A (1899) Remarks on some recent works on the protochordata, with a condensed account of some fresh observations on the enteropneusta. Q J Microsc Sci 42:223–244

Evo-Devo Lessons from the Reproductive Division of Labor in Eusocial Hymenoptera

Claire Ramsay, Paul Lasko, and Ehab Abouheif

Contents

Abstract

The reproductive division of labor between highly fecund queens and non-reproductive workers is the hallmark of eusociality. This division of labor is analogous to the germ-soma divide in multicellular organisms, and in this way, the colonies of eusocial species can be conceptualized as "superorganisms." The developmental mechanisms underlying the nonreproductive phenotype of workers are beginning to be identified in eusocial Hymenoptera (ants, bees, and wasps). The reproductive dimorphism between queens and workers can be understood in terms of "reproductive constraints": developmental mechanisms that reduce or eliminate the ability of workers to reproduce. These constraints can be grouped into five types that act at different stages of development and differentially affect reproductive potential, activity, and success. The degree of

C. Ramsay · P. Lasko · E. Abouheif (✉)
Department of Biology, McGill University, Montreal, QC, Canada
e-mail: claire.ramsay@mail.mcgill.ca; paul.lasko@mcgill.ca; ehab.abouheif@mcgill.ca

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_173

queen-worker dimorphism varies considerably among eusocial insect species, allowing us to explore the developmental mechanisms that underlie the evolutionary origin and elaboration of division of labor in superorganisms. Finally, we highlight several open questions about the evolution of reproductive constraints and their relation to the evolution of complexity at the superorganism level.

Keywords

Eusociality · Reproduction · Hymenoptera · Eco-evo-devo · Superorganisms

Introduction: Eusocial Hymenoptera as Superorganisms

The widespread existence of a reproductive queen caste and nonreproductive worker caste in colonies of eusocial insects may, on the face of it, appear an evolutionary oddity. The question of how natural selection could favor evolution of such a reproductive division of labor, with a subgroup of individuals unable to reproduce and thus unable to maximize their own individual fitness, has fuelled the development of sociobiological theory (Hamilton 1964; Wilson 1971). It continues to be a contentious topic, with tension between the multilevel or group selection perspective and the individual-centric perspective of kin selection (Kramer and Meunier 2016). These continued debates could be seen to evince the evolutionary peculiarity of eusociality. However, in this chapter, we wish to argue that evo-devo studies of eusocial insects do not merely shed light on the proximate mechanisms underlying an extreme kind of social adaptation – to the contrary – these studies offer insight into very general aspects of evolution and development of multicellular organisms. Although eusociality has evolved multiple times among animals, it has evolved most frequently within the hymenopteran insects (the ants, bees and wasps). This chapter will focus on eusociality in the Hymenoptera, a remarkably diverse group that has been studied from an evo-devo perspective.

The "superorganism" concept, proposed by William Morton Wheeler (Wheeler 1911), allows us to draw general eco-evo-devo principles from the studies of eusocial insects. It proposes that the eusocial insect colony can be conceived as an integrated higher-level individual analogous to the multicellular organism. In the same way that individual cells cooperate to sustain and reproduce a multicellular organism, individual insects in a eusocial colony cooperate to sustain and reproduce a superorganism; namely, the colony. Therefore, the integration of free-living unicellular individuals to form a higher-level multicellular individual, or the integration of solitary individuals to form a higher-level eusocial colony or superorganism, represents "major evolutionary transitions" in individuality (Szathmáry and Maynard Smith 1995). Reproductive division of labor is central to both of these major transitions: the existence of nonreproductive castes that support the reproductive individual(s) in the colony is much like the distinction between the somatic and germline cells, where somatic cells support the production of offspring from germline cells. Viewed this way, the division of labor between reproductive and nonreproductive individuals in a eusocial insect colony is not an oddity but rather reflects a general feature of multicellular life.

Furthermore, just as there is variation in the nature of the germline-soma divide among multicellular organisms, there is also great diversity in the manner and degree of reproductive division of labor among ant, bee, and wasp colonies. Colonies are composed of females, with males typically being produced only when it is time for a new colony to be founded. They are generally characterized by some degree of differentiation into reproductive and nonreproductive females, but there is much variation in the degree of worker infertility and ability to take on reproductive functions. At one end of the spectrum, inhibition of reproduction is largely behavioral, and at the other, developmental mechanisms cause workers to completely fail to develop ovaries. Interrogating the eco-evo-devo mechanisms behind this variation in the reproductive division of labor provides a tractable model to study the origins and elaboration of (super)organismal complexity.

Nutrition and Behavior: Key Factors Underlying the Origins of Reproductive Division of Labor

There are many species in which there is no apparent morphological differentiation between reproductive and nonreproductive females, allowing us to explore how the reproductive dimorphism between queens and workers may have originated. In these species, reproductive individuals establish themselves through behavioral dominance, rather than through an evolved developmental switch that determines queen- or worker-caste fate during embryonic or larval development (Wilson 2008). The paper wasps, or Polistinae, are thought to reflect the ancestral condition that led to the evolution of eusociality, where reproductive division of labor is determined by behavioral dominance hierarchies (Caniglia 2015). In the paper wasp *Ropalida marginata*, nutrition appears to play a role in biasing reproductive dominance. Individual adult wasps vary significantly in their degree and timing of ovary activation, and this appears to correlate with their size, which in turn is a function of the amount of nutrition received at the larval stage (Shukla et al. 2013). It is thought that existing nutrition-responsive pathways known to regulate insect reproduction, such as juvenile hormone (JH) and insulin signaling pathways, may have been co-opted to facilitate the origin and elaboration of queen-worker dimorphism in eusocial Hymenoptera (Wheeler 1996). In line with this, larval nutrition and JH titer are important determinants of caste in species with distinct queen and worker castes (Nijhout and Wheeler 1982). Therefore, differential nutrition coupled to behavioral dominance hierarchies is likely to be a key factor in facilitating the origin of the reproductive division of labor in ants, bees, and wasps.

"Reproductive Constraints" Are Developmental Mechanisms That Facilitated the Elaboration of Reproductive Division of Labor

Elaboration of the reproductive division of labor from behavioral hierarchies to morphologically distinct queen and worker castes has been termed "the point of no return" in the evolution of eusociality (Wilson 1971): It is thought to be the point

at which the transition to eusocial living is irreversible. This reproductive dimorphism between queens and workers represents a more distinct germline-soma divide within the colony. The dimorphism is polyphenic, meaning that an egg laid by the queen has the potential to develop into either a queen or a worker and is determined by an environmentally sensitive switch either during embryonic or larval development. If the individual is determined to develop into a worker, then it will develop one or several "reproductive constraints."

"Reproductive constraints" (RCs) are developmental mechanisms that reduce or eliminate the ability of workers to reproduce and are a useful framework for interrogating the developmental bases of queen-worker reproductive dimorphism. Khila and Abouheif (2010) categorized RCs into five different types (Fig. 1a). Here, we will discuss the developmental mechanisms underlying each type of RC and explore its relation to colony-level selection across the eusocial Hymenoptera.

Complete Loss of Ovaries

The most certain way to entirely remove any prospect of worker reproduction is through the complete loss of worker ovaries. However, this is rare among eusocial Hymenoptera. Among the roughly 300 described ant genera, complete worker ovary loss has evolved in only nine (Khila and Abouheif 2010). In *Monomorium emersoni*, for example, ovary development is prevented by degeneration of all the germ cells in the developing worker-fated embryo (Khila and Abouheif 2010). In bees too, complete ovary loss is not widespread. However, one instance of a bee with an entirely infertile worker caste is the stingless bee *Frieseomelitta varia*. Here, the developmental mechanism that leads to ovary loss is entirely different. Worker ovaries develop up until the beginning of pupation; however, during pupation large-scale programmed cell death commences, and by adulthood the ovarian tissues have degenerated into an "amorphic cellular mass" entirely incapable of reproductive function (Boleli et al. 1999).

Spermatheca Loss

Hymenoptera in general have a haplodiploid sex-determination system in which unfertilized haploid eggs develop into males, and fertilized diploid eggs develop into females. Because of this, the ability of workers to mate will affect their ability to produce females, which are potential new queens. Essential for a female to mate is the sperm storage organ, the spermatheca (Fig. 2), and there is reduction or loss of this organ among workers of honeybees and many ant species (Gotoh et al. 2013, 2016).

Within ants, the degree of spermatheca development varies between species, ranging from being fully developed, to a partially-developed vestige, to completely absent – and the degree of loss correlates with the overall degree of queen-worker dimorphism (Gotoh et al. 2016). Within the Ponerine clade of ants, reproductive dimorphism is typically more limited, and workers either have fully developed or

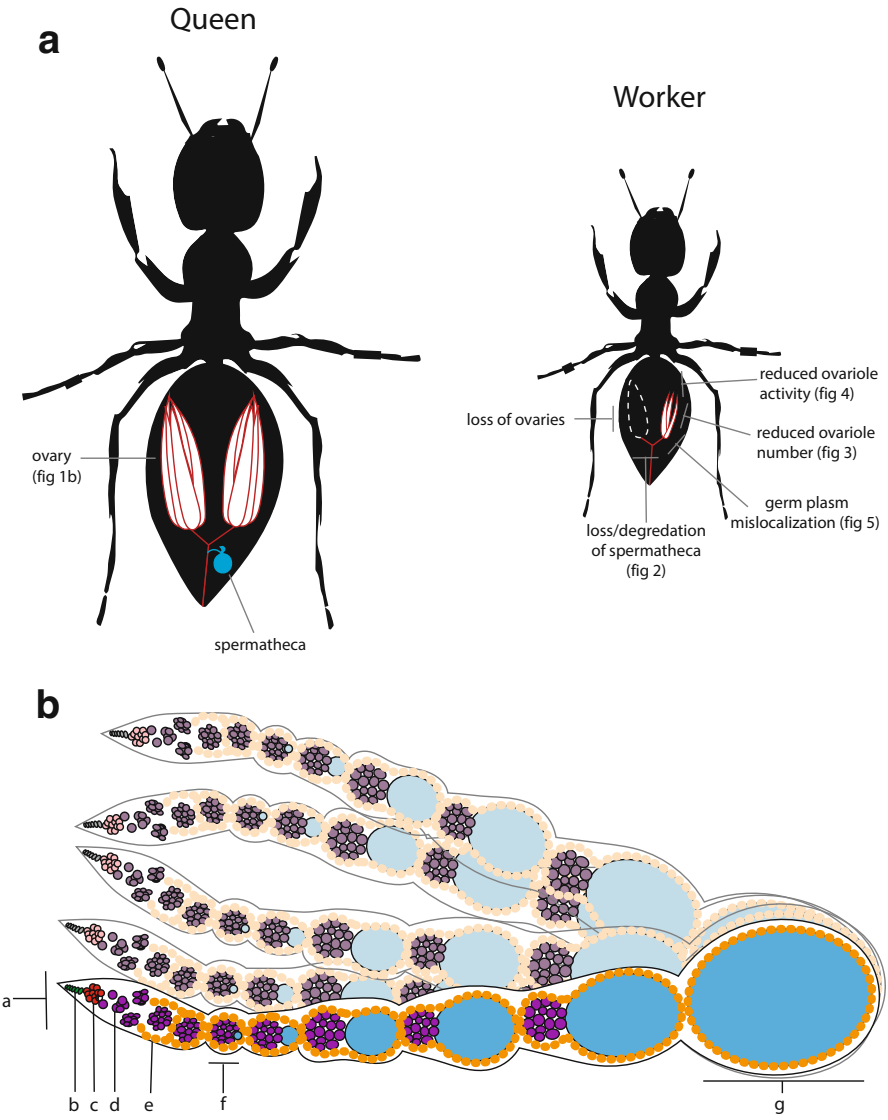


Fig. 1 (**a**) Reproductive anatomy of a hymenopteran queen with large ovaries and spermatheca, and worker with labels indicating the reproductive constraints that can act on reproductive development and oogenesis. (**b**) General anatomy of a polytrophic meroistic ovary, a type of ovary common to hymenopterans and several other insect orders (including the dipteran *Drosophila melanogaster*). A single ovariole within the ovary is highlighted. At the base of the ovariole is the germarium; this region contains various important cell types, including the terminal filament cells and germline stem cells. The germline stem cells differentiate into cystoblast cells, which proliferate to form multicellular cysts. Follicle cells encapsulate the cyst, forming an egg chamber. One of the cells within the cyst is specified as the oocyte, while the others become supportive nurse cells. The continuous production of such egg chambers within the germarium means each egg chamber is pushed posteriorly (toward the oviduct) over time as the oocyte grows and matures. This

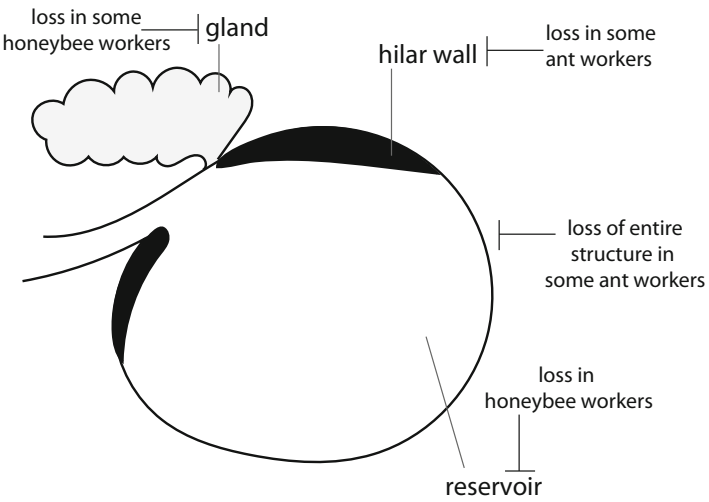


Fig. 2 Spermatheca anatomy, with labels indicating parts of the organ that can be lost in ant and bee workers

only slightly altered spermatheca. In many Ponerine species, workers are capable of mating (becoming gamergates) and producing female offspring (Villet et al. 1991). Gobin et al. (2007) surveyed a number of these Ponerine species and found that the presence of a fully developed, i.e., queen-like, sperm reservoir in workers strictly correlates with the ability of that species to produce gamergates. In species where workers cannot mate, there is degeneration of the epithelium of the sperm reservoir in workers: A thickened region of mitochondria-rich epithelium, called the hilar wall, is converted into a thin layer of squamous cells. This presumably prevents storage of sperm in the reservoir, preventing successful worker mating.

In many other ant species, particularly within the Myrmecine and Formicine clades (Gotoh et al. 2016), spermatheca development is interrupted early and the organ is entirely absent in adult workers. Honeybee workers, on the other hand, do not entirely lack spermatheca (Gotoh et al. 2013). Like many Ponerines, they retain residual structures: However, the points in spermatheca development that are interrupted differ. Rather than degeneration of the reservoir wall, workers of the honeybee species *Apis mellifera* and *Apis cerana* lack the reservoir completely. Most workers also lack the gland associated with the spermatheca. Both the ant and honeybee types of spermatheca degeneration have the same end result in terms of

Fig. 1 (continued) illustration of the cellular organization within an ovariole is based on the well-studied *D. melanogaster* ovary. Evidence suggests that this cellular organization is conserved in ants (Khila and Abouheif 2008), though there is some contention over the existence and position of the germline stem cell population in honeybee ovarioles. (Tanaka and Hartfelder 2004; Peter Dearden, personal correspondence)

loss of the mating ability of workers, indicating that there are multiple developmental processes that have been tinkered with to regulate this RC.

Reduced Ovariole Number

The ovaries of most insects are composed of modular units called ovarioles (Fig. 1b). As each ovariole is an independent oocyte "production line," the total number of ovarioles within the ovaries heavily determines an individual's reproductive capacity (Klepsatel et al. 2013). Ovariole number can differ dramatically between queen and worker ovaries – for instance, honeybee queens can have up to 200 ovarioles per ovary, while worker ovaries can harbor as few as two (Hartfelder et al. 2017). Similar disparities are seen in ant species with strong queen-worker reproductive dimorphism (Khila and Abouheif 2010). In *Drosophila melanogaster* (Sarikaya et al. 2012), honeybees (Hartfelder et al. 2017), and presumably in ants, ovariole number is specified during larval development. In the developing larval ovaries, a population of somatic cells arrange into stacks called "terminal filaments," each of which will develop into a single ovariole (Fig. 3). Thus, following the specification of terminal filament number in the larva, ovariole number is fixed throughout pupation and adulthood.

The developmental mechanisms underlying worker ovariole number are relatively well studied in the honeybee. The larval gonads of worker- and queen-fated larvae are indistinguishable until the fifth instar of larval development. At this stage, apoptosis is induced in the worker ovaries, destroying more than 90% of the terminal filaments (Hartfelder et al. 2017). This caste-specific apoptosis is downstream of JH signaling: Exogenous application of the hormone to worker larvae inhibits terminal filament cell death, indicating that it is the higher JH titer in queens that inhibits apoptosis of the larval ovarioles (Schmidt Capella and Hartfelder 1998). The social regulation of this RC has been demonstrated by orphaning experiments: The earlier

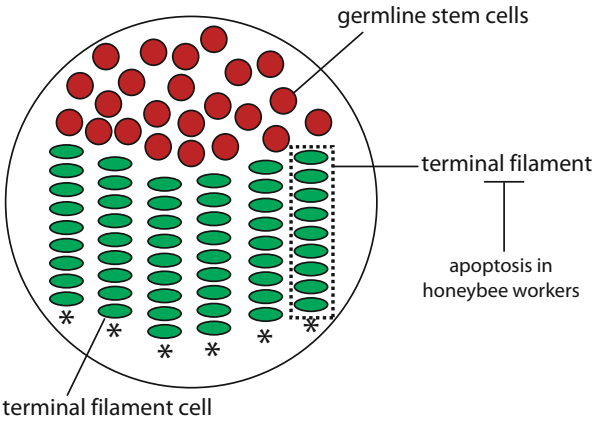


Fig. 3 Late-stage larval ovary, containing germline stem cells, and terminal filaments composed of terminal filament cells. Each terminal filament (indicated with an asterisk) will develop into one ovariole. The number of terminal filaments is known to be reduced in honeybee worker larval ovaries

larvae are removed from the presence of the queen, the more ovarioles they develop (Kuszewska and Woyciechowski 2015).

In ants, the basis of ovariole number determination in workers is unknown. For instance, in the ant *Messor pergandei*, there is some evidence that a subset of the germline-fated cells in the late gastrula degenerate (Khila and Abouheif 2010). Whether this results in the reduced ovariole number of adult workers, or whether there are also mechanisms that come into play during larval ovary development, is an open question. Another set of open questions regards the developmental pathways involved in the specification of caste-specific ovariole number. Recently, the protein network regulating ovariole number in *D. melanogaster* has been constructed (Kumar et al. 2019) which indicates a number of candidate pathways for investigation in eusocial Hymenoptera.

Reduced Ovariole Activity

A common RC is a reduced ovariole activity in workers relative to queens (Fig. 4). This manifests as shorter ovarioles due to a lower number of developing egg chambers per ovariole, stemming from a reduced rate of production of egg chambers in the germarium at the base of the ovaries (Khila and Abouheif 2010).

A proposed mechanism underlying this is reduced proliferation of the GSC pool in the germarium of worker ovarioles, which means a reduced rate of production of egg chambers (Khila and Abouheif 2010). GSC proliferation is known to be plastically responsive to insulin signaling in *D. melanogaster* (Hsu and Drummond-Barbosa 2009), making this a potential candidate pathway for the inhibition of worker ovaries. In line with this, *tor*, a component of the insulin-signaling pathway, is expressed in the very short ovarioles of workers of the ant *Aphaenogaster treate* (Khila and Abouheif 2010).

In addition, the reduced ovariole activity of workers is plastic in both ants and bees: When workers are removed from the presence of the queen, over time they are able to activate their ovaries to some extent. In their study on the Notch pathway in honeybee ovaries, Duncan et al. (2016) showed that when workers are removed from the presence of the queen, the pathway is no longer active in the germarium and oogenesis can proceed. By pharmacological inhibition and analysis of gene and

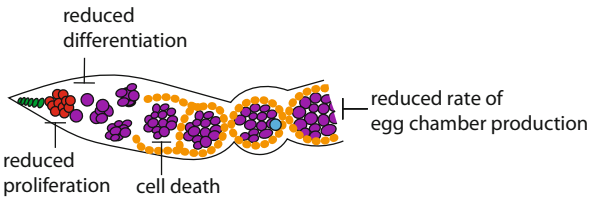


Fig. 4 The germarium at the base of the ovariole, with labels indicating the steps in early oogenesis that may be interfered with in workers to result in a reduced rate of oogenesis and thus a shorter ovariole

protein expression, Notch signaling in the germarium has been implicated as a repressor of oogenesis in worker ovaries (Duncan et al. 2016). The authors suggest that this pathway might act by reducing differentiation of germ cells. There is also evidence for programmed cell death early in oogenesis as a mechanism of worker ovary inhibition in honeybees (Tanaka and Hartfelder 2004; Ronai et al. 2015). It is unclear whether cell death is regulated by Notch signaling, or rather represents an additional means of inactivating ovaries, as well as whether this relates to a reduced rate of GSC proliferation.

Another poorly understood aspect of this RC regards the fact that though ovary activity is clearly regulated in part by a plastic response to queen presence, even in the absence of the queen worker ovarioles do not seem able to reach levels of activity comparable to the queen. This is illustrated most strongly in species such as the ponerine ant *Harpegnathos saltator* (Peeters and Holldobler 1995), which has little queen-worker reproductive dimorphism: Workers even retain full spermatheca and can become gamergates. In the presence of the queen, worker ovaries are largely inactive – however, even when the queen is lost and gamergates are able to take over reproduction for the colony, multiple gamergates are required to achieve a reproductive output equivalent to that of a queen (Peeters et al. 2000). This indicates that there must be some intrinsic constraint to worker ovariole activity beyond the plastic inhibition caused by queen presence.

Oocyte Axis Specification Defects

The latest-acting determinant of an individual's reproductive capacity is the viability of the oocytes they produce. In ants, a mechanism that renders maturing oocytes inviable has been identified (Khila and Abouheif 2008), highlighting that queen-worker reproductive dimorphism can go beyond anatomically visible differences in ovary size. This mechanism involves the mislocalization of a mRNA- and protein-rich cytoplasmic body, the germplasm, from the posterior pole of the developing oocyte while in the ovaries (Fig. 5). In ants, as in *D. melanogaster*, the proper localization of the germ plasm in the oocyte is essential for proper embryonic anterior-posterior axis formation (Ephrussi et al. 1991; Khila and Abouheif 2008). As such, oocytes with mislocalized germ plasm cannot support normal embryonic development.

Khila and Abouheif (2008) investigated a number of ant species and found widespread occurrence of this mechanism in derived ant species past the "point of no return." However, the proportion of oocytes that have germ plasm mislocalization varies considerably between species, suggesting that it is an RC that is selected for under certain contexts. This kind of RC is potentially beneficial in that it allows the ovary to perform alternative functions at the colony level not related to reproduction. A clear example of this is the production of trophic eggs. In many species of ant (Khila and Abouheif 2010) and in certain stingless bees (Hartfelder et al. 2017), worker ovaries can produce a morphologically distinct type of yolk-filled egg that is eaten by other members of the colony. Germ plasm mislocalization allows ovary

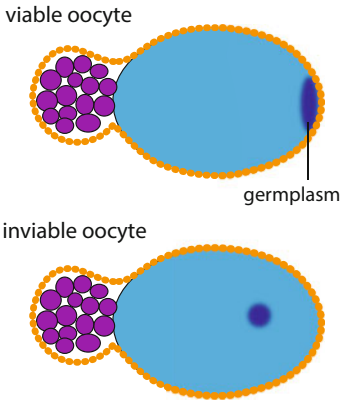


Fig. 5 Egg chamber containing a viable oocyte with germ plasm correctly localized to the posterior pole, versus an egg chamber containing an inviable oocyte in which germplasm has been mislocalized

activity to be maintained in workers for alternative functions such as trophic egg production, while preventing successful worker reproduction. There are numerous open questions regarding the mechanisms underlying germ plasm mislocalization and the production of trophic eggs, and more broadly whether analogous RCs exist in eusocial wasps or bees.

RCs in the Context of Selection

As outlined, there are a number of steps in worker-ovary development and oogenesis that can be interrupted. These RCs exist in different combinations, and to different degrees, among species. Is there any rhyme or reason to RC evolution?

At the level of the individual worker, RCs have been interpreted to be the result of neutral degradation (Bourke 2011). However, queens and workers are derived from the same genome, which retains the full capacity for reproduction in queens. Given this, worker RCs might be more accurately viewed as superorganism-level adaptations that balance the trade-off between minimizing the potential for worker reproduction and allow for nonreproductive colony-level functions that worker ovaries perform. It is helpful to consider that different RCs have different effects on reproduction, regulating it at three hierarchical levels: reproductive potential, reproductive activity, and reproductive success. Reproductive potential refers to the capacity of an individual to reproduce and is determined during development. Reproductive activity is the actual rate of reproduction during adulthood and is regulated by environmental and physiological factors. Finally, reproductive success is determined by whether reproductive attempts actually result in viable progeny.

Considering RCs in this framework helps us understand why certain RCs might be favored under different conditions. Complete ovary loss entirely removes any potential for reproduction, while reducing ovariole number reduces the number of modular units of oocyte production thus reducing reproductive potential. Spermatheca degeneration or loss represents the loss of potential to produce female offspring. Meanwhile, reproductive activity is reflected in the rate of egg production

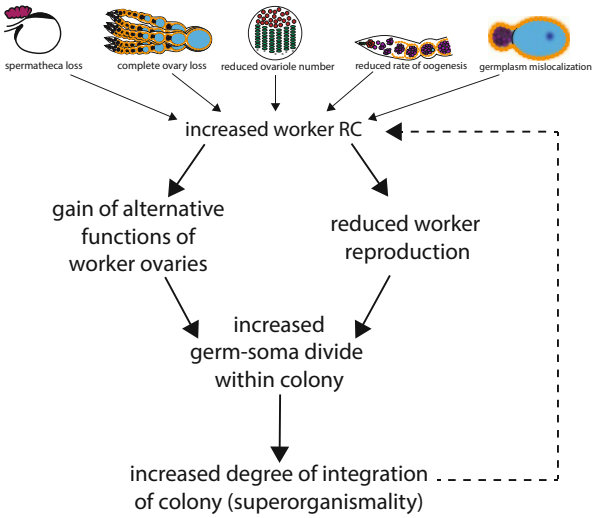


Fig. 6 Summary of how the degree of reproductive constraint in workers (determined by degree and number of RCs in worker development) relates to degree of germline-soma divide within the superorganism, via effects on the extents to which workers can reproduce and that worker ovaries are co-opted for nonreproductive functions. The degree of germline-soma divide might relate to the degree of integration of a eusocial colony into a superorganism (increased "superorganismality"). In turn, increased integration at the colony level might feed back to increase selection for worker RCs, as colony-level selection becomes increasingly powerful relative to individual-level selection

in each ovariole, which is to some degree dependent on the social environment of a worker. Finally, reproductive success is modulated in ants by regulation of germplasm localization in worker-produced oocytes. All of these increase the germline-soma divide to different degrees in different species, leading to different degrees of superorganismality (Fig. 6).

Why, given that it completely removes the potential of worker reproduction, is complete ovary loss so rare? The answer could be that colony-level selection favors the retention of worker ovaries so they can serve nonreproductive functions. One is the aforementioned trophic egg production, which requires retention of a degree of reproductive potential and activity but reduced reproductive success. Another potential function is that ovary-derived signals might be regulators of worker physiology or behavior. Nonreproductive ovaries may still participate in important signaling events between cells and organs. Across a broad sample of ant species, worker subcastes that specialize in brood care tend to have higher ovary activity than subcastes that specialize in foraging (Pamminger and Hughes 2016). In the honeybee too, ovary activity decreases as individual workers transition, with age, from brood care to foraging (Robinson 1992). This suggests that ovary status could be linked to reproduction-associated behavioral syndromes. Another reason for ovary retention could be that maintaining some degree of worker reproductive potential makes the colony more robust to queen loss. Although this has not been directly tested, there is evidence that in some species both queen- and worker-laid males are equally fertile and so might be able to inseminate another queen and found a new colony (Giehr et al. 2020).

Key Lessons and Future Questions

Eusocial Hymenoptera provide tractable systems for investigating elaboration of complexity in biological systems. Through evo-devo studies and perspectives on worker reproductive constraints, we gain insight into how the division of labor originates and is elaborated on. For instance, it seems that many of the mechanisms regulating the division of labor involve developmental processes that are ancestrally plastic, and that have evolved into more or less discrete binary polyphenisms. Another common mechanism underlying RCs seems to be programmed cell death of reproductive tissues, allowing totipotency at the beginning of development and then caste-specific loss of tissues once queen versus worker fate is established. We also see that, much like in multicellular organisms, the degree of reproductive constraint in workers varies, indicating that the evolution of a more distinct germ-soma divide might be important in the elaboration of a (super)organism, following from a major evolutionary transition (Fig. 6). There are some preliminary studies suggesting that the degree of queen-worker reproductive dimorphism is associated with colony size and number of worker subcastes (Villet et al. 1991; Bonner 1993). More evo-devo work is required in order to test these ideas. We know very little about the developmental genetic basis of RCs in general, or even which combination of RCs is present in many species. An important aspect of this work will be to explore how interconnected or modular different RCs are: The modulation of the activity of a given pathway might affect multiple aspects of reproductive development, or each RC may be regulated relatively independently. This will provide insight into how RCs evolve, and into the various combinations we observe in different species. By investigating the developmental and molecular basis of RCs across the phylogeny of ants, bees, and wasps, and viewing these RCs in ecological and evolutionary context, we can uncover more about the relation of the reproductive division of labor to evolution at the level of the superorganism.

Cross-References

- ▶ Devo-Evo of Cell Types
- ▶ Eco-Evo-Devo
- ▶ Evo-Devo Lessons Learned from Honeybees

References

Boleli IC, Paulino-Simões ZL, Bitondi MMG (1999) Cell death in ovarioles causes permanent sterility in Frieseomelitta vari worker bees. J Morphol 242:271–282

Bonner JT (1993) Dividing the labour in cells and societies. Curr Sci 64(7):459–466

Bourke AFG (2011) In: Harvey PH, May RM, Godfray CH, Dunne JA (eds) Principles of social evolution. Oxford series in ecology and evolution. Oxford University Press, Oxford

Caniglia G (2015) Understanding societies form inside the organisms. Leo Pardi's work on social dominance in Polistes wasps (1937–1952). J Hist Biol 48(3):455–486

Duncan EJ, Hyink O, Dearden PK (2016) Notch signalling mediates reproductive constraint in the adult worker honeybee. Nat Commun 7:12427
Ephrussi A, Dickinson LK, Lehmann R (1991) Oskar organises the germ plasm and directs localisation of the posterior determinant nanos. Cell 66:37–50
Giehr J et al (2020) Body size and sperm quality in queen- and worker-produced ant males. J Evol Biol 33:842–849
Gobin B et al (2007) Degeneration of sperm reservoir and the loss of mating ability in worker ants. Naturwissenschaften 95:1041–1048
Gotoh A, Ito F, Billen J (2013) Vestigial spermatheca morphology in honeybee workers, *Apis cerana* and *Apis mellifera*, from Japan. Apidologie 44:133–143
Gotoh A et al (2016) Degeneration patterns in the worker spermatheca during morphogenesis in ants. Evol Dev 18(2):96–104
Hamilton WD (1964) The genetical evolution of social behaviour. J Theor Biol 7:1–16
Hartfelder K et al (2017) The ovary and its genes – developmental processes underlying the establishment and function of a highly divergent reproductive system in the female castes of the honey bee, *Apis mellifera*. Apidologie 49:49–70
Hsu H, Drummond-Barbosa D (2009) Insulin levels control female germline stem cell maintenance via the niche in Drosophila. PNAS 106(4):1117–1121
Khila A, Abouheif E (2008) Reproductive constraint is a developmental mechanism that maintains social harmony in advanced ant societies. PNAS 105(46):17884–17889
Khila A, Abouheif E (2010) Evaluating the role of reproductive constraints in ant social evolution. Philos Trans R Soc Lond Ser B Biol Sci 365(1540):617–630
Klepsatel P et al (2013) Reproductive and post-reproductive life history of wild-caught *Drosophila melanogaster* under laboratory conditions. J Evol Biol 26:1508–1520
Kramer J, Meunier J (2016) Kin and multilevel selection in social evolution: a never-ending controversy? F1000 Res 5:776
Kumar T, Blondel L, Extavour CG (2019) Topology-driven analysis of protein-protein interaction networks detects functional genetic modules regulating reproductive capacity. bioRxiv. https://doi.org/10.1101/852897
Kuszewska K, Woyciechowski M (2015) Age at which larvae are orphaned determines their development into typical or rebel workers in the honeybee (*Apis mellifera* L.). PLoS One 10(4):e0123404
Nijhout HF, Wheeler DE (1982) Juvenile hormone and the physiological basis of insect polymorphisms. Q Rev Biol 57:109–133
Pamminger T, Hughes WO (2016) Testing the reproductive groundplan hypothesis in ants. Evolution 71(1):153–159
Peeters C, Holldobler B (1995) Reproductive cooperation between queens and their mated workers: the complex life history of an ant with a valuable nest. PNAS 92(24):10977–10979
Peeters C, Liebig L, Holldobler B (2000) Sexual reproduction by both queens and workers in the ponerine ant *Harpegnathos saltator*. Insect Soc 47:325–332
Robinson GE (1992) Regulation of division of labor in insect societies. Annu Rev Entemol 37:637–665
Ronai I et al (2015) Regulation of oogenesis in honey bee workers via programmed cell death. J Insect Physiol 81:36–41
Sarikaya DP et al (2012) The roles of cell size and cell number in determining ovariole number in *Drosophila*. Dev Biol 363:279–289
Schmidt Capella IC, Hartfelder K (1998) Juvenile hormone effect on DNA synthesis and apoptosis in caste-specific differentiation of the larval honey bee ovary. J Insect Physiol 44(5–6):385–391
Shukla S, Chandran S, Gadagkar R (2013) Ovarian developmental variation in the primitively eusocial wasp *Ropalidia marginata* suggests a gateway to worker ontogeny and the evolution of sociality. J Exp Biol 216:181–187
Szathmáry E, Maynard Smith J (1995) The major evolutionary transitions. Nature 374:227–232
Tanaka ED, Hartfelder K (2004) The initial stages of oogenesis and their relation to differential fertility in the honey bee castes. Arthropod Struct Dev 33(4):431–442

Villet MH, Crewe RM, Duncan FD (1991) Evolutionary trends in the reproductive biology of ponerine ants. J Nat Hist 25(6):1603–1610
Wheeler WM (1911) The ant-colony as an organism. J Morphol 22(2):307–325
Wheeler DE (1996) The role of nourishment in oogenesis. Annu Rev Entemol 41:407–431
Wilson EO (1971) The insect societies. Harvard University Press, Cambridge, MA
Wilson EO (2008) One giant leap: how insects achieved altruism and colonial life. Bioscience 58(1):17–25

Evo-Devo Lessons Learned from Honeybees

Peter K. Dearden

Contents

Abstract

Honeybees (*Apis mellifera*) are hymenopteran insects of importance both economically and scientifically. Honeybees share much of the basic biology of well-studied insect models, such as *Drosophila* and *Tribolium*, but their sex determination and embryogenesis differ in important ways, which provide some understanding of the way early-acting developmental pathways evolve. Honeybees also display remarkable polyphenisms, critical to their biology. Understanding how these environmentally induced shifts in developmental trajectory occur is critical to our understanding of the evolution of environmental influences on developmental processes.

Keywords

Evolution and development · Honeybee · Caste development · Polyphenisms

P. K. Dearden (✉)
Laboratory for Evolution and Development, Genetics Otago and Department of Biochemistry, University of Otago, Dunedin, New Zealand
e-mail: peter.dearden@otago.ac.nz

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_178

Introduction

Honeybees are remarkable animals of vital importance to human agriculture. Our first references to bees are in cave paintings in Cuevas de la Araña (Arana Caves or Spider Caves) in Valencia, Eastern Spain (Kritsky 2017). These images depict humans robbing beehives for honey from tree-top hives and are dated to the *Epipaleolithic* Period. While the Sumerian Civilisation (c.4500 – c.1900 BC) is recorded as the world's first beekeepers (maintaining wild free-formed hives), manmade bee hives from Ancient Greece have been found (now in the Athens Museum), indicating that by this time, at least, honeybees and humans had begun their long mutualism (Kritsky 2017). Aristotle in his Ἀναλυτικὰ Ὕστερα *(or Prior* and *Posterior Analytics*) performed the first scientific study of honeybees and their behavior, opening the way for future studies of this beloved and beneficial insect.

Honeybees are a critical part of our agricultural infrastructure. Honeybees are valued for producing a number of key products, including honey, wax, propolis, and venom. More importantly honeybee's role in pollination is critical to agricultural production in many countries worth $153 billion US annually (Gallai et al. 2009). Honeybees are not the only pollinators to carry out this vital work, but they are the most manageable and crucial source of pollination capacity.

Honeybees and beekeeping have come under some scrutiny in the past few years, as warnings have been sounded as to a general decline in pollinating insects and increases in diseases that have cause the loss of many bee colonies (Meixner and Le Conte 2016). The loss of this pollination capacity raises questions about the sustainability of modern agricultural systems. Loss of honeybees, in particular, is put down to a combination of an invasive mite (*Varroa destructor*) and the viruses it vectors (e.g., deformed wing virus among many others), changes in land use, and the wide spread use of insecticides, especially neonicotinoids (Meixner and Le Conte 2016). It is not clear at this point which of these is the key issue, but much research is focused on understanding the effect of these factors on honeybees.

Honeybee Biology

Honeybees belong to the Apidae group of the hymenoptera, an order of holometabolous insects (Fig. 1). Phylogenetic studies of the holometabolous insects indicate that the Hymenoptera are the most basally branching of this group, making bees an interesting counterpoint to the more studied *Drosophila* and *Tribolium* (both 325 million years diverged (Hedges et al. 2015). *Nasonia*, a tractable parasitic hymenopteran species that diverged some 200 mya (Hedges et al. 2015) from honeybees, provides an important comparison with honeybee development.

As holometabolous insects, honeybees undergo a larval stage of development, accessible within the hive and pupate in honeycomb in capped cells.

Honeybees are eusocial, providing a key model for our understanding of the evolution of eusocial systems. Honeybee females are split into two castes, workers and queens, with queens (1 per hive) carrying out roles in reproduction, and workers

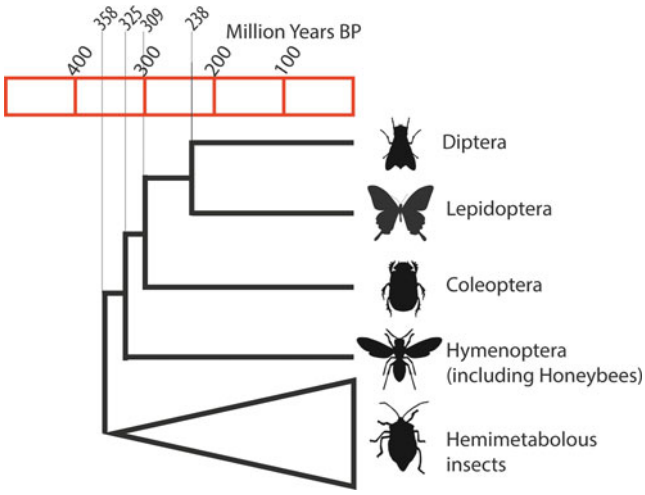


Fig. 1 Phylogenetic relationships between honeybees (Hymenoptera) and other insects. Figures indicate estimated divergence times from Hedges et al. (2015)

roles in caring for offspring, maintaining and guarding the hive, and foraging. Honeybee males (drones) have little to do but mate with the queen, though successful mating is fatal, and remaining drones are killed by workers in autumn. Caste development is triggered by larval feeding, with queen destined larvae being fed large quantities of a rich food source, royal jelly (Tautz 2008).

Honeybees transmit information to each other by means of a symbolic language, the waggle dance, which particularly allows them to direct foragers to food sources (Tautz 2008).

Honeybees display haplodiploid sex determination, with males being haploid and females diploid. Males derive from unfertilized eggs, while females from fertilized ones (reviewed in Gempe and Beye 2011).

Bee Genome and Tools

The honeybee genome was one of the last to be sequenced by Sanger sequencing methods (The Honey Bee Genome Sequencing Consortium 2006), and it has been updated and improved in the past few years (Elsik et al. 2014). The genome is approx. 236 million base pairs, has been super scaffolded into the 16 chromosomes of the honeybee, and is well annotated. Many studies using transcriptomic technologies have provided good annotations of honeybee genes, providing an excellent resource for honeybee researchers. Honeybee genome data are available from the Hymenopteran genome database (Munoz-Torres et al. 2010).

Tools for manipulating gene expression are available for bees, including transgenesis via transposons (Schulte et al. 2014) or CRISPR/cas9 technologies (Kohno

et al. 2016), as well as embryonic (Beye et al. 2002), larval (Kucharski et al. 2008), and adult (Amdam et al. 2003b) RNA interference. Embryonic staging schemes, using light microscopy (Cridge et al. 2017) and electron microscopy (Fleig and Sander 1986), have been published, and the morphology of honeybee embryos and larvae is well studied.

Sex Determination

Sex determination in honeybees has been well studied due to its importance to honeybee culture. As noted Honeybees are haplodiploid, but haplodiplody in this species is affected by inbreeding, a condition that occurs in beekeeping operations. Inbreeding causes the appearance of diploid drones in the hives, aberrations that are detected as larvae and killed by worker bees (reviewed in Heimpel and de Boer 2008). This phenomenon leads to a loss of brood in a hive. As brood loss is a problem for beekeepers, and efforts to identify the cause behind it quickly identified a genetic locus and a gene, named *complementary sex-determiner (csd) (Beye* et al. 2003*)*, as responsible for the effect. Homozygosity at this locus is the cause of the brood-loss effect, and the number of alleles at this locus was critical. If an embryo has two different *csd* alleles, then the embryo must be diploid, if it has only one, the embryo develops as a male, whether the single allele is due to haploidy, or homozygosity. Thus, limited diversity of *csd* in a population can lead to homozygosity and diploid embryos developing as males and being killed.

Once cloned it was found that the *csd* gene encodes a splicing factor (Beye et al. 2003) that is required to produce a female-specific splice variant of a related gene *fem* (Hasselmann et al. 2008). When *csd* is heterozygous, *fem* is spliced to its female form; when hemi or homozygous, a male form of *fem* is produced (Hasselmann et al. 2008), generated by *Am-Tra2*, a homolog of *Drosophila* transformer (Nissen et al. 2012). The female *fem* variant feeds back to splice its own transcript into the female form (Gempe et al. 2009) and acts with *Am-Tra2* (Nissen et al. 2012) to produce a female specific splice variant of *Am-dsx* (Gempe et al. 2009), a homolog of the *Drosophila doublesex* gene. In the absence of the female *fem* splice variant, a male specific variant of *Am-dsx* is formed (Gempe et al. 2009). *Am-dsx* encodes a transcription factor, the variants of which appear to trigger male or female development (Gempe et al. 2009).

The *csd* coding sequence has two hypervariable regions in the encoded protein (Beye et al. 2003). It is not clear how different two alleles need to be at the sequence level to act as different alleles within a strain of bees.

The genetic cascade developed in studies of sex-determination in honeybees clearly feeds into the same key evolutionary conserved transcription factor that controls regulation of the genome in a sex specific way in *Drosophila (Gempe and Beye* 2011*)*, but it is clear that the upstream components of the pathway are diverged. This is supported by the similarities between *fem* and *csd*, and their overlapping functions, implying an evolutionary nascence to this early acting part of the cascade. This evolution of the early acting parts of this and other gene expression cascades in

honeybees supports the theoretical view that such gene regulator cascades evolved backwards, with earliest acting components evolving most recently.

Sex determination in honeybees has illuminated the evolvability of genetic cascades, linking new regulators, tuned to novel signals of gender, into ancient pathways that shape sexual characteristics.

Patterning and Development

Embryonic development in Honeybees has produced a good understanding of morphology and changes during development. One key feature of honeybee embryos is that they are long-germ in form, indeed taken by Klaus Sander as the most long-germ insect (Sander 1976).

Studies of gene expression in honeybee embryos were first undertaken by focusing on Hox and segmentation genes (Fleig 1990; Walldorf et al. 1989). With the advent of the entire honeybee genome sequence, it became clear that some genes known to act early in the development of *Drosophila* embryos were missing from the Honeybee genome (Dearden et al. 2006). How Honeybees carry out these processes in the absence of these genes has been a focus of research (Fig. 2).

Terminal patterning is a key example of a patterning process well studied in *Drosophila* that appears to have components missing in honeybees. In *Drosophila*, terminal patterning is controlled by the trunk/torso signaling pathway, the key signaling event being between a ligand, trunk, and a receptor, torso (reviewed in Duncan et al. 2014). Neither of these genes are encoded in the honeybee genome, despite all the rest of the cascade being present. *Tailless* is expressed, as in *Drosophila*, at both termini of the embryo, but torso signaling does not control its expression (Duncan et al. 2013). Posterior *tailless* is deposited maternally, while anterior *tailless* is activated by a maternal transcription factor, *orthodenticle*. *Orthodenticle* has the same role in *Nasonia*, except in this species it activates *tailless* at both termini of the embryo (Lynch et al. 2006). This pathway shows the same pattern as that seen in sex determination, the grafting of a new set of signals onto the start of a cascade that leads to a conserved process.

Orthodenticle, which has a relatively minor role in *Drosophila* development, appears, with another transcription factor, *hunchback*, to be the key gene in anterior patterning in honeybees, replacing, to some extent, the absent *bicoid* gene (Wilson and Dearden 2011). Caudal has a more extensive function at the posterior end of the embryo (Wilson et al. 2009). These genes, despite having different functions to those seen in *Drosophila* or even *Tribolium*, feed forward to a gap gene network, which differs in details to that of other studied insects (Wilson et al. 2009). This then activates a pair-rule cascade that, while having maternal functions as well, seems relative similar to other insects (Wilson and Dearden 2012), and finally a segment polarity system very similar to that in other insects (Dearden et al. 2006).

Most unexpectedly is the generation of honeybee germ cells. In other Hymenoptera, such as *Nasonia*, the inheritance of the oosome (Olesnicky and Desplan 2007), a cytoplasmic organelle, in syncytial embryos, leads to the definition of the germ-

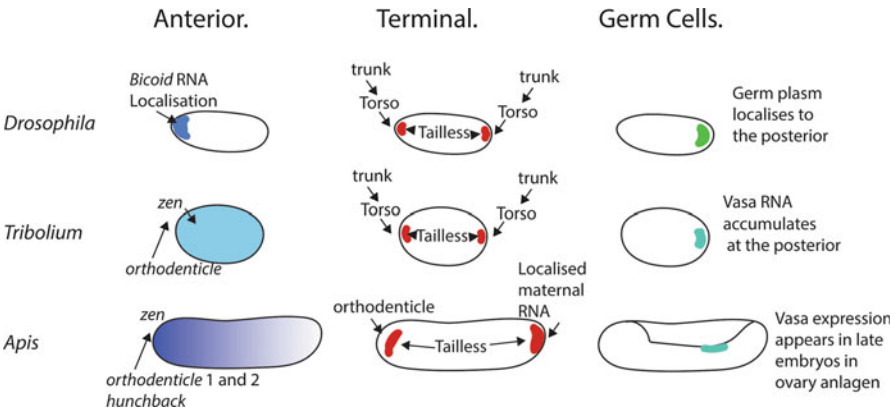


Fig. 2 Differences in early developmental processes between bees and other model insects. During anterior patterning in *Drosophila*, *Bicoid* RNA, localized maternally, is the key anterior determinant. In *Tribolium*, *orthodenticle* and dorso-ventral patterning genes appear to play this role. In Apis, two *orthodenticle* genes and *hunchback* pattern much of the anterior –posterior axis (Wilson and Dearden 2011). During terminal patterning, trunk and torso activate *tailless* at each end of the embryo in *Drosophila* and *Tribolium*, whereas in Apis, *orthodenticle* activates anterior tailless expression, and posterior *tailless* RNA is placed maternally (Wilson and Dearden 2009). During germ cell development, localized germplasm (*Drosophila*) or vasa RNA (*Tribolium*) marks the developing germ anlagen. In *Apis*, germ cells appear to form late in development in the developing ovary (Dearden 2006)

cells, similar to the specification of germ cells by germ plasm in *Drosophila*. In honeybees, no oosome or germ plasm is visible, and markers of germ-cell fate only occur later in development, in cells that develop near the location of the ovaries (Dearden 2006). While it is not yet proved that there is no early specification of germ-cells in this species, it does appear that they form by epigenesis, through some cell signaling process.

Polyphenisms

Honeybees display a number of polyphenisms that have been intensively studied. The principle of these is caste development. In this process, worker bees secret a substance named Royal Jelly, which is then fed to specific larvae, causing them to change their developmental trajectory to produce queens (Tautz 2008). This example of a dietary-driven change in phenotype, which is accessible and manipulable, has provided an opportunity to understand how diet interacts with the genome, how developmental plasticity is encoded in the genome, and how gene regulation can be switched to produce alternative phenotypes.

Larvae destined to develop as queens are fed royal jelly, in amounts that far outweigh the diet fed worker destined larvae. Gene expression studies of developing worker and queen larvae have been carried out, and a number of key pathways identified (de Azevedo and Hartfelder 2008; Mutti et al. 2011; Cameron et al. 2013).

The problem with most of these experiments is the difficulty in testing the function of identified genes and pathways.

Caste development can occur in lab rearing experiments, but such experiments are very difficult, and linking manipulating larvae by gene knock-down or drug treatment adds another layer of difficulty. Few candidate genes from gene expression studies have been tested, though there are identified roles for Target of Rapamycin signaling (TOR) (Mutti et al. 2011), hexamerins (Cameron et al. 2013), and DNA methyl-transferase 3 (DNMT3) (Kucharski et al. 2008b). This last gene is interesting as the honeybee, unlike *Tribolium* and *Drosophila*, has a fully functioning DNA methylation system (Wang et al. 2006), and knocking down DNMT3 leads to a reduction in the ability to make workers implying that de-novo methylation is required to form the evolutionary novel worker caste.

The remarkable results from knocking down DNMT3 imply that DNA methylation might be key in polyphenism control (Kucharski et al. 2008b). The role of methylation in caste development also raised the possibility that other epigenetic processes might be involved. Indeed, royal jelly contains a histone deactylase activity (Spannhoff et al. 2011), and miRNAs (Guo et al. 2013), some of which are plant derived (Zhu et al. 2017), all of which may have some caste development activity.

The consensus has been that multiple components of royal jelly act together in a complex way to alter a wide range of metabolic and developmental processes leading to caste development, this complexity making it difficult to isolate a single factor. It came as somewhat of a surprise when a study was published indicating that one of the proteins in Royal Jelly, Major Royal Jelly Protein 1 (now named Royalactin), was the key component that triggers queen development (Kamakura 2011).

While Royalactin seemed like an important step forward (Kamakura 2011), questions have been raised about the repeatability of the experiment (Buttstedt et al. 2016), and the proposed mechanism (Kucharski et al. 2015). Extensive experiments on the protein and its effects have been unable to replicate Royalactin's key role in caste development (Buttstedt et al. 2016), though the author of the original study states that these experiments are flawed (Kamakura 2016). It is unclear at this time what role Royalactin plays, but the single component model that Royalactin represents is not consistent with previous thought on caste development.

Honeybees biology relies on other polyphenisms, including the repression of worker ovaries and reproduction by the queen bee. Queen bees express a well-characterized pheromone, named Queen Mandibular Pheromone (QMP) (Tautz 2008), that acts on workers to repress their ovaries, among other effects. When the queen is removed, some workers can overcome this repression, active their ovaries, and lay unfertilized eggs, which develop as drones. Ovary activation can be triggered in the lab, by removing workers from the influence of a queen, and synthetic QMP is available, providing a reasonable experimental system to discover mechanisms of ovary repression.

Quantitative trait locus mapping studies of bee colonies, where workers bees escape repression even in the presence of a queen, indicated particular areas of the

bee genome that may contain key parts of the repression mechanism (Oxley et al. 2008). These experiments led researchers to apoptosis as a key mechanism for ovary repression (Ronai et al. 2015), though how this is linked to QMP is not described, nor have manipulative experiments been performed that allow experimental proof that this mechanism is important. A study has also identified Notch cell signaling, an ancient and conserved cell signaling system most often associated with lateral inhibition during neurogenesis, as a key regulator of ovary activation (Duncan et al. 2016). A mix of manipulative experiments (using the feeding of small molecule Notch inhibitors), and imaging, indicates that QMP activates Notch signaling in the germanium region of the ovary and that when QMP is removed Notch signaling is turned off. Blocking Notch signaling makes worker bees resistant to QMP, indicating that Notch is a key pathway responding to QMP in ovary repression (Duncan et al. 2016) (Fig. 3).

Finding Notch signaling as a key factor in repression is interesting as it supports the idea that the novel evolutionary character of repression of the ovary does not necessarily require the evolution of a whole new set of processes and pathways but the re-purposing of an ancient one. It may even be that such conserved pathways are preadapted to be co-opted into roles like this, as the widespread and vital function of Notch signaling in the embryo and larvae reduces the chance that worker bees might evolve resistance to QMP. If the evolution of ovary repression is, as suggested, an

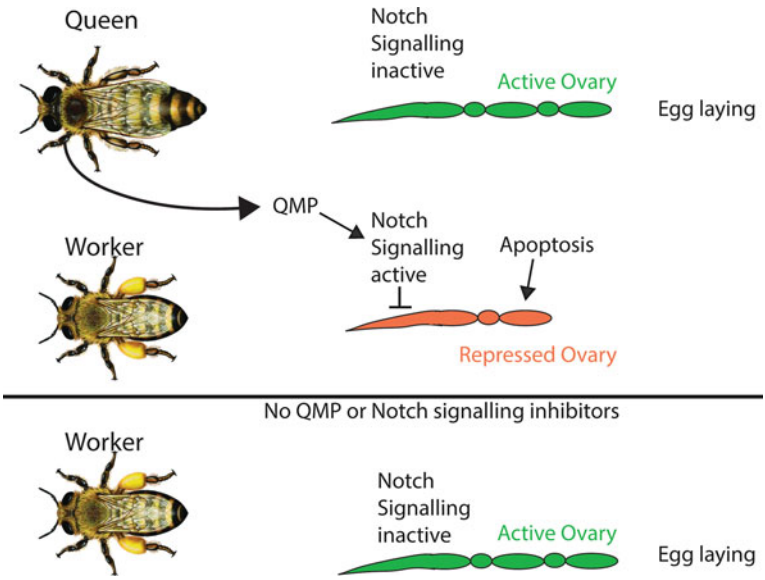


Fig. 3 Queen bees produce Queen Mandibular Pheromone (QMP) which represses worker reproduction. QMP induces Notch cell signaling in the germarium of the ovary, blocking reproduction (Duncan et al. 2016), possibly through apoptosis. In queens, or workers in the absence of QMP (or in the presence of Notch inhibitors), Notch signaling is not active and the ovaries are active

evolutionary arms race, then manipulating Notch signaling may be an evolutionary response to avoid resistance.

Eusociality

Honeybees are the poster child for eusocial species, and as such some research has been undertaken to try to understand the evolution and genetic underpinnings of eusociality. Eusociality is defined as a level of social behavior in a species that includes division of labor into reproducing and nonreproducing groups and overlapping generations. In honeybees, perhaps the caste difference between workers and queens is the most obvious sign of eusociality, but worker bees also display age-related task allocation, with the youngest workers taking on tasks inside the hive, and only the oldest leaving for foraging duties (Tautz 2008). Gene expression and methylation studies have been undertaken on the brains of bees performing different tasks, and a number of candidate genes have been identified. Problematically, none of the technologies used to manipulate gene expression has been able to show that these candidates have a role in these processes.

Perhaps the most compelling is the case of vitellogenin, a phospholipoglycoprotein critical to reproduction due to its role as an egg yolk protein (Amdam et al. 2003a; Amdam et al. 2003b). In workers, however, levels of vitellogenin differ between worker bees, with higher levels correlating with increased longevity, pollen (protein) foraging, and slower progression between worker tasks (Nelson et al. 2007).

Understanding the genetics of eusociality, and its evolution, is challenging. In honeybee, more tools to manipulate gene expression in specific cells and at specific times of development will be required to unravel this complex phenomenon.

Summary and Prospects

Honeybees are an accessible model system for a wide range of remarkable biology, as well as being economically important. While the development of tools and technologies to modify gene expression and perturb biology has occurred, much remains to be learnt about these astounding insects and what they can teach about the evolution of developmental mechanisms.

Cross-References

- ► Convergence
- ► Developmental Innovation and Phenotypic Novelty
- ► Developmental Plasticity and Evolution
- ► Developmental System Drift
- ► Evo-Devo of Social Behavior

References

Amdam GV, Norberg K, Hagen A, Omholt SW (2003a) Social exploitation of vitellogenin. Proc Natl Acad Sci U S A 100(4):1799–1802

Amdam GV, Simões ZL, Guidugli KR, Norberg K, Omholt SW (2003b) Disruption of vitellogenin gene function in adult honeybees by intra-abdominal injection of double-stranded RNA. BMC Biotechnol 3(1):1

Beye M, Hartel S, Hagen A, Hasselmann M, Omholt SW (2002) Specific developmental gene silencing in the honey bee using a homeobox motif. Insect Mol Biol 11(6):527–532

Beye M, Hasselmann M, Fondrk MK, Page RE, Omholt SW (2003) The gene *csd* is the primary signal for sexual development in the honeybee and encodes an SR-type protein. Cell 114(4): 419–429

Buttstedt A, Ihling CH, Pietzsch M, Moritz RF (2016) Royalactin is not a royal making of a queen. Nature 537(7621):E10–E12

Cameron R, Duncan E, Dearden P (2013) Biased gene expression in early honeybee larval development. BMC Genomics 14(1):903

Cridge A, Lovegrove M, Skelly J, Taylor S, Petersen G, Cameron R, Dearden P (2017) The honeybee as a model insect for developmental genetics. Genesis 55(5):e23019

de Azevedo SV, Hartfelder K (2008) The insulin signaling pathway in honey bee (*Apis mellifera*) caste development—differential expression of insulin-like peptides and insulin receptors in queen and worker larvae. J Insect Physiol 54(6):1064–1071

Dearden PK (2006) Germ cell development in the honeybee; vasa and nanos expression. BMC Dev Biol 6(6):1–14

Dearden PK, Wilson MJ, Sablan L, Osborne PW, Havler M, McNaughton E, Kimura K, Milshina NV, Hasselmann M, Gemp T, Schioett M, Brown SJ, Elsik CG, Holland PW, Kadowaki T, Beye M (2006) Patterns of conservation and change in honey bee developmental genes. Genome Res 16:1376–1384

Duncan EJ, Benton MA, Dearden PK (2013) Canonical terminal patterning is an evolutionary novelty. Dev Biol. https://doi.org/10.1016/j.ydbio.2013.02.010

Duncan EJ, Johnson TK, Whisstock JC, Warr CG, Dearden PK (2014) Capturing embryonic development from metamorphosis: how did the terminal patterning signalling pathway of *Drosophila* evolve? Curr Opin Insect Sci 1:45–51

Duncan EJ, Hyink O, Dearden PK (2016) Notch signalling mediates reproductive constraint in the adult worker honeybee. Nat Commun 7:12427

Elsik CG, Worley KC, Bennett AK, Beye M, Camara F, Childers CP, de Graaf DC, Debyser G, Deng J, Devreese B (2014) Finding the missing honey bee genes: lessons learned from a genome upgrade. BMC Genomics 15(1):86

Fleig R (1990) *Engrailed* expression and body segmentation in the honeybee, *Apis mellifera*. Rouxs Arch Dev Biol 198:467–473

Fleig R, Sander K (1986) Embryogenesis of the honeybee *Apis mellifera L.* (Hymenoptera: Apidae): an SEM study. Int J Insect Morphol Embryol 15(5):449–462

Gallai N, Salles JM, Settele J, Vaissiere BE (2009) Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol Econ 68(3):810–821

Gempe T, Beye M (2011) Function and evolution of sex determination mechanisms, genes and pathways in insects. BioEssays 33(1):52–60. https://doi.org/10.1002/bies.201000043

Gempe T, Hasselmann M, Schiøtt M, Hause G, Otte M, Beye M (2009) Sex determination in honeybees: two separate mechanisms induce and maintain the female pathway. PLoS Biol 7(10):e1000222

Guo X, Su S, Skogerboe G, Dai S, Li W, Li Z, Liu F, Ni R, Guo Y, Chen S (2013) Recipe for a busy bee: microRNAs in honey bee caste determination. PLoS One 8(12):e81661

Hasselmann M, Gempe T, Schiott M, Nunes-Silva CG, Otte M, Beye M (2008) Evidence for the evolutionary nascence of a novel sex determination pathway in honeybees. Nature 454(7203): 519–522. https://doi.org/10.1038/nature07052

Hedges SB, Marin J, Suleski M, Paymer M, Kumar S (2015) Tree of life reveals clock-like speciation and diversification. Mol Biol Evol 32(4):835–845. https://doi.org/10.1093/molbev/msv037

Heimpel GE, de Boer JG (2008) Sex determination in the Hymenoptera. Annu Rev Entomol 53:209–230

Kamakura M (2011) Royalactin induces queen differentiation in honeybees. Nature 473(7348): 478–483

Kamakura M (2016) Royalactin is not a royal making of a queen Reply. Nature 537(7621):E13–E13

Kohno H, Suenami S, Takeuchi H, Sasaki T, Kubo T (2016) Production of knockout mutants by CRISPR/Cas9 in the European honeybee, *Apis mellifera L*. Zool Sci 33(5):505–512

Kritsky G (2017) Beekeeping from antiquity through the middle ages. Annu Rev Entomol 62:249–264

Kucharski R, Maleszka J, Foret S, Maleszka R (2008) Nutritional control of reproductive status in honeybees via DNA methylation. Science 319(5871):1827–1830

Kucharski R, Foret S, Maleszka R (2015) EGFR gene methylation is not involved in Royalactin controlled phenotypic polymorphism in honey bees. Sci Rep 5:14070

Lynch JA, Brent AE, Leaf DS, Pultz MA, Desplan C (2006) Localized maternal *orthodenticle* patterns anterior and posterior in the long germ wasp *Nasonia*. Nature 439(7077):728–732

Meixner MD, Le Conte Y (2016) A current perspective on honey bee health. Apidologie 47(3): 273–275

Munoz-Torres MC, Reese JT, Childers CP, Bennett AK, Sundaram JP, Childs KL, Anzola JM, Milshina N, Elsik CG (2010) Hymenoptera genome database: integrated community resources for insect species of the order Hymenoptera. Nucleic Acids Res 39(suppl_1):D658–D662

Mutti NS, Dolezal AG, Wolschin F, Mutti JS, Gill KS, Amdam GV (2011) IRS and TOR nutrient-signaling pathways act via juvenile hormone to influence honey bee caste fate. J Exp Biol 214(23):3977–3984

Nelson CM, Ihle KE, Fondrk MK, Page RE, Amdam GV (2007) The gene *vitellogenin* has multiple coordinating effects on social organization. PLoS Biol 5(3):e62

Nissen I, Müller M, Beye M (2012) The *Am-tra2* gene is an essential regulator of female splice regulation at two levels of the sex determination hierarchy of the honeybee. Genetics 192(3): 1015–1026

Olesnicky EC, Desplan C (2007) Distinct mechanisms for mRNA localization during embryonic axis specification in the wasp *Nasonia*. Dev Biol 306(1):134–142

Oxley PR, Thompson GJ, Oldroyd BP (2008) Four quantitative trait loci that influence worker sterility in the honeybee (*Apis mellifera*). Genetics 179(3):1337–1343

Ronai I, Barton DA, Oldroyd BP, Vergoz V (2015) Regulation of oogenesis in honey bee workers via programed cell death. J Insect Physiol 81:36–41

Sander K (1976) Specification of the basic body pattern in insect embryogenesis. Adv Insect Physiol 12:125–238

Schulte C, Theilenberg E, Müller-Borg M, Gempe T, Beye M (2014) Highly efficient integration and expression of piggyBac-derived cassettes in the honeybee (*Apis mellifera*). Proc Natl Acad Sci 111(24):9003–9008

Spannhoff A, Kim YK, Raynal NJM, Gharibyan V, Su MB, Zhou YY, Li J, Castellano S, Sbardella G, Issa JPJ (2011) Histone deacetylase inhibitor activity in royal jelly might facilitate caste switching in bees. EMBO Rep 12(3):238–243

Tautz J (2008) The buzz about bees: biology of a superorganism. Springer Science & Business Media. Berlin, Heidelberg

The Honey Bee Genome Sequencing Consortium (2006) Insights into social insects from the genome of the honeybee *Apis mellifera*. Nature 443:931–949

Walldorf U, Fleig R, Gehring WJ (1989) Comparison of homeobox-containing genes of the honeybee and *Drosophila*. Proc Natl Acad Sci U S A 86(24):9971–9975

Wang Y, Jorda M, Jones L, Maleszka R, Ling X, Robertson HM, Mizzen CA, Peinado MA, Robinson GE (2006) Functional CpG methlyation system in a social insect. Science 314:645–647

Wilson MJ, Dearden PK (2009) Tailless patterning functions are conserved in the honeybee even in the absence of Torso signaling. Dev Biol 335(1):276–287. https://doi.org/10.1016/j.ydbio.2009.09.002. S0012-1606(09)01174-9 [pii]

Wilson MJ, Dearden PK (2011) Diversity in insect axis formation: two orthodenticle genes and hunchback act in anterior patterning and influence dorsoventral organization in the honeybee (Apis mellifera). Development 138(16):3497–3507

Wilson MJ, Dearden PK (2012) Pair-rule gene orthologues have unexpected maternal roles in the honeybee (Apis mellifera). PLoS One 7(9):e46490

Wilson MJ, Havler M, Dearden PK (2009) Giant, Kruppel, and caudal act as gap genes with extensive roles in patterning the honeybee embryo. Dev Biol 339(1):200–211

Zhu K, Liu M, Fu Z, Zhou Z, Kong Y, Liang H, Lin Z, Luo J, Zheng H, Wan P (2017) Plant microRNAs in larval food regulate honeybee caste development. PLoS Genet 13(8):e1006946

Evo-Devo Lessons Learned from Aphids

Gregory K. Davis, Jennifer A. Brisson, and Ryan D. Bickel

Contents

Abstract

Aphids possess several features that have uniquely expanded our understanding of how reproduction and development evolve. The complex life cycles of aphids, for example, are unusual in that they include cyclical parthenogenesis, seasonal host alternation, and multiple polyphenisms. Their close interactions with other organisms such as host plants and symbiotic bacteria likewise present instances of fascinating biology. As dramatic examples of developmental plasticity, the polyphenisms are perhaps the most intrinsically interesting from the standpoint of evolutionary developmental biology or evo-devo. The wing and reproductive polyphenisms in particular have garnered the most attention, providing insight into the mechanism and evolution of developmental responses to environmental cues. Additionally, the bacterial endosymbiosis and the induction of galls in host plants by some aphid species present opportunities to better understand

G. K. Davis (✉)
Department of Biology, Bryn Mawr College, Bryn Mawr, PA, USA
e-mail: gdavis@brynmawr.edu

J. A. Brisson · R. D. Bickel
Department of Biology, University of Rochester, Rochester, NY, USA
e-mail: jennifer.brisson@rochester.edu; ryan.bickel@rochester.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_182

how interactions between species can impact development and how interspecific coordination or control of development, respectively, has evolved. Here we briefly introduce aphids and describe the wing polyphenism, reproductive polyphenism, bacterial endosymbiosis, and gall formation, in each case indicating what we know and what we hope to learn.

Keywords

Plasticity · Polyphenism · Endosymbiosis · Buchnera · Gall

Introduction

Commonly known as true aphids, members of the Family Aphididae (or the Superfamily Aphidoidea, depending on taxonomic scheme) are small (1–10 mm) hemimetabolous insects which, along with true bugs, cicadas, and leafhoppers, are members of the Order Hemiptera. They spend most of their lives on host plants feeding on phloem sap, relying on bacterial endosymbionts housed in specialized cells to provide essential amino acids. Their small heads, equipped with mouthparts specialized for piercing-sucking and a pair of long antennae, attach to a thorax and larger, soft abdomen that possesses a pair of upward pointing appendages known as siphunculi, which secrete chemical defenses and alarm pheromones. Aphids are unusual among insects in that a majority of the more than 5,000 known species (Favret 2018) live in temperate regions. Most aphid species are also unusual among plant-feeding insects in that they are specialists, feeding on just one or a few closely related host plants. Almost all aphid species have a life cycle that involves cyclical parthenogenesis, typically alternating between several female asexual generations during the spring and summer, and a sexual generation in the fall in which sexual females and males mate to produce fertilized eggs. These eggs can be frost resistant and often overwinter to hatch as specialized asexual females (known as fundatrices) in the spring. A defining feature of true aphids is that asexual females are viviparous and give live birth to progeny, in contrast to the closely related adelgids and phylloxerans, groups in which both sexual and asexual females are oviparous. Adding to this complexity, some aphid species also alternate between unrelated host plants during their life cycles. Typically, after several asexual generations on a herbaceous host plant during the spring and summer, specialized winged forms migrate to a woody host in the fall, whereupon sexual females and males mate and lay overwintering eggs (Fig. 1). After hatching, fundatrices give rise to either winged daughters or granddaughters that migrate back to the herbaceous host. Both paleontological and molecular studies indicate that the ancestors of aphids appeared in the early Mesozoic on gymnosperms and true aphids subsequently diversified, along with angiosperms, into the major aphid subfamilies in the Cretaceous. Host-alternation among true aphids evolved at least twice, perhaps having the greatest impact on Aphidinae where it may have contributed to a Miocene radiation of this subfamily to comprise more than half of all living species. Although a

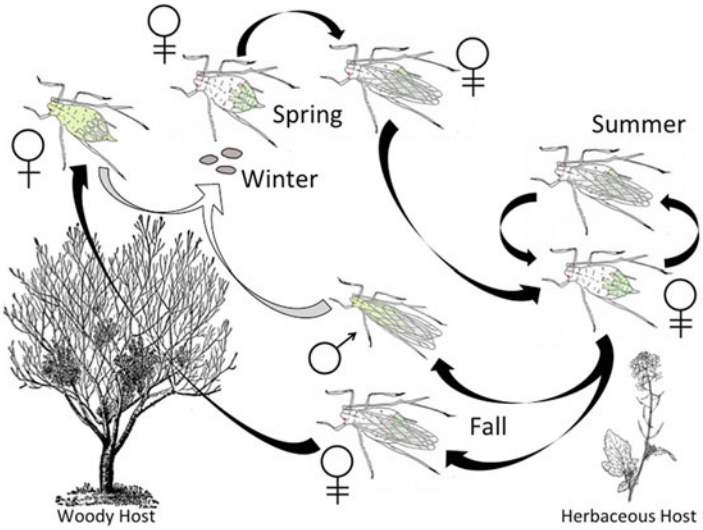


Fig. 1 A typical host-alternating aphid life cycle (e.g., *Myzus persicae*). Summer months are spent on a herbaceous host as asexual viviparous females (double-stroke female symbol), which can be winged or unwinged. In the fall, special migratory (winged) asexual viviparous females and males are asexually produced (black arrows) and migrate to a woody host. These asexual viviparous females will asexually produce sexual oviparous females that then mate with males to sexually produce (white merged arrow) diapausing eggs that overwinter. In the spring, from these eggs hatch a special asexual viviparous female, whose winged asexual viviparous offspring migrate back to herbaceous hosts. (Aphids drawn by Maiko Sho. Mustard plant use license CC BY-SA 2.5, https://en.wikipedia.org/w/index.php?curid=10534762)

minority of aphid species host-alternate, it is generally thought that many nonalternating species are derived from host-alternating ancestors, particularly in Aphidinae. For reviews on the evolutionary history of aphids and their associations with plants, see Moran (1992) and Peccoud et al. (2010).

Polyphenism

A primary task of evo-devo, or more specifically eco-evo-devo, is to better understand adaptive developmental plasticity, or the process by which environmental factors effect changes in development that lead to adaptive changes in phenotype. Evo-devo researchers are particularly interested in polyphenism, an extreme form of adaptive phenotypic plasticity wherein an evolved, developmental response to an environmental cue allows organisms to acquire a discrete, alternative adult phenotype (often referred to as a "morph"). Such developmental responses and their resulting morphs are adaptive in being able to meet environmental challenges that are coincident with, or predicted by, the inducing cue. Polyphenisms are important from an evolutionary perspective because, once evolved, they allow populations to

match varying environments over short, non-generational timescales. From a developmental perspective, polyphenisms provide striking examples of how a single genome can direct more than one ontogeny.

Aphids are excellent models for studying polyphenism; not only do they exhibit multiple polyphenisms – up to eight alternative morphs in some species – but the form of parthenogenesis practiced by summer females does not generally involve recombination. The resulting genetically identical populations, known as "clones," provide an advantage, allowing those who study plasticity to avoid the influence of genetic variation on phenotype. Given their clonal nature and the opportunity for kin selection, is it perhaps not surprising that some aphid species are social. The latter all induce galls, host-plant growths that provide a protective environment. Associated with these galls are polyphenic castes that possess specialized morphology and engage in altruistic behavior at a cost to their own reproductive success, similar to the better-known castes of social hymenopterans and termites. Soldier aphids, for example, are likely cued by colony density and typically appear as sterile, modified nymphs that defend a clone against predators and perform other tasks such as gall-cleaning (Pike and Foster 2008).

Most evolutionary and developmental insights into aphid phenotypic plasticity, however, concern the more common wing and reproductive polyphenisms, both of which figure prominently in the complex life cycles of aphids. These differ from other well-known insect polyphenisms in three respects. First, in all aphid species, the reproductive polyphenism – and in some, the wing polyphenism as well – is transgenerational in that environmental cues are sensed and their influence mediated by the mother (Ogawa and Miura 2014). This maternal sensing of the environment and transmission of information to offspring is facilitated by the fact that, among all true aphids, asexual females are viviparous, with embryonic development of offspring occurring within mothers prior to birth. Second, in contrast to many well-known polyphenisms in which morph identity is determined postembryonically, in the pea aphid at least, the morphs for both the wing and reproductive polyphenisms are determined earlier, during embryogenesis. Third, unlike some polyphenisms in which the environmental cue is nutritional or dietary, aphids tend to respond to cues (e.g., population density or photoperiod) that predict the onset of environmental challenges (e.g., dwindling resources or winter temperatures). Among the many aphids that exhibit both the wing and reproductive polyphenisms, the pea aphid, *Acyrthosiphon pisum* , has emerged as a model for their study, in part because of the availability of genomic and transcriptomic resources, as well as the fact that this nonhost alternating species is able to complete its life cycle in the lab on one of several, easily cultivated host plants.

The Wing Polyphenism

In the asexual, summer phase of a typical life cycle, genetically identical female aphids can be winged or wingless depending on the environment in which they develop. This plastic response is known as the wing polyphenism. The two morphs

differ not only in the possession of wings but also in a number of behavioral, physiological, and morphological traits. High population densities cue the development of winged aphids, while low densities result in wingless aphids (Sutherland 1969). The two morphs and the roles they play are important adaptations in aphid ecology. Consider a single wingless asexual female pea aphid on a single host plant. She will give birth to as many as 10 offspring a day, and each daughter takes only 10 days to reach reproductive maturity. The plant will soon be overrun with aphids, and this high density will cue a shift toward producing winged asexual females, which can disperse to colonize a new host. Since wingedness is facultative, one might expect that this ability to migrate comes at a cost and, indeed, winged females are less fecund than wingless females (reviewed by Zera and Denno 1997). Thus, like all polyphenisms, the wing polyphenism provides a means to navigate the competing demands of a predictably variable environment: winged morphs, cued by high density, are able to escape inevitably poor conditions while wingless morphs, the result of low density, can instead devote more resources toward reproduction.

While the mechanical stimulation associated with crowding (aphids bumping into and touching each other) appears to be the primary environmental cue determining wing morph, depending on the aphid species, other factors, such as host-plant quality or the presence of predators, may contribute as well (reviewed in Müller et al. 2001). Also depending on the species, the developmental time period of environmental sensitivity to crowding can be prenatal, as in the pea aphid, or extend postnatally into the early nymphal stages. All aphids have wingless nymphal stages and wings do not manifest until adulthood. Interestingly, all asexual aphid females are born with wing buds. In species that have been examined thus far (most thoroughly in the pea aphid), wing buds in wingless morphs degenerate or cease growing around the second nymphal instar, after which morph-specific ontogenies diverge (Ishikawa et al. 2008). In particular, winged morphs grow slower, especially in the penultimate instar when their wings and flight muscles gain impressively in size. While wingless adults are marked by abdomens chock-full of developing embryos, winged adults possess well-developed wings attached to a muscled thorax specialized for flight. Intermediates are rare, consistent with the discrete nature of polyphenism. By the penultimate instar and as adults, the two morphs are distinguishable by their dramatically different morphologies.

In sum, we know much about the aphid wing polyphenism, including the ecological roles of the two morphs, the environmentally sensitive time period, and how and when morph-specific development and gene expression diverge. That said, we know less about the molecular mechanisms that underlie wing induction. For example, exactly how is the winged or wingless fate determined in an aphid embryo or nymph? In other words, what is the nature of the "switch" that directs individuals down a winged or wingless developmental pathway? In species where the winged or wingless fate is determined prenatally, including the pea aphid, how do aphid mothers first sense high density, what is the nature of the signal by which this information is sent to developing embryos, and how do embryos receive the signal? Even in the more direct cases where fate can be determined in early nymphal stages, how do nymphs sense crowding?

Transcriptomic profiling of pea aphid adult females in high- and low-density environments has revealed a large number of genes that are differentially expressed (Vellichirammal et al. 2016), which in turn have helped generate hypotheses as to mechanism. In particular, processes related to odorant binding, neurotransmission, chromatin remodeling, and hormonal activity have been implicated as mediating the maternal response to crowding. Bolstering the idea that neurotransmitters play an important role in the process, whole body titers of dopamine, serotonin, and octopamine were reduced in response to crowding. Moreover, a follow-up study manipulating maternal levels of ecdysone signaling found that increasing maternal ecdysone decreases the percentage of winged offspring and vice versa, suggesting that ecdysone signaling plays a key role in regulating the wing polyphenism (Vellichirammal et al. 2017).

Now that we have a foothold in our understanding of the developmental mechanisms that underlie the wing polyphenism, we can investigate how this plastic response has evolved. Two obvious areas of opportunity exist. First, extensive variation in the propensity to produce winged offspring in response to crowding exists in natural populations. For example, some pea aphid genotypes characteristically produce nearly 100% winged offspring when crowded and some close to 0%, with everything in between (Grantham et al. 2016). But what is the nature of this variation in the response to crowding? At what points in the wing induction pathway does the variation lie? Are certain steps in the pathway more constrained than others? These questions are currently unanswered. Second, as indicated above, we observe interspecific variation in the timing of wing morph determination: in some species determination occurs during embryogenesis, whereas in others, it occurs during nymphal development, and still others it can be either (Müller et al. 2001). Prima facie, the evolutionary transition between species where the nymphs are induced directly and the indirect, maternally mediated induction of embryos would appear far from trivial and it is surprising this transition has evolved repeatedly. It remains an open question to what extent these distinct processes share features of their mechanisms, if they share any at all.

A potential advantage of the aphid wing polyphenism from an evo-devo perspective is that, in pea aphids at least, the environmentally cued polyphenism coexists with a genetically controlled wing polymorphism in a different part of the life cycle. Most of the thousands of aphid species produce winged and wingless females during the asexual portion of their life cycle via the wing polyphenism, as described above. But in an estimated 10% of the species in the subfamily Aphidinae, males, which typically appear in the fall, can also be winged or wingless. In the case of the pea aphid, this second (male) wing dimorphism is under strict Mendelian control by the presence of a winged or wingless allele at a single locus on the X chromosome (Caillaud et al. 2002). The specific gene or genes that control this dimorphism are, as yet, unknown. The presence of both a wing polyphenism and polymorphism in female and male pea aphids, respectively, presents an exciting opportunity to compare and contrast the molecular mechanisms underlying the environmental and genetic control of wing development. This approach may inform our understanding of genetic accommodation, a process whereby a novel trait, induced either by genetic

mutation or environmental change, is refined through selection on additional quantitative genetic variation (West-Eberhard 2003).

The Reproductive Polyphenism

Except in cases where the sexual phase of the life cycle has been lost, all aphids are cyclically parthenogenetic, switching between asexual reproduction in the spring and summer and sexual reproduction in the fall. Genetically identical females can thus be either asexual or sexual, depending on whether they developed under summer or fall conditions, respectively. This plastic response is known as the reproductive polyphenism, but asexual and sexual females differ by more than just whether they require males to reproduce. Among true aphids, all asexual females are also viviparous. While sexual females reproduce by laying eggs fertilized by sperm, asexual females reproduce, first by dispensing with meiotic reduction and fertilization (asexuality) and second by allowing oocytes to complete embryogenesis prior to birth (viviparity). The principal difference between sexual and asexual females is thus a pair of ovaries filled with either large, yolk-filled, haploid oocytes or much smaller, developing diploid embryos. In the case of asexuals, the ability to begin embryogenesis inside of one's mother without the need for sperm allows for the telescoping of generations—that is, an asexual mother possesses not only daughter embryos within her ovaries but also granddaughter embryos within ovaries of her daughter embryos.

In the pea aphid and other aphidine species that have been examined, the developmental decision to adopt either a sexual or asexual fate is made during embryogenesis based on the photoperiod-induced state of the asexual mother (reviewed by Ogawa and Miura 2014). Specifically, an asexual mother produces sexuals in the fall when she is exposed to long nights over the course of her own development, the sexual fate of her embryonic progeny being determined by the *loss of an asexual-promoting maternal signal*, rather than the gain of a sexual-promoting signal. This model is supported by the fact that microcautery of neurosecretory cells in the protocerebrum of the vetch aphid, *Megoura viciae*, results in the production of sexual progeny under short nights (Steel and Lees 1977).

While there is room for debate on the nature of the maternal signal, a lead contender is juvenile hormone (JH), known for its classic role of maintaining the juvenile character of nymphal and larval instars prior to metamorphosis, as well as regulating polyphenisms. A role for JH in mediating the aphid reproductive polyphenism in particular is supported by two pieces of evidence – one functional, one correlational. Functionally, topical administration of JH or JH analogs to what would normally be a sexuals-producing mother causes it instead to produce asexual daughters, demonstrating that JH is sufficient to induce the asexual fate (Corbit and Hardie 1985; Hardie and Lees 1985). Correlationally, JH III titers are generally higher in aphids exposed to short nights than in aphids exposed to long nights (Ishikawa et al. 2012). Both pieces of evidence are consistent with a model wherein exposure to short nights allows endogenous titers of JH in the maternal hemolymph

to rise, signaling – directly or indirectly – the naïve germaria of daughter embryos to produce embryos instead of oocytes (i.e., adopt an asexual instead of a sexual fate).

In order to better understand how photoperiod is received and processed by the mother, as well as the nature of the developmental switch that occurs in developing embryos, a number of studies in the pea aphid have described changes in transcript abundance associated with sexual induction, both in the sexuals-producing mother (e.g., Le Trionnaire et al. 2012) and in sexual-fated embryos (e.g., Gallot et al. 2012). Although these approaches have identified candidate genes to pursue, questions remain about the process of sexual induction, including aspects as fundamental as the maternal signal. The suggestion that the signal is asexual-promoting, for example, is based on an ablation study conducted over four decades ago in the vetch aphid (Steel and Lees 1977). Thus, a fresh attempt at disrupting these same neurosecretory cells, predicted to mediate the effect of short nights, in the pea aphid would be well worth the effort. In addition, although current evidence supports JH as the maternal signal, a requirement for JH in specifying asexual fate has not yet been clearly demonstrated, in part because JH's classic role in regulating the timing and nature of molts can complicate such efforts. In this regard, it is worth noting that Steel (1977) described how the axons of the required neurosecretory cells in the vetch aphid project ventrally and abdominally, past the corpora allatum, the endocrine gland that secretes JH, potentially terminating near the ovaries and the developing embryos held within.

Beyond the proximal mechanisms of sexual induction, one can also ask how the process has evolved. Sexual reproduction is the only way for true aphids to produce a diapausing, frost-resistant egg. Thus, aside from the long-term benefits of sexual reproduction, in the short-term sex in aphids appears to be maintained by selection for the ability to survive winter (Simon et al. 2010). An essential feature of some polyphenisms is a delay between the environmental cue and the environmental challenge, which allows enough time to mount the adaptive response and meet the challenge on time (Nijhout 2003). In aphids, the advance warning of long nights allows enough time to produce a diapausing, frost-resistant egg before the first killing frost of winter. At northern latitudes within the temperate zone, freezing winter temperatures tend to occur earlier in the season, when nights are shorter (despite increasing night length due to increasing latitude). In order to meet, on time, a killing frost with sexually produced eggs, one would predict that populations have changed the timing of the environmental cue by evolving a change in the night length threshold for inducing sexuals. As it turns out, for pea aphid populations, the required night length is indeed shorter for northern populations than for southern populations (MacKay et al. 1993), suggesting local adaptation by genetic accommodation. In what could be considered an example of environmental canalization, wherein a phenotype is buffered against environmental variation, pea aphid populations in the most southern portions of this latitudinal cline are reported to be *anholocyclic* – that is, they do not produce sexual females or males in response to long nights. This is presumably because the relative fitness of any sexually reproducing clone, regardless of timing, is lower than that of exclusively asexual clones in environs where aphids can overwinter without the need for eggs. In France,

so-called *androcyclic* pea aphid clones have been isolated that produce asexual females and males but not sexual females. The production of males allows one to cross these clones to cyclically parthenogenetic clones, and a quantitative trait locus (QTL) analysis has identified a region of the X chromosome responsible for the loss of sexual females, which is recessive and shows a strong signature of selection (Jaquiéry et al. 2014). Further investigation into the mechanism of these evolved changes to the ancestral polyphenism – whether a shifted threshold, anholocycly, or androcycly – should shed light on the process of genetic accommodation.

Downstream of the induction, a close examination of the two reproductive morphs as adults should help us understand how aphids have solved the challenges that accompanied the evolutionary acquisition of both cyclical parthenogenesis and viviparity (Davis 2012). Although the resulting phenotypes (fundatrix vs. summer asexual) can differ in important ways, the oviparous and viviparous development of progeny from sexual and asexual mothers, respectively, can be viewed as an example of developmental system drift, wherein a resulting phenotype remains relatively stable despite changes in development (True and Haag 2001). The differences between these developmental modes are often stark, particularly for oogenesis, which is truncated in the viviparous case with the inclusion of developing embryos within the ovaries. Comparative studies can thus ask the question: Are the observed morphological differences in development accompanied by differences in patterning? The approach is akin to comparative molecular embryology, an early evo-devo mainstay, with the twist that the comparison is within the same species, comparing developmental programs directed by the same genome. If the first forays into this subject are any indication (e.g., Bickel et al. 2013; Duncan et al. 2013), the ways in which patterning of early viviparous development has been modified from the ancestral, and presumably more conserved, oviparous condition have much to teach us about how development evolves.

Interspecific Coordination and Control of Development

The literature concerning the ecology of aphids and their interactions with other species is vast. Probably the best-known example is the symbiotic providing of sugar-rich honeydew to ants in exchange for protection. From an evo-devo perspective, however, the bacterial endosymbiosis and the induction of galls in host plants by some aphid species represent opportunities to understand how early aphid development has been modified to house needed bacteria and how certain aphids hijack plant development to accommodate their lifestyles.

Bacterial Endosymbiosis

Because aphids consume phloem sap, a diet poor in amino acids, they rely on bacterial endosymbionts (*Buchnera aphicola*) to help them meet their nutritional needs. The bacteria synthesize amino acids for the aphid, and the aphid, in turn, feeds

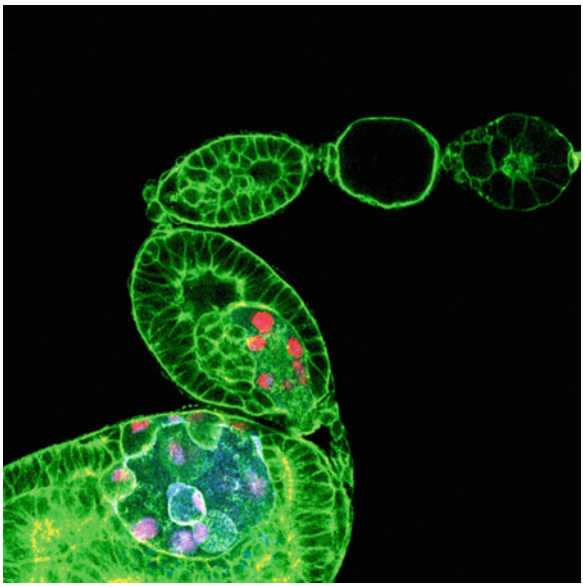


Fig. 2 A single ovariole from a viviparous asexual female aphid showing a germarium and diploid oocyte (upper right) followed by embryos in various stages of development. Bacteriocytes are stained for filamentous actin (green), the transcription factors Distalless (red) and Engrailed (blue). (Image provided by David Stern and Toru Miura)

and houses the bacteria in aphid cells called bacteriocytes, which are polyploid and cluster together as an organ in the aphid abdomen. This aphid-bacterial relationship is ancient, the product of stable maternal transmission for over a hundred million years. One intriguing question, from an evo-devo perspective, is the origin of the aphid-derived bacteriocytes. Prior to this mutualistic relationship, bacteriocytes did not exist in the aphid; they are, therefore, a novel cell type that has evolved to facilitate the symbiosis. The cell type from which they are derived is unknown. In pea aphids, the bacteriocytes are specified early in development (Fig. 2), prior to gastrulation, and it is not clear what, if any, germ layer they are derived from. The mechanisms that specify bacteriocyte identity and promote their differentiation also remain poorly characterized, although they appear to include the co-option of conserved transcription factors (Braendle et al. 2003). One of the most intriguing is the homeotic gene, *Ultrabithorax*, which appears to be expressed in pea aphid bacteriocytes (Braendle et al. 2003) and has been shown to be essential for bacteriocyte development in the heteropteran hemipteran insect *Nysius plebeius* (Matsuura et al. 2015). Indeed, similar bacteria-housing cells have independently evolved in multiple insect orders, providing a rich framework for investigating the evolution and development of this intriguing cell type.

Gall Formation

The most familiar gall-forming insects belong to the orders Hymenoptera, Diptera, and Hemiptera. Although only a minority of aphid species induces galls, the process is as fascinating as it is mysterious (Wool 2004). A gall is an induced hollow growth

Fig. 3 Galls induced on leaves of elm by the aphid *Tetraneura ulmi*. (Image provided by David Stern)

at a specific site on the primary host plant in the spring and serves as a protective structure for multiple asexual generations (Fig. 3). They tend to be outfitted with a network of phloem elements and ducts in the gall wall, providing residents with easy access to sap, often expropriating nutrients needed for host organs such as fruits. From a developmental perspective, it is important to note that galls are the result of patterned growth and are stereotyped; galls induced by individuals of the same species are very similar whereas galls induced by different species, even if induced on same organ of the same plant, are different. Typically, only the fundatrix, the female hatching out of a sexually produced egg, can induce gall formation, while genetically identical daughters cannot. Thus the ability to induce galls is a feature of aphid polyphenism. While it has long been presumed that aphids inject signals to instruct gall formation, we remain profoundly ignorant of the nature of such signals and how they have evolved to hijack plant development to produce such ordered and species-specific structures. Needless to say, gall formation is in dire need of investigation, but as the pea aphid does not induce galls, we will first need to develop a model aphid system for its investigation.

Cross-References

- ▶ Canalization: A Central but Controversial Concept in Evo-Devo
- ▶ Developmental Plasticity and Evolution
- ▶ Developmental System Drift
- ▶ Eco-Evo-Devo
- ▶ Evo-Devo Lessons Learned from Honeybees

References

Bickel RD, Cleveland HC, Barkas J, Jeschke CC, Raz AA, Stern DL, Davis GK (2013) The pea aphid uses a version of the terminal system during oviparous, but not viviparous, development. Evodevo 4:10

Braendle C, Miura T, Bickel R, Shingleton AW, Kambhampati S, Stern DL (2003) Developmental origin and evolution of bacteriocytes in the aphid-Buchnera symbiosis. PLoS Biol 1:E21

Caillaud MC, Boutin M, Braendle C, Simon JC (2002) A sex-linked locus controls wing polymorphism in males of the pea aphid, *Acyrthosiphon pisum* (Harris). Heredity 89:346–352

Corbit TS, Hardie J (1985) Juvenile hormone effects on polymorphism in the pea aphid, *Acyrthosiphon pisum*. Entomol Exp Appl 38:131–135

Davis GK (2012) Cyclical parthenogenesis and viviparity in aphids as evolutionary novelties. J Exp Zool B Mol Dev Evol 318:448–459

Duncan EJ, Leask MP, Dearden PK (2013) The pea aphid (*Acyrthosiphon pisum*) genome encodes two divergent early developmental programs. Dev Biol 377:262–274

Favret C (2018) Aphid species file. Version 5.0/5.0 [2018.07]. http://Aphid.SpeciesFile.org

Gallot A, Shigenobu S, Hashiyama T, Jaubert-Possamai S, Tagu D (2012) Sexual and asexual oogenesis require the expression of unique and shared sets of genes in the insect *Acyrthosiphon pisum*. BMC Genomics 13:76

Grantham ME, Antonio CJ, O'Neil BR, Zhan YZ, Brisson JA (2016) A case for a joint strategy of diversified bet hedging and plasticity in the pea aphid wing polyphenism. Biol Lett 12:20160654

Hardie J, Lees AD (1985) The induction of normal and teratoid viviparae by a juvenile hormone and kinoprene in two species of aphids. Physiol Entomol 10:65–74

Ishikawa A, Hongo S, Miura T (2008) Morphological and histological examination of polyphenic wing formation in the pea aphid *Acyrthosiphon pisum* (Hemiptera, Hexapoda). Zoomorphology 127:121–133

Ishikawa A, Ogawa K, Gotoh H, Walsh TK, Tagu D, Brisson JA, Rispe C, Jaubert-Possamai S, Kanbe T, Tsubota T, Shiotsuki T, Miura T (2012) Juvenile hormone titre and related gene expression during the change of reproductive modes in the pea aphid. Insect Mol Biol 21:49–60

Jaquiéry J, Stoeckel S, Larose C, Nouhaud P, Rispe C, Mieuzet L, Bonhomme J, Mahéo F, Legeai F, Gauthier JP, Prunier-Leterme N, Tagu D, Simon J-C (2014) Genetic control of contagious asexuality in the pea aphid. PLoS Genet 10:e1004838

Le Trionnaire G, Jaubert-Possamai S, Bonhomme J, Gauthier JP, Guernec G, Le Cam A, Legeai F, Monfort J, Tagu D (2012) Transcriptomic profiling of the reproductive mode switch in the pea aphid in response to natural autumnal photoperiod. J Insect Physiol 58:1517–1524

MacKay PA, Lamb RJ, Smith MAH (1993) Variability in life history traits of the aphid, *Acyrthosiphon pisum* (Harris), from sexual and asexual populations. Oecologia 94:330–338

Matsuura Y, Kikuchi Y, Miura T, Fukatsu T (2015) Ultrabithorax is essential for bacteriocyte development. Proc Natl Acad Sci USA 112:9376–9381

Moran NA (1992) The evolution of aphid life cycles. Annu Rev Entomol 37:321–348

Müller CB, Williams IS, Hardie J (2001) The role of nutrition, crowding, and interspecific interactions in the development of winged aphids. Ecol Entomol 26:330–340

Nijhout HF (2003) Development and evolution of adaptive polyphenisms. Evol Dev 5:9–18

Ogawa K, Miura T (2014) Aphid polyphenisms: trans-generational developmental regulation through viviparity. Front Physiol 5:1

Peccoud J, Simon JC, von Dohlen C, Coeur d'acier A, Plantegenest M, Vanlerberghe-Masutti F, Jousselin E (2010) Evolutionary history of aphid-plant associations and their role in aphid diversification. C R Biol 333:474–487

Pike N, Foster WA (2008) The ecology of altruism in a clonal insect. In: Korb J, Heinze J (eds) Ecology of social evolution. Springer, Berlin, pp 37–56

Simon J-C, Stoeckel S, Tagu D (2010) Evolutionary and functional insights into reproductive strategies of aphids. C R Biol 333:488–496

Steel CGH (1977) The neurosecretory system in the aphid *Megoura viciae*, with reference to the unusual features associated with long distance transport of neurosecretion. Gen Comp Endocrinol 31:307–322

Steel CGH, Lees AD (1977) The role of neurosecretion in the photoperiodic control of polymorphism in the aphid *Megoura viciae*. J Exp Biol 67:117–135

Sutherland ORW (1969) The role of crowding in the production of winged forms by two strains of the pea aphid, *Acyrthosiphon pisum*. J Insect Physiol 15:1385–1410

True JR, Haag ES (2001) Developmental system drift and flexibility in evolutionary trajectories. Evol Dev 3:109–119

Vellichirammal NN, Madayiputhiya N, Brisson JA (2016) The genome-wide transcriptional response underlying the pea aphid wing polyphenism. Mol Ecol 25:4146–4160

Vellichirammal NN, Gupta P, Hall TA, Brisson JA (2017) Ecdysone signaling underlies the pea aphid transgenerational wing polyphenism. Proc Natl Acad Sci USA 114:1419–1423

West-Eberhard MJ (2003) Developmental plasticity and evolution. Oxford University Press, New York

Wool D (2004) Galling aphids: specialization, biological complexity, and variation. Annu Rev Entomol 49:175–192

Zera AJ, Denno RF (1997) Physiology and ecology of dispersal polymorphism in insects. Annu Rev Entomol 42:207–230

Part VIII

Vertebrate Evo-Devo

Shifting the Black Box: Approaches to the Development and Evolution of the Vertebrate Mesoderm

Ann C. Burke

Contents

Abstract

The genotype was an abstraction at the time of the Modern Synthesis, though its direct causation of the phenotype was comfortably assumed. The cellular and developmental processes that actually build the phenotype were relegated to a black box and largely considered irrelevant to evolutionary biology. Techniques for manipulating DNA and sequencing of whole genomes have now given the genotype physical presence, and dramatic correlations of genetic and phenotypic perturbation reinforce the earlier assumptions. The last four decades have brought development back into the mainstream of evolutionary biology with the formalization of evo-devo. However, the genocentric perspective persists. A majority of evo-devo studies focus on evolution of developmental genes, and we still lack adequate knowledge of the rich phenomena between genotype and phenotype. Here I present data and perspectives from the vertebrate mesoderm and

A. C. Burke (✉)
Biology Department, Wesleyan University, Middletown, CT, USA
e-mail: acburke@wesleyan.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_188

its musculoskeletal derivatives. The conserved embryonic cell populations of somitic and lateral plate mesoderm interact to generate the enormous range of diversity within the phylum, and understanding changes in these interactions can explain and predict both innovation and constraint. A wealth of work has mapped the expression and interactions of genes at play in the mesoderm, but while the musculoskeletal system varies widely, the genetic picture is highly conserved. Here I focus on the embryological, cellular context in which the genes take their actions, and argue that this context is the source of morphological variation leading to evolution. Correlations between genetic perturbations and morphological outcomes are valuable data but rarely address *what actually goes on* during morphogenesis. Cell behaviors like shape change, proliferation, growth, migration, and adhesion are activities that build structure in the embryo. These essential phenomena are often still stuck under the black box, which must be lifted to understand the dynamics that are the source of variation for evolutionary change.

Keywords

Vertebrate mesoderm · Lateral somitic frontier · Morphogenesis · Descriptive embryology · Evo-devo

Introduction

Understanding how complex animal morphology evolves remains a motivating curiosity for biologists, a curiosity fueled by two scales of the fourth dimension. On one scale is the fossil record and the deep history of both static and dynamic forms that it reveals. On another scale, the study of ontogeny/embryology provides an exquisite view of how complex form is reproduced from a single cell in each generation. Traditionally, both of these views were concentrated on the phenotype. Its essential counterpoint, the genotype, was originally entirely abstract but has acquired structure as molecular genetics exposed a rich layer of phenomena, building a detailed picture of the genetic circuits and pathways involved in the long path from egg to organism. As a result of those discoveries, developmental biology has largely become the study of developmental genes. Despite the intricacy of these data describing the genetic basis of animal diversity, a yawning gap persists between knowledge of genetic output and the emergent phenotype, exposing the limited explanatory power of a genocentric view. The morphologies generated by individual ontogenies and their visible histories in the fossil record cannot be properly integrated with studies of developmental genetics without an appreciation of the intervening plane of morphogenesis. The musculoskeletal system of vertebrates offers an example where studies of gene expression and interaction make up the vast bulk of recent published studies, while description and understanding of cell behavior and fates are almost exclusively found in the twentieth-century literature. I argue here that a full understanding of morphological evolution is currently stalled by our neglect of descriptive embryology.

A Century of Changing Perspectives

During the "New Synthesis" of the 1940s and 1950s, the central position of the phenotype was usurped by a focus on the genotype and the rigorous, quantitative power of statistical models of stasis and change. The collective focus on population genetics sets the proverbial black box squarely between genotype and phenotype, effectively removing the developmental process from the equation. Despite the clear advantages of the new synthetic approach for microevolutionary studies, the shift of focus has been much lamented as reductionist for the simple reason that it largely eliminates the organism as a factor in its own evolution. Recently, several evolutionary biologists have argued for an extended view that does not dismiss the tenets of population genetics and classic evolutionary analysis but broadens the range of phenomena considered causal in organismic evolution (see chapter ▶ "Evo-Devo's Contributions to the Extended Evolutionary Synthesis"), emphasizing a shift in focus from factors *extrinsic* to the organism – the population and natural selection – to more *intrinsic* forces that produce the essential variation for extrinsic forces to work upon. Morphological variations are generated in the embryo by developmental mechanisms, which have accumulated and altered through the unique history of a lineage. This essential component of the phenotype is reclaiming a position in current evolutionary biology, but ultimate causation is still expected at the level of the genotype.

A broader perception of causality has always been part of the venerable field of comparative morphology. The important role of embryology in the evolution of morphology was championed through the 1970s and 1980s by a minority of authors' intent on understanding the connections between ontogeny and phylogeny (see, for instance, the chapters on ▶ "Conrad Hal Waddington (1905–1975)"; ▶ "Stephen Jay Gould (1941–2002)"; ▶ "Pere Alberch (1954–1998)").

In the last 30 years, the continuous explosion of techniques and data from molecular biology has rekindled an interest in the underpinnings of animal form. The recognition that developmental genes initially discovered in *Drosophila* were virtually universal among metazoans came as a surprise to many biologists. Despite the accepted homology of basic cell architecture and most of our biochemistry, it still seemed shocking that such a diverse array of phenotypes could be generated by a common set of genes orchestrating the developmental process (Nagy 1998). The discovery of Hox genes and the later recognition of the colinear Hox code in both arthropods and vertebrates generated incredible excitement across biological disciplines. There was a clear parallel between genotype and phenotype that had previously been only perceived in a handful of genetic diseases.

The comparative analysis of these "toolkit" genes and their regulatory networks (GRNs) beyond standard model systems is the origin and motivation for the modern field of evo-devo. The molecular revolution swept up newly minted developmental biologists and pursuit of developmental genes, and their interactions became the major focus of studies of plant and animal development and evolution. Most evo-devo studies are firmly on the gene side of the genotype-phenotype divide, focused at the level of the genes and their networks. The graphics used to depict this level of

complexity are stunningly abstract compared to the animal morphology they are at some level responsible for. Despite the often exquisite models of molecular interactions, these data are essentially two-dimensional from the perspective of cells, tissues, and organisms. We have learned much about the evolutionary volatility, indeed capriciousness of these toolkit genes, but very little about how they directly affect cell behavior and morphogenesis.

In the pre-molecular form of evo-devo, development was seen as playing both a regulatory and a generative role in the evolutionary process (see chapter ▶ "Pere Alberch (1954–1998)"). The appreciation of development as an active rather than passive component of evolution is at the heart of this approach. It shifts the black box from its traditional position over the physical dynamics of cell and tissue interactions and lets it rest instead over the genetic networks which, we can safely assume, underlie these phenomena. By concentrating on the interrelated properties of cell size, shape, and cytoskeletal organization, extracellular matrix composition, adhesion, and mechanical stresses, common laws of morphogenesis are sought that may provide causal linkage between the molecular, cellular, and tissue levels. The regulatory nature of animal development buffers against both genetic and environmental perturbations in ways that minimize phenotypic upset. However, and most importantly in the context of this chapter, developmental phenomena are also the source of morphological change.

This essay seeks to bring emphasis to a perspective eclipsed in the New Synthesis and still largely ignored by mainstream trends in the field of evo-devo (see chapters ▶ "Evo-Devo's Contributions to the Extended Evolutionary Synthesis"; ▶ "Inherency"; ▶ "Mechanisms of Pattern Formation, Morphogenesis, and Evolution"). A genocentric view of both development and evolution dominates the field, dangerously limiting to our understanding of what is actually going on. As the lament that development was left out of the Modern Evolutionary Synthesis has started to fade, this is a caution not to let the embryo drop out of developmental biology. I present an example of how descriptive embryology of vertebrate body wall formation provides an essential context for exploring the consequence of gene regulation on cell behavior. Changes in that regulation or its consequences during development fuel morphological evolution. Elucidating the epistatic features of more and more genes cannot fill the persisting gap between genotype and phenotype. The medium for understanding this essential polarity is the embryo itself, the framework for the mechanisms leading to morphological innovation. Now that the genetic elements are identified, their output in terms of cellular-level phenomena is the critical step to get morphogenesis out from under the black box.

Development and Evolution of the Vertebrate Mesoderm

The genomes of vertebrates have become larger and more complex since the Cambrian. The range of cell types (>250) doesn't necessarily reflect that complexity and appear to diversify in a pattern independent of extensive changes in organismal morphology (see chapter ▶ "Devo-Evo of Cell Types"). Novelties in the deployment

of certain proteins (i.e., collagens or keratin) can lead to novel structures at stages of terminal differentiation (e.g., scales, hair, feathers, horn). However, most variation within the vertebrate body plan stems from morphological variation of the musculoskeletal system arising from allometric growth of various components, changes in proportions, or more rarely the relationships between common components (e.g., turtles (Burke 1989)).

Vertebrate Musculoskeletal System

The vertebrate musculoskeletal system comprises two basic systems, the axial and the appendicular. Within the gnathostome clade, the postcranial axial system consists of the vertebra, ribs, unpaired median fins, and associated muscles: the appendicular system comprises muscles and skeleton of the paired appendages (fins/limbs) and their respective girdles. The morphology of both individual parts of this body plan, as well as the proportions and arrangements of the parts, has undergone tremendous variation during vertebrate history, producing a remarkable array of locomotor adaptations. It is well-accepted that all of this variation, from fruit bats to hammerhead sharks, evolved from a common ancestor that lived sometime during the late Cambrian or early Ordovician. It is also understood that these variations are the result not of new genes but of new gene regulation changing the translation of local information at the cellular level into the global pattern of the body plan.

On the phylogenetic scale, a simple example of the impact of change in gene regulation is the phenomenon called vertebral transposition. A change in the number of segments in various axial body regions in vertebrates is a common form of variation between taxa. The Hox genes are known to influence the morphology of different vertebral types during development. They are also a target of evolutionary change resulting in transposition. Change in their regulation determines at what AP level along the axis they are expressed, and Hox expression boundaries correlate with the anatomical transitions and the position of the limbs in different taxa, despite different numbers of individual vertebrae in particular regions (Burke et al. 1995).

Descriptive Embryology: Phenomenology of the Lateral Somitic Frontier

The cells that form all of the key parts of the musculoskeletal system during embryonic development arise from two populations of embryonic mesoderm, the somites and somatic lateral plate (abbreviated here as LPM, Fig. 1a, b). Decades of fate-mapping work with avian embryos reveal that the somites are the source of cells that form the axial skeleton (vertebra and ribs), as well as all the striated muscles in the body (reviewed in Le Douarin 1984; Ordahl 2000). The limb skeleton arises from cells in the lateral plate, which also makes the sternum and provides all the connective tissue in the limb and ventral body wall. Thus, somitic myoblasts that will form appendicular and body wall muscles migrate extensively away from their original

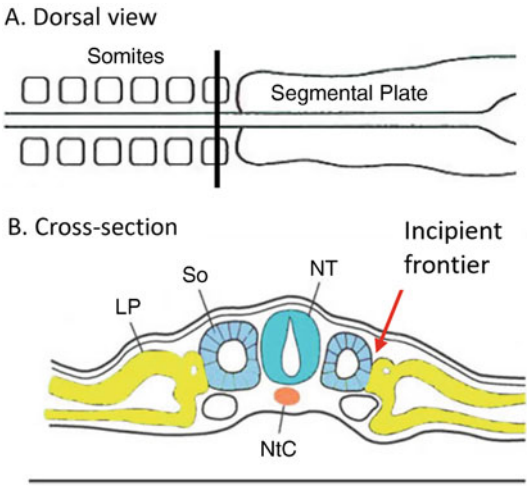


Fig. 1 Schematic representation of the mesoderm in an early chick embryo. (**a**) Dorsal view of early somites and segmental plate, anterior to the left. (**b**) Cross-section at the level indicated in **a**. NT, neural tube; NtC, notochord; So, somite; and LP, somatic lateral plate

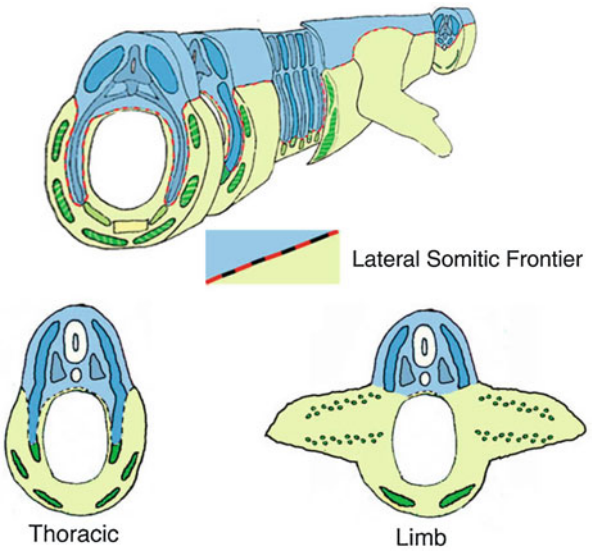


Fig. 2 Schematic amniote embryo in late stages of body wall formation. Sections show different topologies of the frontier at thoracic and limb levels. Primaxial domain is blue. Abaxial is green

segmental position and into the mesenchyme of the LPM where they differentiate. Proper function of locomotion, feeding, and respiration requires proper integration of the axial and appendicular systems during embryonic development and subsequent growth. As noted this requires extensive and orchestrated mixing of somitic and LPM cell populations. This mixing of cell lineages produces two domains in the developing vertebrate embryo (Nowicki et al. 2003, Fig. 2). The *primaxial* domain includes the axial skeleton, its muscles, and investing connective tissue, all

composed entirely of somitic cells. The *abaxial* domain is made up of somitic myoblasts that will differentiate within lateral plate-derived connective tissue. The dynamic boundary between the two domains is called the *lateral somitic frontier* (LSF). Originally based on quail-chick chimera fate maps (Nowicki et al. 2003), Cre-transgenic mice that activate a reporter gene exclusively in the lateral plate mesoderm in embryos younger than E15 (Logan et al. 2002) provide a more complete description of the frontier in the mouse embryo (Durland et al. 2008).

The traditional terms of hypaxial and epaxial distinguish between regions in the body, either above (epi-) or below (hyp-) the axis, as well as the innervation of the resident muscles from dorsal or ventral rami of the spinal nerves respectively. Primaxial and abaxial specifically refer to the dynamic embryonic tissue context of structures as they develop, rather than their adult position. The primaxial domain is the site of formation of all the epaxial muscles plus the hypaxial, ventral vertebral muscles, and intercostal muscles. The abaxial domain is the embryonic context for the appendicular system and includes LPM-derived sternum, girdle and limb bones, and all the rest of the hypaxial muscles that are invested in LPM connective tissue. Most and possibly all abaxial muscles derive from the migrating muscle precursors (MMPs), the somitic myoblasts that delaminate from the somite and migrate into the lateral plate mesenchyme.

Many forms of experimental data suggest that cells on either side of the LSF are patterned independently. Classical chick studies have demonstrated that somites from any axial level will form (abaxial) limb muscles when transplanted to limb levels (extensive reviews in Ordahl 2000). Heterotopic transplants with non-limb-level somites show similar results; cervical or thoracic somites will form abaxial abdominal muscles when transplanted to the appropriate level (Murakami and Nakamura 1991; Nowicki and Burke 2000). In the latter experiments, the primaxial structures derived from the transplanted somites (vertebrae, vertebral ribs, and associated epaxial *and* hypaxial muscles) maintain the morphological identity and Hox expression of their original AP level. The abaxial derivatives of those same transplanted somites adapt to their new location in the host lateral plate, forming host-appropriate abaxial muscles (Nowicki et al. 2003). Molecular genetic studies corroborate the physical perturbation experiments. The phenotypes of mice carrying mutations in either Hox genes or genes involved in the myogenic pathway show perturbations to combined epaxial and hypaxial elements. These defects segregate neatly however as either abaxial or primaxial (reviewed in Burke and Nowicki 2003).

To reiterate, the terminology of primaxial and abaxial domains is explicitly based on the lineage of the connective tissue. The experimental data summarized above supports the idea that control of muscle patterning resides in the connective tissue. In the context of the head and branchial region, experimental work demonstrates that connective tissue – in this case neural crest derived – determines the pattern of the branchiomeric muscles (Noden and Trainor 2005). Extensive work on the limb demonstrates a pattern-forming role for the lateral plate-derived connective tissue in limb muscle formation (Murphy et al. 2011). The repeated observation that somitic myoblasts will generate muscle patterns depending on the context (lineage)

of their investing connective tissue supports the hypothesis that the lateral somitic frontier is a boundary where pattern information changes along with the lineage of connective tissue from somitic to lateral plate. We have hypothesized that the primaxial and abaxial environments contain their own, independently regulated patterning information.

"Patterning information" is an admittedly vague term, often used in company with the concept of "positional information" (Wolpert 2016). Both of these terms are used here to describe, a posteriori, phenomena where pattern appears from non-pattern. These terms do not describe causal mechanisms. In truth, they should be recognized as euphemisms for the black box that covers all the molecular gymnastics and cell behaviors that result in a pattern.

Modularity in the Vertebrate Mesoderm

Gastrulation results in a gradient of mesodermal populations from medial to lateral, paraxial, intermediate, and lateral plate, reflecting the sequence of ingression. As development proceeds, the intermediate mesoderm sinks into the coelom, and a border is formed between the somitic and lateral plate mesoderm. This boundary is the incipient frontier and becomes clearly visible after segmentation (Fig. 1a).

Colinear Hox expression linked to anterior-posterior patterning is active before gastrulation as expression in the epiblast has a causal influence on the timing of ingression of cells destined for both paraxial and LP mesoderm (Moreau et al. 2019). The full dynamics is far from being understood, but by the pharyngula stage, the Hox code is offset between embryonic cell populations. AP Hox expression boundaries are more anterior in the CNS than in the paraxial mesoderm, and there is another offset of Hox expression between paraxial and lateral mesoderm (reviewed in Wellik 2007). Thus, the Hox expression profile of cells on either side of the frontier is not equivalent.

Such a boundary between cell populations of different history and gene activity conforms to Meinhardt's description of an organizing region: "Boundaries create new positional information, and its interpretation leads to new boundaries, etc." (Meinhardt 1983, p. 384). The resident Hox code in each population provides some degree of positional information that we assume influences the behavior/potential of cells on either side. Cell behaviors affecting changes in shape, adhesion, proliferation, growth, expansion, and migration vary along the AP axis. The regionalization of the axis into cervical, thoracic, etc. is accomplished by different behaviors of cells acting in the dorsoventral or mediolateral plane. Cell behavior and the gene expression that provoke or respond to it are manifest at the frontier. In the thoracic region, for instance, the frontier retreats as the primaxial anlage of ribs and intercostal muscles advance from the dorsal midline to approach the ventral midline. In the limb regions, the frontier stands its ground relative to axial structures and is transgressed by migrating myoblasts (MMPs) that will form abaxial muscles.

To summarize this development using Meinhardt's language, gastrulation sets up an initial set of boundaries between mesodermal populations. The resident Hox code

in each population provides positional information that influences different behaviors of cells at different AP levels along the incipient frontier. As the topography of the frontier changes in these regionalized ways, secondary embryonic fields come into play providing new positional information. The key element here is that the embryonic environment of one cell population can provide new positional information to another. The embryonic environment is in part determined by the resident molecular fruit salad in the nuclei of its cells, a product of their history. As somitic myoblasts cross the frontier they interact and migrate within a population of mesenchymal cells with a different lineage and history of gene regulation. The primaxial and abaxial domains fit the structural and molecular definition of developmental modules. Their relative independence across Meinhardt-type developmental boundaries provides a site for viable developmental variations enhancing evolvability of the musculoskeletal system.

Evolution on the Frontier: Innovation in the Abaxial Domain

The experiments and observations on mesodermal development provide a perspective on the evolution of the morphologies they produce. The most extensive development of the abaxial domain is in the paired appendages. Fin/limb buds arise as outgrowths of the embryonic somatopleure (the LPM and overlying ectoderm) that are then infiltrated by somitic MMPs that cross the frontier to form the abaxial domain. Comparative studies show variation in MMP behavior across the jawed vertebrates, and these data contribute to changing views of key evolutionary events at major nodes in the vertebrate phylogeny (see Wotton et al. 2015; Okamoto et al. 2019; Tani-Matsuhana et al. 2018). The dramatic evolutionary variability of the appendicular system arises from coordinated development across the lateral somitic frontier leading to innovation.

Origin of Paired Appendages

Paired appendages appear at the base of the gnathostomes and clearly had a profound influence on the subsequent radiation of vertebrate taxa. The origin of paired appendages is a fascinating field. Traditionally focused on the fossil record, recent hypotheses include both developmental and paleontological data and concepts (see Nuño de la Rosa et al. 2013; Johanson 2010; Charest et al. 2018). The extant jawless fish, the lamprey and hagfish, have sophisticated cranial features in keeping with other craniates, but along with the absence of jaws, they also completely lack paired appendages. Thus, lampreys appear to retain the plesiomorphic condition for vertebrates and offer a proxy view of ancestral postcranial body wall development, in the absence of an appendicular system.

The lamprey has been extensively studied, and there is a recent wealth of evo-devo studies on the molecular genetics of lamprey development (reviewed by York et al. 2019). Like other vertebrates, lamprey somites appear structurally

homogeneous, but there is spatial heterogeneity in the expression of several muscle-specific structural genes (Kusakabe et al. 2011). In early descriptions, the ventrolateral edge of the lamprey "mesoblast" advances between the ectoderm and endoderm to provide the mesodermal component of the body wall. These classical embryological studies are challenging to interpret and sometimes difficult to reconcile with modern terminology. The nature of the lateral plate is particularly murky, leaving open the question of what role this tissue plays in a creature that never evolved an appendicular system.

To directly explore the role of the LPM in lamprey, Tulenko et al. (2013) confirmed the expansion of a thin cell layer advancing between the ectoderm and endoderm visible in plastic sections. This tissue is in the position of presumptive LPM (PLPM), segregated from the somites by pronephric tubules from the intermediate mesoderm. DiI injections to either somites or PLPM in early embryos were employed to fate-map these tissues. The results show that after the advancement of the PLPM around the yolk, this tissue is displaced from the ectoderm by the leading edge of somites. Thus, the somatopleure (the ectoderm and somatic lateral plate together) is disrupted by the advancing somites, which extend to meet at the ventral midline closing the body cavity. The resulting muscular body wall is thus entirely primaxial. The PLPM is not infiltrated by myoblasts and is confined to the inner lining of the coelom. Compared to the histological data from shark, and fate-mapping data from axolotl and amniotes, the lamprey is unique in this regard (Tulenko et al. 2013).

Comparative studies of GRNs now associated with outgrowth and patterning of the fin/limb suggest they were co-opted from the embryonic fields of preexisting structures such as the median fins, the gill arches, and the heart (Freitas et al. 2006; Gillis et al. 2009). Gene expression leading to novel patterning requires tissue territory in which to deploy, and any hypothesis on the evolution of paired appendages must explain changes in distributions and behavior in the cell lineages involved. The fin/limb buds, as mentioned, arise from the somatopleure. The formation of the body wall in lamprey disrupts the somatopleure and sequesters the LPM into the coelom.

The initial steps in the evolution of paired appendages from an ancestral lamprey-like, primaxial body wall could have been extremely simple. A change in proliferation rates in the PLPM or a shift in adhesion between the advancing somite and the ectoderm during body wall closure could result in a portion of somatopleure remaining intact on the surface of the body wall. This "error" in tissue distribution would provide a novel canvas for evolutionary innovation (Tulenko et al. 2013). The new domain would be developmentally independent from the long-established axial system, and new genetic and cellular interactions could play out without immediate compromise to ancestral structures. The axial Hox code and its axial corollaries could remain unchanged, but the axial code's interpretation at the frontier would be a site for developmental experimentation, presenting variation to natural selection (Shearman and Burke 2009).

Once the abaxial domain and an appendicular system were established, the frontier continued to provide a site for innovation (Fig. 3). For instance, the amniotes

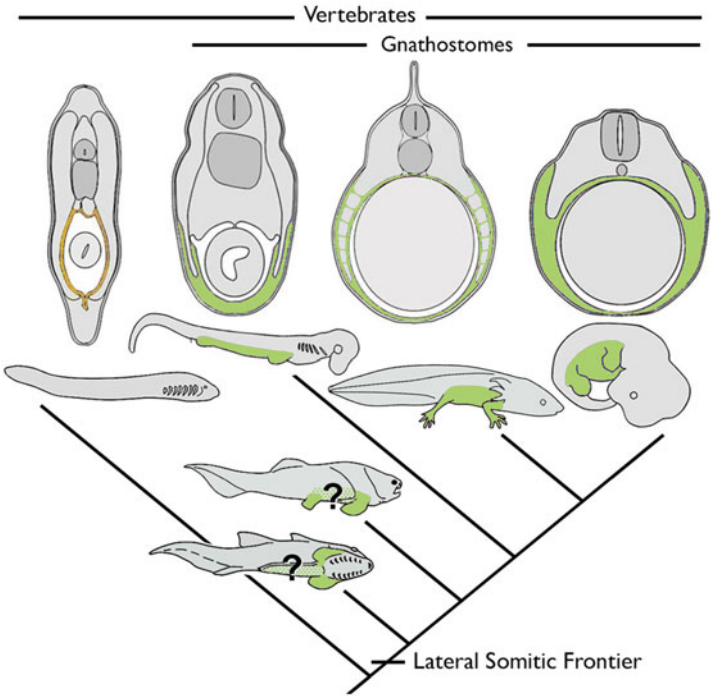


Fig. 3 Cladogram of selected vertebrate taxa. The internalized in the lamprey is shown in yellow. The green illustrates the abaxial domain. The lateral somitic frontier and potential for abaxial regions are hypothesized to be an innovation at the base of the gnathostome lineage and to have expanded considerably on tetrapods. (modified from Tulenko et al. 2013)

display many class-based innovations of abaxial structures. The plastron of turtles, the extensive keel of the avian sternum, and the mammalian diaphragm are all structures arising in the abaxial domain. The odd number of "cervical" vertebrae in sloths (Buchholtz and Stepien 2009) as well as limblessness in snakes and lizards (Head and Polly 2015) has been attributed to changes along the frontier primarily affecting the abaxial domain.

Toward the Future

The molecular revolution sparked major advances in our knowledge of development and evolution. Our conception of how morphology evolves however is too often expressed in the two-dimensional language of gene expression and interaction. Such a view sharply focused at one level of biological complexity does little to populate the four-dimensional space between genotype and phenotype, risking a teleological myopia regarding causation.

The recent 100-year anniversary of D'Arcy Thompson's *On Growth and Form* has inspired a number of special volumes reassessing the impact of Thompson's focus on physical rules of morphogenesis (*Journal of International Development*, vol 50, 2006; *Developmental Dynamics*, vol. 245:3, 2016; *Mechanisms of Development*, vol 145, 2017; *Development*, vol 144:23, 2017). What is abundantly clear from the papers in these volumes is the powerful integration of physics, mathematics, computing, and cell biology. The integration grows from the expansion of tools and technologies to model, manipulate, and visualize morphogenetic processes in vivo. Perhaps especially, the ability to make visible the dynamic properties of cells is essential for bringing together physical and molecular aspects of development to address the questions framed in *On Growth and Form*. Interestingly, a majority of the papers in the volumes cited above barely mention genes.

The discovery and exploration of the axial Hox code and how it is "read" in the lateral plate to regionalize the AP axis provide a case in point regarding the explanatory limits of developmental genetic approaches. There is a great wealth of careful descriptive and elegant experimental work that has identified and tested the impact of Hox and other genetic players on morphogenetic events that occur along the axis, from somitogenesis to the muscle regulatory cascade and limb positioning. The majority of these genes are transcription factors whose presence or absence can be correlated with aberrant phenotypes, but don't reveal any clear physical connection to cell behavior. The homeodomain itself is a notoriously promiscuous binding site, and understanding anything discrete about Hox effects has proven elusive (see papers in Gofflot et al. 2018). That cells do not migrate from specific somites into the limb buds in the absence of a specific transcription factor (e.g., Lbx) is an important observed fact, but doesn't say anything about actual cell behavior other than they fail to migrate. The knowledge that they normally *do* migrate is all important and arises from classic embryological studies (Le Douarin 1984).

The universal phenomenon of mesodermal segmentation in vertebrate embryos provides another example of how gene expression and GRNs have come to dominate our explanations of development. The formation of epithelial somites from a uniform mesenchyme in ordered progression has been studied by generations of developmental biologists and remains an active area of research. In the 1970s and early 1980s, the literature was full of studies aimed at understanding the interactions between embryonic structures and the effects of chemical or physical perturbation on the segmentation process (reviewed by papers in French et al. 1988). These studies were focused on cells and tissues and the possible forces generated by and responded to during the rearrangement of cells resulting in individual segments. For instance, Belousov (Naumidi and Belousov 1977) proposed a model based on Cooke and Zeeman's (1976) "clock and wavefront" model of segmentation. Cells of the segmental plate, subject to some kind of cellular oscillator, form basally connected, fan-shaped groups that succumb to bending forces at a certain size dictated by the physical stretching limit of the cells, hence "rolling up" as epithelial balls – somites. The clock depends on a cell's autonomous behavior, the wavefront is determined

by physical forces, and together they "control" an ordered process producing equally sized somites of the appropriate number. In a rare and satisfying instance of confirming old models with new data, work initiated in the Pourquie lab revealed a beautifully oscillating array of gene expression consistent with the clock and wavefront model (Pourquie in Ordahl 2000). Causality is indicated by mutant phenotypes, and there is no doubt this elaborate regulatory system, involving the interaction of dozens of toolkit genes, has evolved to produce reliable segmentation. However, how these genes directly affect cell behavior to physically produce segments from non-segments is still unknown. New work studying mechanical processes and self-organizing behavior of cellular mechanics of segmentation might fill this gap by providing cellular-level phenomena along the lines of Belousov's model (see Adhyapok et al. 2019).

Clearly there is no ignoring the importance of genes. However, it is higher levels of organization that provide essential context that influences gene action. In the ontogenetic time frame, products of the same gene are deployed heterogeneously in time and space by complex regulation that itself is context dependent. As far as we understand it, the context is multidimensional, involving many levels of the biological hierarchy. Pleiotropy is the norm in complex organisms, where a single gene can have multiple roles within the same individual. As a result, change is necessarily generated at a higher hierarchical level than the gene itself, through epigenetic interactions (in the broadest sense of *anything* above the level of the code itself). Within the nucleus, molecules converge to generate a cocktail of transcription factors, cofactors, microRNAs, and no doubt players yet to be described. The cytoplasm offers its own world of posttranslational modifications, the cytoskeleton and organelles, and outside an individual cell, the influential context expands to the physiochemical properties inherent in different tissues and environments.

Despite the often-derogatory use of the term in current usage, "descriptive studies" of embryos are still a cornerstone of our collective knowledge of the dynamic process of embryogenesis. Knowledge of cell lineage and behavior are essential for designing experiments to test hypotheses of mechanism. Once the pattern of cell distribution is clear, the regulation and expression of relevant genes can be viewed in perspective and linked specifically to the morphogenetic events that characterize cellular events. Just as it is obvious that the same gene can be used to different effects at different times and places in the embryo, it is also obvious that a full and accurate description of those times and places is necessary to understand gene function.

Evolutionary change requires variation – the embryo reflects both the history of a lineage and its potential futures, because this is the place where morphological variation is generated. As comparative genomics can expose the origin of novel genetic networks, common or unique developmental patterns in extant organisms can be explored as factors that influenced the evolutionary trajectory of a lineage into the present. An explicit recognition of the importance and complexity of the embryonic context in which genes act, including the physical details of communication, adhesion, attraction, inhibition, etc., is necessary before a useful causality can be understood between genotype and phenotype.

Cross-References

- ▶ Computational Modeling at the Cell and Tissue Level in Evo-Devo
- ▶ Devo-Evo of Cell Types
- ▶ Evo-Devo's Contributions to the Extended Evolutionary Synthesis
- ▶ Evolution of Skeletal Tissues
- ▶ Inherency
- ▶ Mechanisms of Pattern Formation, Morphogenesis, and Evolution
- ▶ Pere Alberch (1954–1998)
- ▶ Stephen Jay Gould (1941–2002)

References

Adhyapok P, Piatkowska AM, Clendenon SG, Stern CD, Glazier JA, Belmonte JM (2019) A Mechanical Model of Early Somite Segmentation. bio Rxiv 804203

Buchholtz EA, Stepien CC (2009) Anatomical transformation in mammals: developmental origin of aberrant cervical anatomy in tree sloths. Evol Dev 11(1):69–79

Burke AC (1989) Development of the turtle carapace: implications for the evolution of a novel bauplan. J Morphol 199(3):363–378

Burke AC, Nowicki JL (2003) A new view of patterning domains in the vertebrate mesoderm. Dev Cell 4(2):159–165

Burke AC, Nelson CE, Morgan BA, Tabin C (1995) Hox genes and the evolution of vertebrate axial morphology. Dev 121(2):333–346

Charest F, Johanson Z, Cloutier R (2018) Loss in the making: absence of pelvic fins and presence of paedomorphic pelvic girdles in a Late Devonian antiarch placoderm (jawed stem-gnathostome). Biol Lett 14(6):20180199

Cooke J, Zeeman EC (1976) A clock and wavefront model for control of the number of repeated structures during animal morphogenesis. J Theor Biol 58(2):455–76

Durland JL, Sferlazzo M, Logan M, Burke AC (2008) Visualizing the lateral somitic frontier in the Prx1Cre transgenic mouse. J Anat 212(5):590–602

Freitas R, Zhang G, Cohn MJ (2006) Evidence that mechanisms of fin development evolved in the midline of early vertebrates. Nature 442(7106):1033

French V, Ingham P, Cooke J, Smith J (1988) Mechanisms of segmentation. Dev sup 104:1–249

Gillis JA, Dahn RD, Shubin NH (2009) Shared developmental mechanisms pattern the vertebrate gill arch and paired fin skeletons. Proc Natl Acad Sci 106(14):5720–5724

Gofflot F, Jeannotte L, Rezsohazy R, Krumlauf R, Gaunt SJ, Gentile C, Kmita M, Casaca A, Hauswirth GM, Bildsoe H, Mallo M (2018) Hox genes: past, present and future of master regulator genes. Int J Dev Biol 62:653–876

Head JJ, Polly PD (2015) Evolution of the snake body form reveals homoplasy in amniote Hox gene function. Nature 520(7545):86

Johanson Z (2010) Evolution of paired fins and the lateral somitic frontier. J Exp Zool B 314:347–352

Kusakabe R, Kuraku S, Kuratani S (2011) Expression and interaction of muscle-related genes in the lamprey imply the evolutionary scenario for vertebrate skeletal muscle, in association with the acquisition of the neck and fins. Dev Biol 350(1):217–227

Le Douarin N (1984) Chimeras in developmental biology. Academic Press San Diego, CA

Logan M, Martin JF, Nagy A, Lobe C, Olson EN, Tabin CJ (2002) Expression of Cre recombinase in the developing mouse limb bud driven by a Prxl enhancer. Genesis 33(2):77–80

Meinhardt H (1983) Cell determination boundaries as organizing regions for secondary embryonic fields. Dev Biol 96(2):375–385

Moreau C, Caldarelli P, Rocancourt D, Roussel J, Denans N, Pourquie O, Gros J (2019) Timed collinear activation of Hox genes during gastrulation controls the avian forelimb position. Curr Biol 29(1):35–50

Murakami G, Nakamura H (1991) Somites and the pattern formation of trunk muscles: a study in quail-chick chimera. Arch Histol Cytol 54(3):249–258

Murphy MM, Lawson JA, Mathew SJ, Hutcheson DA, Kardon G (2011) Satellite cells, connective tissue fibroblasts and their interactions are crucial for muscle regeneration. Development 138(17):3625–3637

Nagy L (1998) Changing patterns of gene regulation in the evolution of arthropod morphology. Am Zool 38(6):818–828

Noden DM, Trainor PA (2005) Relations and interactions between cranial mesoderm and neural crest populations. J Anat 207(5):575–601

Nowicki JL, Burke AC (2000) Hox genes and morphological identity: axial versus lateral patterning in the vertebrate mesoderm. Dev 127(19):4265–4275

Nowicki JL, Takimoto R, Burke AC (2003) The lateral somitic frontier: dorso-ventral aspects of anterior-posterior regionalization in avian embryos. Mech Dev 120(2):227–240

Okamoto E, Moriyama Y, Kuraku S, Kai KI, Tanaka M (2019) Involvement of HGF/MET signaling in appendicular muscle development in cartilaginous fish. Develop Growth Differ 61(1):97–103

Ordahl CP (ed) (2000) Somitogenesis, vol 1 & 2. Academic Press, San Diego

Shearman RM, Burke AC (2009) The lateral somitic frontier in ontogeny and phylogeny. J Exp Zool B Mol Dev Evol 312(6):603–612

Tani-Matsuhana S, Kusakabe R, Inoue K (2018) Developmental mechanisms of migratory muscle precursors in medaka pectoral fin formation. Dev Genes Evol 228(5):189–196

Tulenko FJ, McCauley DW, MacKenzie EL, Mazan S, Kuratani S, Sugahara F, Kusakabe R, Burke AC (2013) Body wall development in lamprey and a new perspective on the origin of vertebrate paired fins. Proc Natl Acad Sci 110(29):11899–11904

Wolpert L (2016) Positional information and pattern formation. Curr Top Dev Biol 117:597–608

Wotton KR, Schubert FR, Dietrich S (2015) Hypaxial muscle: controversial classification and controversial data? In: Brand-Saberi B (ed) Vertebrate myogenesis. Results and problems in cell differentiation, vol 56. Springer, Berlin/Heidelberg

York JR, Lee EMJ, McCauley DW (2019) The lamprey as a model vertebrate in evolutionary developmental biology. In: lampreys: biology, conservation and control. Springer, Dordrecht, pp 481–526

Development and Evolution of the Neck Muscles

Rie Kusakabe and Shigeru Kuratani

Contents

Abstract

The neck, which is conspicuous especially in amniotes, is a vertebrate body part found between the head and shoulder (pectoral) girdle. This domain is associated with a complicated set of skeletal muscles that play crucial roles in respiration, swallowing, vocalization, and movement of the head. Although the neck is less obvious in anamniotes, it can be identified by developmental and morphological patterns of postpharyngula embryos. Embryological and experimental approaches have shown complex behavior of neck muscle precursors, especially in amniotes. Modern genetic engineering has made it possible to label a restricted population of muscle precursor cells to trace their developmental fates, and the neck muscles are now shown to be of both somite origin and head mesodermal origin. Studies of the cyclostomes and the chondrichthyes have shown that the

R. Kusakabe (✉)
Laboratory for Evolutionary Morphology, RIKEN Center for Biosystems Dynamics Research (BDR), Kobe, Japan
e-mail: rie.kusakabe@riken.jp

S. Kuratani
Laboratory for Evolutionary Morphology, RIKEN Center for Biosystems Dynamics Research (BDR), Kobe, Japan

Evolutionary Morphology Laboratory, RIKEN Cluster for Pioneering Research (CPR), Kobe, Japan
e-mail: shigeru.kuratani@riken.jp

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_191

invention of the neck with overt hypobranchial and cucullaris muscles appears to have taken place roughly at the origin of gnathostomes.

Keywords

Vertebrates · Development · Skeletal muscle · Neck

Introduction

Vertebrates are defined by possession of an overt head, and for this reason, they are often regarded as forming an independent phylum distinctly different from other chordates (Irie et al. 2018). In jawed vertebrates, the head is connected to the trunk by way of a domain called the "neck," defined as the body part between the occipital region and the shoulder girdle. Morphologically, actively movable neck is most conspicuous only in amniotes (birds, reptiles, and mammals) as a coelom-free body part connecting the head and trunk (Fig. 1a), enveloping visceral structures including the esophagus and trachea, and serving as a path for food intake and respiration. The aim of this chapter is to overview the history and current insights of evolutionary developmental studies on the neck, with a particular emphasis on the muscles.

Cartilage and muscle connective tissues of the head and neck primarily derive from the cephalic neural crest (NC) cells (Fig. 1a; reviewed by Noden and Francis-West 2006). In particular, the postotic NC, which gives rise to both the vagal and cardiac NC cells, exhibits a complex pattern of distribution, since they contain both trunk- and cranial types of NC cells (Fig. 1c and d); the major population of trunk-type NC cells migrate along the ventrolateral pathway (Fig. 1d; within somites) to form the so-called Froriep's ganglia (Fig. 1d; the vestigial spinal dorsal root ganglia; a trunk component) and to support cells associated with the developing hypoglossal nerve (CNXII), whereas the cephalic component of the postotic NC cells – that is, the circumpharyngeal NC cells – migrate dorsolaterally beneath the surface ectoderm (Fig. 1c and d), is excluded by the rostral somites to form a posteriorly open arch-like distribution and populates ventrally to form the ectomesenchyme in postotic pharyngeal arches (PAs 3-6), the cardiac ectomesenchyme, and the enteric ganglia (Kuratani 1997).

Skeletal myoblasts are also specified under distinct regulatory mechanisms in the head and trunk. The cranial mesoderm and somites, the embryonic sources of skeletal muscle cells, form bilaterally along the body axis, with the former arising as an unsegmented mesenchyme and the latter as transitory epithelial blocks. In the trunk skeletal muscle development, *Pax3*, a gene encoding a paired class homeodomain transcription factor, is expressed in early dermomyotomes, or the lateral epithelial layer of somites, and plays a key role in the specification of muscle lineage. Later, within the *Pax3*-positive ventral dermomyotomal cells, myoblasts at the limb levels are marked with expression of *Lbx1*, encoding a lady bird class of homeodomain transcription factors. These cells delaminate from the ventral edge of the dermomyotomes and migrate into limb buds to differentiate into the limb

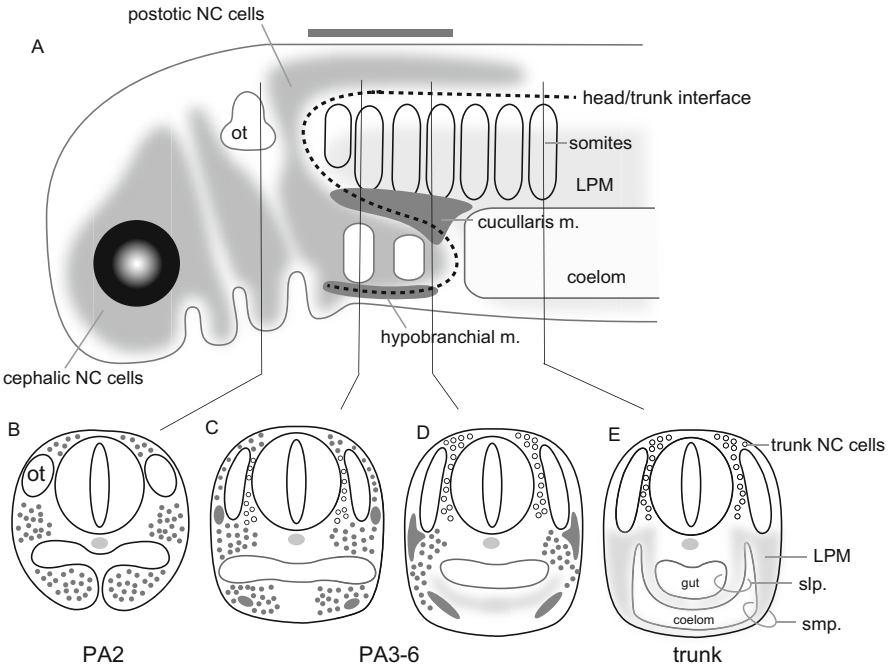


Fig. 1 Schematic distribution of embryonic domains in the vertebrate head/neck region. (**a**) S-shaped head/trunk interface represents the posterior limit of distribution of cranial neural crest (NC) cells, which migrates along the dorsolateral pathway. The neck corresponds to the region where the cephalic, somatic, and lateral plate mesoderm (LPM) come into contact. The region that gives rise to the developmental components of the "neck" is indicated by the horizontal bar (top). (**b**-**e**) Transverse views at the levels of the second pharyngeal arch (PA2, **b**), of the posterior pharyngeal arches (PA3-6, **c**, and **d**) and of the trunk (**e**). Gray dots indicate the distribution of the cranial type of NC cells, whereas open dots indicate the trunk type of NC cells. LPM, lateral plate mesoderm; NC, neural crest; slp., splanchnopleura; smp., somatopleure. (Based on Kuratani 1997)

musculature. *Pax3* upregulates *c-Met*, encoding a receptor of the HGF ligand, a signaling component required for the migration of the myoblasts. The chemokine receptor CXCR4 and its ligand SDF1 also play roles in directed movement of the migratory muscle precursors (see Vasyutina et al. 2005 and references therein).

In contrast, cranial myoblasts are specified by *Mesp1, Islet1,* and *Tbx1* (reviewed in Sambasivan et al. 2011). This mesoderm is also termed the cardiopharyngeal mesoderm, since it contains cardiomyogenic progenitors derived from an early embryonic domain called the second heart field (SHF). Cranial mesoderm derivatives include extraocular muscles derived from the paraxial mesoderm, and other branchiomeric muscles. *Pax3* has not been suggested to function in cranial myogenesis, but its paralogue, *Pax7,* plays a major role in the regulation of persistent muscle stem cells.

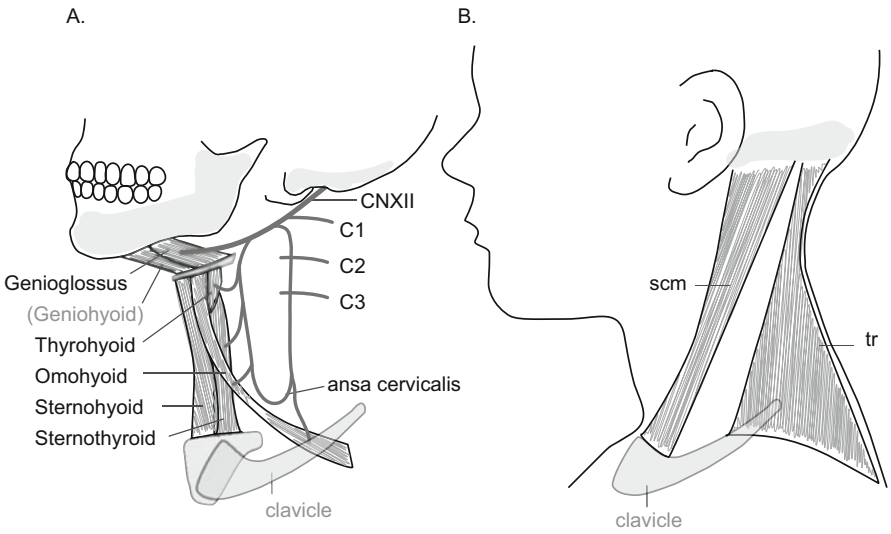


Fig. 2 Overview of human neck muscles. (**a**) A schematized view of the hypobranchial muscles. The labeled muscles belong to the hypobranchial muscles, except geniohyoid, which is innervated by C1 of CNXII. (**b**) The cucullaris muscles, represented by the sternocleidomastoid (scm) and trapezius (tr). Scm and tr connect the occipital region of the skull, the clavicle, and scapula (not shown), but its evolutionary precursor, the cucullaris, used to connect primarily the trunk, cranium, and the visceral skeleton (see Fig. 3)

In the pharyngula, since the rostralmost somites and caudal pharyngeal arches overlap each other dorsoventrally, these two embryonic environments overlap to form the S-shaped interface (Fig. 1a). This interface represents a unique and peculiar embryonic domain where different embryonic components meet each other to form a highly elaborated body part of vertebrates. The representative muscles developing at this interface, the *hypobranchial* and *cucullaris* muscles (Fig. 2), connecting the head (occipital part of the cranium, visceral arch skeletons, and their derivatives) and shoulder girdle, have always been a major target of investigation in evolutionary studies of jawed vertebrates (reviewed by Kuratani 1997; Ericsson et al. 2013; Tada and Kuratani 2015).

Development of the Amniote Hypobranchial Muscles

The tongue and infrahyoid muscles are collectively called **hypobranchial muscles**, which are characterized by their connection and positions (Fig. 2a): these muscles are connected to visceral skeletal elements, and they are not found in the body wall like other hypaxial trunk muscles but rather directly cover the visceral organs. The hypobranchial muscles comprise most of the muscles of the tongue and the muscles attaching the branchial or hyoid apparatus and their derivatives to the pectoral girdle (infrahyoid muscles). The neurons controlling the hypobranchial muscles

(occipitospinal nerves, including CNXII and cervical ansa in mammals; Fig. 2a) are generally somatic motor in nature, similar to spinal motoneurons.

Edgeworth (1935) described histological observations of developing neck muscle in a variety of vertebrate embryos and proposed presence of premyogenic condensations ("muscle plates") that give rise to conserved sets of head and neck muscles (Edgeworth 1935; reviewed by Miyake et al. 1992). In this theory, the hypobranchial muscle develops from "epi- and hypobranchial muscle plates," with the premyogenic condensation originating from a few anterior somites. Thus, the hypobranchial muscles in jawed vertebrates are derived from anterior somites (Fig. 3a)

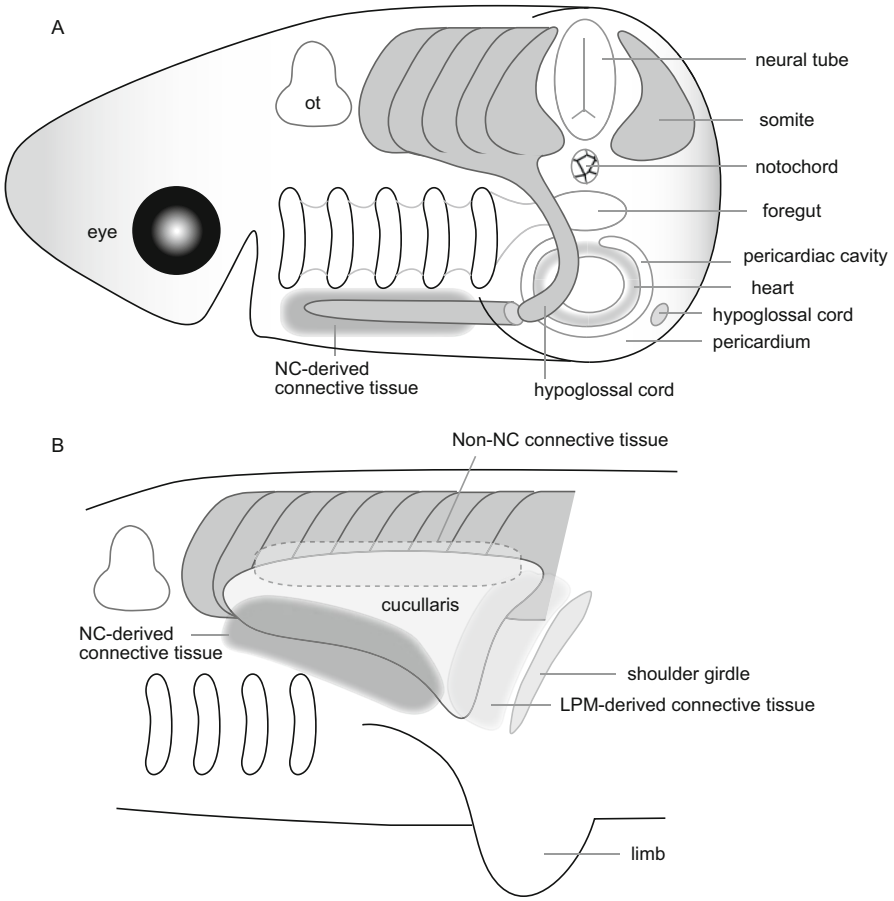


Fig. 3 Spatial relationships of neck muscles with the surrounding embryonic tissues based on Heude et al. 2018. (**a**) Development of the hypobranchial muscles. Precursors of hypobranchial muscles emerge from the ventral side of the rostral somites and proceed on the hypoglossal cord that posteriorly traces the pharyngeal region. The connective tissue of hypobranchial muscles is derived from NC cells. (**b**) Cucullaris muscle, which develops under a branchiomeric myogenic program, is associated with connective tissues of mixed origin. Both NC and LPM provide connective tissues of cucullaris. *ot* otic vesicle

(somite 2–5; reviewed in Noden and Francis-West 2006). The hypobranchial myoblasts migrate along the caudal aspect of the postotic pharyngeal arches, rostrally within the pericardium, to enter the oral floor in the anterior direction as a tightly condensed strand called the hypoglossal cord. Thus, the somatic neck muscles also grow within the lateral body wall during their development, in the same manner as other ventral skeletal muscles (Fig. 3a).

In the ventral portion of the dermomyotome, hypobranchial muscle precursors reside as the *Lbx1*-positive myoblasts and undergo long-distance migration, which led to the idea that these muscles are specified in a way similar to that of limb muscles. However, in mice carrying deficiency in the *c-met* or *Lbx1* genes that results in loss of the limb muscles, the tongue muscles are partially present, although reduced in size (Bladt et al. 1995; Brohmann et al. 2000). Recent analysis showed that *Tbx1 and Mesp1*, but not *Pax3*, are required for normal tongue muscle formation, suggesting a head muscle-like regulation in the development of hypobranchial muscles (Heude et al. 2018).

Directed movement of the hypoglossal cord is also enigmatic. HGF ligand is not present along the hypoglossal cord, and HGF- or SDF1-soaked beads cannot interrupt its normal migratory pathway. These experiments suggest that the hypobranchial muscle precursors are specified and guided by a distinct mechanism from that of the limb muscles, such as collective cell movements driven by surrounding tissues (as suggested by Lours-Calet et al. 2014). However, the relationships of these surrounding tissues and the future nonmuscle tissues associated with hypobranchial muscles, that is, cartilage and connective tissues, remain to be elucidated.

As for the cell lineage of the cartilage and connective tissues attaching the hypobranchial muscle, the chick-quail transplantation showed that the connective tissue component of the rostral hypobranchial muscles are of NC-origin (Fig. 3a) (Köntges and Lumsden 1996). Detailed information has been obtained in mice, in an analysis using Cre-mediated conditional labeling. The caudal side the intrinsic tongue musculature, as well as the rostral side, was associated with *Wnt1-/Sox10*-positive NC-derived connective tissues (Matsuoka et al. 2005). This notion has also been supported by a recent analysis defining the hypobranchial muscles as one of the *Mesp1/Pax3*-positive mesodermal derivatives associated with *Wnt1*-positive, NC-derived connective tissues (Heude et al. 2018). Thus, the hypobranchial muscles have a composite nature, originating from somites but completing their development within the craniofacial environment and patterned by NC cells (Noden and Francis-West 2006).

Development of the Amniote Cucullaris Muscles

Cucullaris muscles represent another group of head/trunk interfacial components located between the branchiomeric series of cephalic muscles (innervated by special visceral efferent neurons) and the rostral somitic muscles (innervated by general somatic efferent neurons). In the mammalian neck, the counterparts to the cucullaris are the sternocleidomastoid and trapezius muscles located in the superficial layer (Fig. 2b). The former has its origin in the occipital bone and is inserted in the sternum

and clavicles, whereas the latter is a large, triangular muscle found at the back of the shoulder and is further divided into three rostrocaudal domains associated with different origins and insertions. These muscles, innervated by the spinal accessory nerve (CNXI) as well as by the ventral rami of some cervical spinal nerves, together form an extensive connection among the occipital bone, the shoulder girdle, and the cervical/thoracic vertebrae.

In contrast, nonmammalian gnathostomes possess only a single cucullaris muscle, which is termed variously in numerous species (reviewed in Miyake et al. 1992), and supposed to represent the ancestral musculature that was split into two parts (trapezius and sternocleidomastoid) in the mammalian lineage. This muscle is located between the visceral arches and the trunk, and its evolutionary origins have long been in dispute (reviewed by Noden and Francis-West 2006; Sambasivan et al. 2011; Ericsson et al. 2013; Tada and Kuratani 2015). The cucullaris muscles once were described as one of the derivatives of the "branchial muscle plate" by Edgeworth (reviewed by Miyake et al. 1992). This cell population contributes to both dorsal and ventral components of the branchial muscles, and the cucullaris muscles are the most dorsal component. However, no apparent myogenic condensation was observed, leaving the existence of the actual "muscle plate" differentiating into cucullaris in question.

Later embryological studies, such as those mapping anterior somites in chicken and axolotl (described below), demonstrated a major somitic contribution to cucullaris muscles (Noden 1983; Huang et al. 2000). Based on their genetic lineage tracing in mice, Matsuoka and colleagues also proposed that the mouse trapezius muscles were partially somite-derived (Matsuoka et al. 2005). This view is consistent with innervation of the trapezius by the spinal accessory nerve (XI) and, in many cases, by C3 and C4 spinal nerves. It has also been suggested that the nature of the spinal accessory motoneurons is intermediate between branchial and somatic motor (reviewed by Tada and Kuratani 2015).

On the other hand, some recent studies have provided insights that appear to oppose the somitic origin of cucullaris muscles. In both mice and chick, the cucullaris muscles are free of expression of *Pax3/Pax7*, the major driver of somitic muscle proliferation (Amthor et al. 1999). Genetic cell lineage tracing in mice has also shown that the trapezius muscle is associated with solely NC-derived connective tissues, although the scapular spines they attach undergo endochondral ossification, a conventional ossification pattern of the mesodermal skeleton (Matsuoka et al. 2005). Moreover, some shoulder bones, such as the clavicle, were revealed to have a dual origin – the posterior margin of the clavicle, attached by somite-derived pectoral and deltoid muscles, is mesodermal in origin, although it undergoes dermal ossification, which was previously thought to indicate NC origin. In other words, some bones in the shoulder girdle are derived from a cryptic combination of NC and mesoderm. This study revealed that a boundary exists between these two cell lineages exists even within a seemingly single bone, which corresponds well with the origin of connective tissues through which the muscle attached. These insights raised the possibility that cucullaris muscles would differentiate in concert with NC-derived connective tissues and skeletons.

Another nonsomitic theory of cucullaris was proposed based on transplantation experiments in chick embryos, which suggested that cucullaris muscles were derived from an "occipital lateral plate mesoderm (LPM)" adjacent to somites 1-3 (Theis et al. 2010). It was also revealed that the occipital LPM expresses head-muscle-related genes, such as *Islet1 and Tbx1*, but not somitic genes such as *Pax3*. These observations led to the conclusion that the "occipital LPM" deploys the program of head development, which has been proposed to facilitate late onset of muscle differentiation (Theis et al. 2010).

In mice, clonal analysis showed that trapezius muscles are clonally related to the SHF-derived myocardium (Lescroart et al. 2015). Trapezius muscles, as well as the laryngeal muscles, are absent following mutation of the *Tbx1* gene, a key regulator of cephalic muscle and SHF development. Moreover, Heude and colleagues added persuasive evidence for the cardiopharyngeal origin of cucullaris muscles (Heude et al. 2018). Their study proposed three distinct myogenic programs involved in the formation of the neck/shoulder region: branchiomeric, anteriormost-somitic, and posterior somitic. They also defined a new boundary between the NC and mesoderm contributing connective tissues of the neck musculature, in which the connective tissues of the cucullaris muscles are of a mixed origin (Fig. 3b). The cucullaris muscles develop in a cranial NC domain in the early stages, then expand to incorporate connective tissues from both the NC and LPM populations, but the cucullaris muscles themselves did not develop from the LPM.

Thus, although questions still remain as to the precise classification of cucullaris muscle precursors, both hypobranchial and cucullaris muscles seem to achieve complex connectivity with associated tissues through interactions with both the NC and (cranial plus lateral plate) mesoderm (Kuratani et al. 2018). In the head/trunk interface, these mesenchymal cell populations are juxtaposed to each other uniquely in the vertebrate body plan. Difficulty in precise identification of developmental origin of mesenchymal cells would have underlain the conflicting insights with respect to the developmental origin of cucullaris muscles.

It is noteworthy that amniote neck also contains a seemingly trunk-derived musculature (innervated by cervical spinal nerves) and has long been assumed to be of myotomal origin. Among these muscles, the splenius muscle has recently been suggested to have dual embryonic origin (cephalic and somitic; Lescroart et al. 2015) and to share its developmental program with that of the hypobranchial muscles (described below; Heude et al. 2018). Thus, the neck region harbors numerous musculoskeletal elements with composite or as-yet-unidentified developmental backgrounds, reflecting the embryonic complexity of the head/trunk interface.

Comparison of the Neck Among Extant Anamniote Vertebrates

The definition of neck is ambiguous in anamniotes, in which the head and thorax are not clearly compartmented, and forelimbs, or pectoral fins, are located adjacent to the most posterior pharyngeal arch. Nevertheless, members of the skeletal muscles in the postotic region can be categorized as "neck muscle" counterparts, especially by

their topography and developmental patterning (Kuratani 1997). Specifically, muscles located ventral to the pharyngeal arches and those caudally adjacent to the head in anamniotes have been compared with hypobranchial and cucullaris muscles of amniotes, respectively, taking the skeletal connection and innervation patterns into account (reviewed in Tada and Kuratani 2015). The definition of the hypobranchial muscle is relatively clear in major clades of anamniotes, based on the innervation (by the hypoglossal or most rostral spinal nerves), which also facilitates the straightforward comparison of origin and migratory behavior of precursor cells during development. The cucullaris muscles have also been identified in major clades of gnathostomes, with some exceptions such as snakes and caecilians (Edgeworth 1935). However, the muscles of the cucullaris group have been given a wide variety of names (summarized in Ericsson et al. 2013).

Amphibian hypobranchial muscles have been anatomically documented in numerous species (reviewed in Ziermann 2019), and hypobranchial development has been analyzed in the axolotl *Ambystoma mexicanum* and the anuran *Xenopus laevis* (Martin and Harland 2006; Piekarski and Olsson 2007). Amphibian hypobranchial muscles consist of geniohyoideus and rectus cervicus, which is a plesiomorphic larval state (Ziermann 2019). These muscles derive from ventrolateral processes of occipital somites, moving ventrally on a pathway similar to that of the amniote hypoglossal cord. In most of the species (excluding *Xenopus*), the tongue structure, in which the tongue muscles (genioglossus and hyoglossus) develop, forms during metamorphosis. In *Xenopus*, somitic cells generating hypaxial muscles express *Pax3*, and the function of *Lbx1* was shown to be necessary for formation of hypobranchial muscles, similarly to the case in amniotes (Martin and Harland 2006). However, recent genomic research in the axolotl *A. mexicanum* showed that this species lost the *Pax3* locus in its genome, and the major function of *Pax3* is complemented by its paralogue *Pax7*; in *Pax7*-depleted individuals, limb muscle was completely lost (Nowoshilow et al. 2018). Thus, major players upstream of the myogenic pathway might have undergone alterations within the amphibian clade.

As for the cucullaris-related muscles, urodele axolotl possess the protractor pectoralis, a pair of large, triangular "trapezius-like" muscles, that connect the head and shoulder girdle. Cell lineage tracing analysis using fluorescent dyes in the axolotl indicated that the protractor pectoralis develops with major contributions from somites (Piekarski and Olsson 2007), similarly to the classical indication obtained from chick-quail chimera. The possible LPM-origin of the cucullaris muscle was also tested in axolotl, with labeling of the corresponding tissue in early embryo (Sefton et al. 2016). The results showed that the axolotl cucullaris derives from the mesoderm adjacent to the three anterior somites, but in the genetic context, this mesoderm was the posterior cranial mesoderm, expressing *Tbx1* and *Islet1,* which is consistent with the latest insights from mice (described above).

Actinopterygians exhibit dramatic divergence in both hypobranchial and cucullaris muscle morphology (reviewed in Huby and Parmentier 2019). Teleosts have a single hypobranchial muscle, such as the sternohyoid muscle in zebrafish (Schilling and Kimmel 1997), whereas basal actinopterygians such as bichir possess the separate geniohyoideus-equivalent (coracomandibularis or branchiomandibularis) and the

sternohyoid muscles (Noda et al. 2017). Only a few works have documented the developmental origin of hypobranchial muscles in actinopterygians (e.g., myomere 2nd to 5th giving rise to the sternohyoid in Salmo; Winterbottom 1973). In zebrafish, the *Pax3* gene is required for the formation of sternohyoideus muscle (Minchin et al. 2013). As an equivalent of the cucullaris muscles, teleost fishes possess the protractor pectoralis, which is innervated by a branch of the vagus (X) nerve, a branch also named as the accessory nerve in some studies (Winterbottom 1973; Greenwood and Lauder 1981). The developmental origin of this muscle remains unclear, due to the reduced bulk and late differentiation, as discussed by Hinits et al. (2011).

In chondrichthyans (sharks and rays), geniohyoideus and rectus cervicus have also been documented as somite derivatives, although they have been given different names in different species (Edgeworth 1935). In the dogfish *Scyliorhinus,* the rectus cervicus is termed coracoarcualis, which originates at scapulocoracoid cartilage and inserts to the anterior, medially located hypobranchial muscle. This medial muscle is the geniohyoideus counterpart, termed coracomandibularis. Coracoarcualis also inserts to another bilateral hypobranchial muscle, the coracohyoideus. Overall, the hypobranchial anatomy of chondrichthyans is similar to that found in the urodele amphibians mentioned above. Recent reports on the development of paired appendages in the shark also suggested the involvement of *Pax3* and *Lbx1* in the hypobranchial myogenesis (Okamoto et al. 2017). The cucullaris muscle is also conspicuous in chondrichthyans, where it is often termed as the protractor pectoralis muscle (Miyake et al. 1992). However, the developmental origin of this muscle has long been debated; as in the case of other vertebrates, the protractor pectoralis develops late, and the status of the premyogenic primodium has never been clearly documented. In the shark, the cucullaris is observed as a thin single muscle covering the dorsal side of the posterior branchial region (Edgeworth 1935 and others). Edgeworth categorized this muscle as a branchial muscle plate-derivative. Later studies in skate, however, showed that it is composed of multiple musculatures, each of which receives innervation from the vagus or the rostral spinal nerves (Boord and Sperry 1991). Thus, the cucullaris (protractor pectoralis) muscle of chondrichthyans, although morphologically conspicuous, exhibits variations in the number of subdomains and innervation patterns among species.

Since the neck intervenes between the head and the pectoral girdle, it cannot be morphologically defined in animals that completely lack the pectoral girdle. Nevertheless, there has been a series of studies seeking the developmental and genetic insights for the head-trunk interface in cyclostomes. Cyclostomes are a monophyletic sister group to gnathostomes consisting of lampreys and hagfish, both of which lack the paired appendages as well as the shoulder girdle. In the adult lamprey, the muscle bilaterally covering the ventrolateral aspect of the pharynx is termed the hypobranchial muscle, as it is innervated by the hypoglossal nerve (XII) (Kuratani 1997; Kusakabe and Kuratani 2005). This muscle has its developmental origin in anterior somites in the embryo, which express the lamprey cognate genes of *Pax3* and *Lbx1* (Kusakabe et al. 2011). Lampreys develop no paired appendage, and no cucullaris-equivalent muscle has been observed at any stage of their life cycle. However, a possible linkage between the anterior somites of the larval lamprey,

which extend into the head region, and the cucullaris has been discussed (Kusakabe et al. 2011). During lamprey development, the most anterior somites form the supra- and infraoptic muscles, the latter of which is innervated by a part of the spinal nerve plexus located caudal to the vagal (X) motoneuron. This spinal nerve is characteristic of the lampreys and might reflect the location of an ancestral spinal accessory nerve (Tada and Kuratani 2015). These insights suggest that the hypobranchial and cucullaris muscles plus their innervation constitute a vertebrate synapomorphy that appeared prior to the acquisition of the paired limbs and the morphologically apparent neck during evolution.

Evolutionary Schemes of Acquisition of the Neck

Vertebrates have diversified from a jawless ancestor (Janvier 1996). Several groups of stem gnathostomes, such as jawless ostracoderms (including osteostracans), possessed pectoral fin-like structures posterior to the head, which were continuous with the head shield rostrally. The heart is also encased in the head shield, implying that the neck domain of ostracoderms was very restricted if present. In contrast, the placoderms, a group of fossil jawed stem gnathostomes, possessed jointed paired appendages (both pectoral and pelvic) and separate trunk bony plates surrounding the anterior part of the body, including the pectoral girdle. This trunk shield directly articulates with the bony plates of the head shield.

In fossil placoderms with well-preserved musculatures, cucullaris-equivalent muscles were identified (Trinajstic et al. 2013; reviewed in Kuratani et al. 2018). These cucullaris muscle equivalents spanned the neck-trunk joint, extending from the lateral head shield to the anterolateral trunk shield, and seemed to actively depress and elevate the head (Trinajstic et al. 2013), a morphology reminiscent of the lamprey infraoptic muscle. The hypobranchial muscles were also found in the interfacial region of the head and trunk and thus are adjacent to the pharyngeal arches and the most anterior part of the thoracic cavity (Johanson 2003). Together with the presence of hypobranchial muscle in the cyclostomes, these insights suggest the establishment of a developmental basis for the neck prior to the divergence of gnathostomes. These observations also imply that the earliest cucullaris may have arisen as somitic muscles, which then acquired a branchiomeric nature in crown lineages, as seen in some amniotes.

The postotic- or circumpharyngeal ectomesenchyme is a cryptic embryonic domain where unique and novel structures arise. The neck muscles are representative of these structures; the somite-derived muscles (hypobranchial muscles) differentiate in concert with the NC-derived (i.e., nonsomitic) connective tissues. The cucullaris muscles (whose developmental origin remains in dispute) also develop in the same region. An equivalent domain of the circumpharyngeal region does not exist in amphioxus or tunicates, the closest sister lineage of vertebrates (reviewed in Irie et al. 2018). In amphioxus, somites overlap with the pharyngeal region, extending throughout the rostrocaudal axis of the body. In other words, the head/trunk interface, which divides the somatic and visceral arch domains, is elongated to span most

of the body. One possible hypothesis is that shortening, or localization, of the head-trunk interface occurred during the chordate evolution, and the remaining interface served as the developmental environment in which the neck, one of the most intriguing morphological features of the jawed vertebrates, was acquired (Kuratani et al. 2018).

Cross-References

- ▶ Evolution and Development of the Vertebrate Cranium
- ▶ Shifting the Black Box: Approaches to the Development and Evolution of the Vertebrate Mesoderm

References

Amthor H, Christ B, Patel K (1999) A molecular mechanism enabling continuous embryonic muscle growth – a balance between proliferation and differentiation. Development 126(5):1041–1053

Bladt F, Riethmacher D, Isenmann S, Aguzzi A, Birchmeier C (1995) Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud. Nature 376(6543):768–771

Boord RL, Sperry DG (1991) Topography and nerve supply of the cucullaris (trapezius) of skates. J Morphol 207(2):165–172

Brohmann H, Jagla K, Birchmeier C (2000) The role of Lbx1 in migration of muscle precursor cells. Development 127(2):437–445

Edgeworth FH (1935) The cranial muscles of vertebrates. Cambridge University Press, Cambridge

Ericsson R, Knight R, Johanson Z (2013) Evolution and development of the vertebrate neck. J Anat 222(1):67–78

Greenwood PH, Lauder GV (1981) Protractor pectoralis muscle and the classification of teleost fishes. Bull Br Mus Nat Hist 41:213–234

Heude E, Tesarova M, Sefton EM, Jullian E, Adachi N, Grimaldi A, Zikmund T, Kaiser J, Kardon G, Kelly RG, Tajbakhsh S (2018) Unique morphogenetic signatures define mammalian neck muscles and associated connective tissues. eLife 7:e40179

Hinits Y, Williams VC, Sweetman D, Donn TM, Ma TP, Moens CB, Hughes SM (2011) Defective cranial skeletal development, larval lethality and haploinsufficiency in Myod mutant zebrafish. Dev Biol 358(1):102–112

Huang R, Zhi Q, Patel K, Wilting J, Christ B (2000) Contribution of single somites to the skeleton and muscles of the occipital and cervical regions in avian embryos. Anat Embryol 202(5):375–383

Huby A, Parmentier E (2019) Actinopterygians: head, jaws and muscles. In: Heads, jaws, and muscles. Springer, Cham, pp 93–117

Irie N, Satoh N, Kuratani S (2018) The phylum Vertebrata: a case for zoological recognition. Zool Lett 4:32

Janvier P (1996) Early vertebrates. Oxford University Press, Oxford

Johanson Z (2003) Placoderm branchial and hypobranchial muscles and origins in jawed vertebrates. J Vertebr Paleontol 23(4):735–749

Köntges G, Lumsden A (1996) Rhombencephalic neural crest segmentation is preserved throughout craniofacial ontogeny. Development 122(10):3229–3242

Kuratani S (1997) Spatial distribution of postotic crest cells defines the head/trunk interface of the vertebrate body: embryological interpretation of peripheral nerve morphology and evolution of the vertebrate head. Anat Embryol 195(1):1–13

Kuratani S, Kusakabe R, Hirasawa T (2018) The neural crest and evolution of the head/trunk interface in vertebrates. Dev Biol 444:S60–S66

Kusakabe R, Kuratani S (2005) Evolution and developmental patterning of the vertebrate skeletal muscles: perspectives from the lamprey. Dev Dyn 234(4):824–834

Kusakabe R, Kuraku S, Kuratani S (2011) Expression and interaction of muscle-related genes in the lamprey imply the evolutionary scenario for vertebrate skeletal muscle, in association with the acquisition of the neck and fins. Dev Biol 350(1):217–227

Lescroart F, Hamou W, Francou A, Theveniau-Ruissy M, Kelly RG, Buckingham M (2015) Clonal analysis reveals a common origin between nonsomite-derived neck muscles and heart myocardium. Proc Natl Acad Sci U S A 112(5):1446–1451

Lours-Calet C, Alvares LE, El-Hanfy AS, Gandesha S, Walters EH, Sobreira DR, Wotton KR, Jorge EC, Lawson JA, Kelsey Lewis A, Tada M, Sharpe C, Kardon G, Dietrich S (2014) Evolutionarily conserved morphogenetic movements at the vertebrate head-trunk interface coordinate the transport and assembly of hypopharyngeal structures. Dev Biol 390(2):231–246

Martin BL, Harland RM (2006) A novel role for lbx1 in Xenopus hypaxial myogenesis. Development 133(2):195–208

Matsuoka T, Ahlberg PE, Kessaris N, Iannarelli P, Dennehy U, Richardson WD, McMahon AP, Koentges G (2005) Neural crest origins of the neck and shoulder. Nature 436(7049):347–355

Minchin JEN, Williams VC, Hinits Y, Low S, Tandon P, Fan CM, Rawls JF, Hughes SM (2013) Oesophageal and sternohyal muscle fibres are novel Pax3-dependent migratory somite derivatives essential for ingestion. Development 140(20):4296–4296

Miyake T, McEachran JD, Hall BK (1992) Edgeworth's legacy of cranial muscle development with an analysis of muscles in the ventral gill arch region of batoid fishes (Chondrichthyes: Batoidea). J Morphol 212(3):213–256

Noda M, Miyake T, Okabe M (2017) Development of cranial muscles in the actinopterygian fish Senegal bichir, *Polypterus senegalus* Cuvier, 1829. J Morphol 278(4):450–463

Noden DM (1983) The embryonic origins of avian cephalic and cervical muscles and associated connective tissues. Am J Anat 168(3):257–276

Noden DM, Francis-West P (2006) The differentiation and morphogenesis of craniofacial muscles. Dev Dyn 235(5):1194–1218

Nowoshilow S, Schloissnig S, Fei J-F, Dahl A, Pang AWC, Pippel M, Winkler S, Hastie AR, Young G, Roscito JG, Falcon F, Knapp D, Powell S, Cruz A, Cao H, Habermann B, Hiller M, Tanaka EM, Myers EW (2018) The axolotl genome and the evolution of key tissue formation regulators. Nature 554(7690):50–55

Okamoto E, Kusakabe R, Kuraku S, Hyodo S, Robert-Moreno A, Onimaru K, Sharpe J, Kuratani S, Tanaka M (2017) Migratory appendicular muscles precursor cells in the common ancestor to all vertebrates. Nat Ecol Evol 1(11):1731–1736

Piekarski N, Olsson L (2007) Muscular derivatives of the cranialmost somites revealed by long-term fate mapping in the Mexican axolotl (*Ambystoma mexicanum*). Evol Dev 9(6):566–578

Sambasivan R, Kuratani S, Tajbakhsh S (2011) An eye on the head: the development and evolution of craniofacial muscles. Development 138(12):2401–2415

Schilling TF, Kimmel CB (1997) Musculoskeletal patterning in the pharyngeal segments of the zebrafish embryo. Development 124(15):2945–2960

Sefton EM, Bhullar BA, Mohaddes Z, Hanken J (2016) Evolution of the head-trunk interface in tetrapod vertebrates. eLife 5:e09972

Tada MN, Kuratani S (2015) Evolutionary and developmental understanding of the spinal accessory nerve. Zool Lett 1:4

Theis S, Patel K, Valasek P, Otto A, Pu Q, Harel I, Tzahor E, Tajbakhsh S, Christ B, Huang R (2010) The occipital lateral plate mesoderm is a novel source for vertebrate neck musculature. Development 137(17):2961–2971

Trinajstic K, Sanchez S, Dupret V, Tafforeau P, Long J, Young G, Senden T, Boisvert C, Power N, Ahlberg PE (2013) Fossil musculature of the most primitive jawed vertebrates. Science 341(6142):160–164

Vasyutina E, Stebler J, Brand-Saberi B, Schulz S, Raz E, Birchmeier C (2005) CXCR4 and Gab1 cooperate to control the development of migrating muscle progenitor cells. Genes Dev 19(18):2187–2198

Winterbottom R (1973) A descriptive synonymy of the striated muscles of the Teleostei. Proc Acad Natl Sci Phila 125:225–317

Ziermann JM (2019) Diversity of heads, jaws, and cephalic muscles in amphibians. In: Heads, jaws, and muscles. Springer, Cham, pp 143–170

Evolution of Skeletal Tissues

Tatsuya Hirasawa and Shigeru Kuratani

Contents

Abstract

The vertebrate skeletal system is a central subject of research in evolutionary developmental biology. Morphological homologies of vertebrate skeletal elements can be traced in two separate systems: exoskeletons and endoskeletons. This separation is not necessarily linked to differences in histogenesis or to cell lineage origins: homologous bones can be formed through different types of ossification or from cells of different origin. The earliest skeleton that appeared in evolution was an endoskeleton consisting of a notochord and cartilage, likely having evolved through the elaboration of an ancestral developmental

T. Hirasawa (✉)
Department of Earth and Planetary Science, Graduate School of Science, The University of Tokyo, Tokyo, Japan
e-mail: hirasawa@eps.s.u-tokyo.ac.jp

S. Kuratani
Laboratory for Evolutionary Morphology, RIKEN Center for Biosystems Dynamics Research (BDR), Kobe, Japan

Evolutionary Morphology Laboratory, RIKEN Cluster for Pioneering Research (CPR), Kobe, Japan
e-mail: shigeru.kuratani@riken.jp

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_190

mechanism. The exoskeleton consisting of dermal bones first evolved in basal members of the gnathostome lineage, prior to the acquisition of the jaw. From this lineage, a group including jawed vertebrates newly acquired endoskeletal bones. Of the latter group, the chondrichthyans, or "cartilaginous fishes," underwent a secondary reduction of these bony tissues. Endochondral ossification, in which osteoblasts produce bones inside of a cartilage template, was a new type of ossification and evolved in the osteichthyan lineage. These evolutionary processes based on the fossil record provide a framework for an understanding of the evolutionary developmental biology of vertebrate skeletal tissues based on developmental genetics.

Keywords

Bone · Cartilage · Extracellular matrix (ECM) · Fossil record · Homology

Introduction

The vertebrate skeleton is a central subject of research in various fields including medicine, taxonomy, functional morphology, paleontology, and developmental biology. Because the skeletons of extant and extinct species are easily preserved as dry specimens in museums or as fossils in rocks, variations in their morphology and histology have been analyzed in considerable detail, contributing to our current knowledge of vertebrate evolution. In developmental biology, skeletal morphology has been one of the major phenotypes analyzed by genetic experiments, such as studies using genetically engineered mice. Under these circumstances, it is not surprising that the vertebrate skeleton has been, and will continue to be, actively studied in evolutionary developmental biology.

The skeleton supports the body, helping it to resist forces from the environment as well as transmitting muscular forces. In vertebrates, the skeleton consists of the notochord, cartilage, and bone. These three types of skeletal element show a wide spectrum of histological variation and are classified into many subcategories. To be noted is that most are classified on the basis of the variations observed in the most extensively studied taxon, namely the mammal. In reality, however, there are many more tissue types in the vertebrate evolution, classified as intermediates between canonical classifications (Hall 2015).

The *notochord* consists of vacuolated cells encapsulated by a fibrous extracellular matrix (notochordal sheath), extending along the anteroposterior axis to provide mechanical support of the body. In many extant vertebrates, bony or cartilaginous elements (the centrum) develop around the notochord and then replace it during ontogeny. On the other hand, in the cyclostomes and some fishes, such as coelacanths and lungfishes, the notochord persists as the main axial skeleton of the adult. In the fossil record, members of the tetrapod stem group, including *Eusthenopteron*, possessed the notochord instead of the centrum; thus, the centrum evolved in tetrapods and modern fishes independently (Arratia et al. 2001).

As the persistence of the notochord is evidenced by a hole in the centrum (the notochordal foramen) in some fossil tetrapods including basal synapsids and diapsids, the disappearance of the persistent notochord in adult forms seems to have taken place independently in mammalian and diapsid lineages. In extant mammals, the notochord becomes transformed into the nucleus pulposus of the intervertebral disc during embryonic development (Pattappa et al. 2012); thus, a derivative of the notochord is still a part of the skeletal system. The centrum of teleosts evolved differently from that of tetrapods, and the bony tissue of the centrum is deposited on the surface of the notochordal sheath (Hall 2015).

Cartilage is a type of skeletal tissue that contains a large amount of extracellular matrix (ECM). ECM-secreting cells are distributed at the periphery of the cartilage (chondroblasts of the perichondrium) as well as within the cartilage (chondrocytes). Composition of ECM can vary not only interspecifically, but also among cartilaginous tissues within the body of a single species. In gnathostomes, the ECM of cartilaginous tissues typically contain proteoglycans and type II collagen, and it maintains its elasticity to a certain extent, whereas in some cases, such as the centrum of sharks, ECM becomes mineralized to gain stiffness (calcified cartilage). Cartilaginous tissues of cyclostomes are different in their ECM composition. For example, the lamprey cartilage contains a specific fibrous protein called lamprin, instead of type II collagen. While lamprin evolved from a type of protein different from collagens, it provides lamprey cartilages with mechanical properties similar to those of gnathostome cartilages (see chapter ► "Convergence").

Bone is a solid skeletal tissue, where the ECM is mineralized with calcium phosphate and also contains type I collagen. Cells remodeling the bone matrix (osteoblasts and osteoclasts for secreting and resorbing the matrix, respectively) are distributed in the periphery (periosteum) and within the bone, and most osteoblasts become surrounded by the bone matrix to become isolated cells (osteocytes). Unlike the notochord and cartilage, the bone is invaded by blood vessels. Spaces enclosing osteocytes (lacunae) often encircle the canal enclosing a blood vessel (Haversian canals), forming a metabolic unit of the bone (the osteon). On the other hand, bones not containing osteocytes (acellular bones) are also found in some gnathostome groups, which has become dominant in teleosts.

Tendons and *ligaments* are generally not classified as skeletal tissues, but nevertheless they are not clearly separable from the skeletal tissues. A tendon is a connective tissue developing at the junction between a muscle and either bone or cartilage, differentiating from a cell lineage shared with that of skeletal tissues (Hirasawa and Kuratani 2018). In normal development, some tendons are accompanied by mineralization at the edges where they are connected to bones or throughout their length (Haines and Mohuiddin 1968; Hall 2015). The latter example – the ossified tendon – is present in epaxial muscle systems of some ornithischian dinosaurs and birds. Some ossified tendons are not simply mineralized, but also contain Haversian canals like bony tissues do. Also, it is sometimes difficult to draw a clear line between ligaments and cartilages, as the former can be transformed from the latter. For example, in some mammals, the cartilage connecting the pubic symphysis

of pregnant females becomes transformed into a ligament, to enable widening of the interpubic joint at childbirth (Hall 2015).

Among these skeletal tissues, the notochord was acquired prior to the origin of vertebrates (Lauri et al. 2014) and the other types of skeletal tissue arose and received modifications to establish morphological diversity during vertebrate evolution. The bones were particularly indispensable for the high levels of mobility and to support the large body sizes seen in vertebrate diversity. This chapter explains the practical classifications and the evolutionary processes of these vertebrate skeletal tissues, to provide a framework for evolutionary developmental biological research into vertebrate skeletons.

Classification of Skeletal Elements in Vertebrates

Exoskeleton and Endoskeleton

There are at least four systems of classification formulated to analyze vertebrate skeletal elements. The first one, the classification system of exoskeleton and endoskeleton (Fig. 1), focuses on the evolutionary derivation of skeletal elements (Hirasawa and Kuratani 2015). This classification is independent of types of developmental mode.

The term *exoskeleton* (or, dermoskeleton; Donoghue and Sansom 2002) is assigned to skeletal elements formed directly underneath the basement membrane of the ectoderm and to their evolutionary derivatives (i.e., homologues). The skeletal elements of the skull vault and dermal scales (the latter of which differ from the epidermal scales seen in reptiles and birds) fall into this category. Exoskeleton tissues consist typically of bone and dentine, which are sometimes covered by enamel or enameloid. On the other hand, in some amniotes including mammals and birds, diminutive cartilaginous tissues (adventitious, or secondary cartilage) are formed at the periphery of some exoskeletal bones.

The term *endoskeleton* is assigned to those skeletal elements that are formed deep within the body, internal to the dermis. In other words, the endoskeleton develops primarily at the same depth as the skeletal muscles develop in an embryonic body. During development, most endoskeletal elements are initially formed as cartilaginous tissue or as a notochord, and some of those elements subsequently become replaced with bone. Some endoskeletal elements, nevertheless, deviate from this developmental process and can directly develop into bones without cartilaginous templates. The centrum of teleosts and sesamoid bone can be regarded as the major examples of such endoskeletal elements.

As a general rule during vertebrate evolution, the elements of exo- and endoskeletons have not interchanged with each other. Although some exoskeletal elements, such as the clavicle and gastralium of amniotes, become located deep in the body (sunken exoskeleton; Hirasawa and Kuratani 2015), their primordia develop apart from the endoskeleton. Conversely, the ribs and vertebrae of turtles are not covered by skeletal muscles and form the bony dorsal shell or

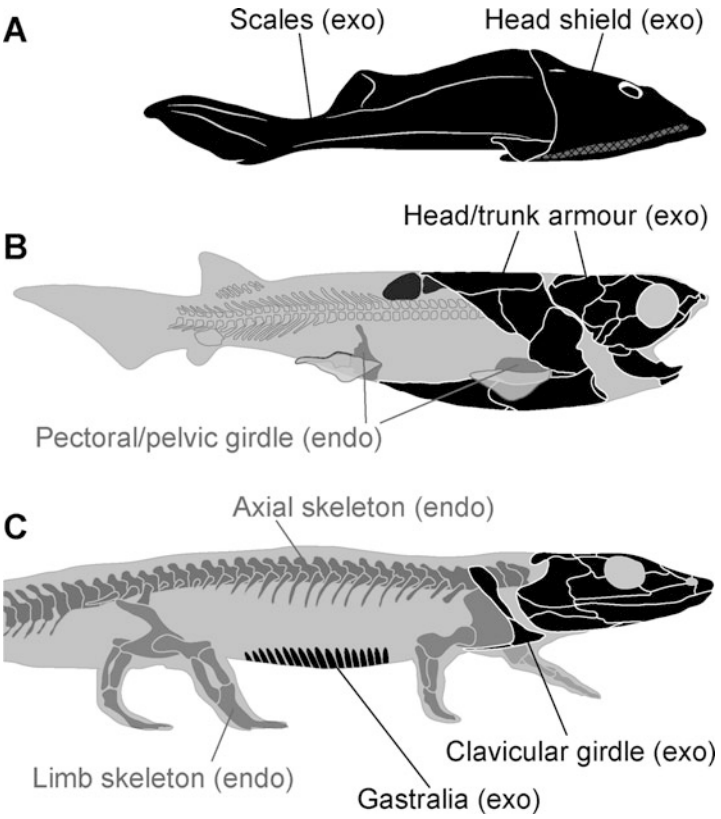


Fig. 1 Exo- and endoskeletons in the vertebrate body. (**a**) Osteostracan *Cephalaspis*. (**b**) Stem jawed vertebrate ("placoderm") *Compagopiscis*. (**c**) Tetrapod *Dendrerpeton*. (Modified from Hirasawa and Kuratani 2015 based on Janvier 2007, Trinajstic et al. 2013, and Clack 2012)

carapace (exposed endoskeleton; Hirasawa and Kuratani 2015), although they develop at the same depth as the axial muscles during embryonic development (Hirasawa et al. 2013). On the other hand, some exo- and endoskeletal elements, which initially develop as separate entities, become fused to each other later in ontogeny. Such ontogenetic fusions can be observed in skulls of many gnathostome taxa, for example. In any of these cases, detailed analyses have supported the scenario that exo- and endoskeletons can be traced in two separate systems in evolution (Hirasawa and Kuratani 2015).

Intramembranous and Endochondral Bone

The above classification of exo- and endoskeletons follows the evolutionary successions, or homologies, but does not necessarily correspond to differences in the developmental process. Focusing on the developmental process, vertebrate bones

are divided into two main classes, namely intramembranous and endochondral bones.

Intramembranous bones (or, membranous bones) are formed without cartilaginous templates, whereas *endochondral bones* are formed through replacements from cartilaginous templates. The exoskeleton consists almost exclusively of intramembranous bones, for which the term *dermal bone* is used. One exception is the antlers of the Cervidae, which are exoskeletal elements newly evolved in this lineage and develop through endochondral ossification (bone formation), at least partly (Hall 2015).

Most endoskeletal elements are formed by endochondral ossification in addition to intramembranous ossification occurring at the periphery of cartilaginous templates (perichondral ossification; Fig. 2a, b). In the latter process, osteoblasts derived from the perichondrium deposit a bone tissue, or a periosteal bone collar, on the surface of a cartilaginous template, and the bone does not replace the cartilage. On the other hand, some endoskeletal elements develop solely through intramembranous ossification. Such intramembranous bones in the endoskeletal system are called *membrane bones*, to distinguish them from dermal bones, which are exoskeletal as the name implies (Patterson 1977). The centrum of teleosts, which is an endoskeletal element, develops as an intramembranous bone, thus falling into the category of membrane bones histologically. Also, sesamoid bones and the orbitosphenoid of the Amphisbaenia represent membrane bones.

The difference between intramembranous and endochondral ossifications can also be seen in the types of stem cells later differentiating into osteoblasts (Debnath et al. 2018). In intramembranous ossifications within both endoskeletons (long bones) and exoskeletons (calvarium) of mice, a specific type of cells called periosteal stem cells (PSCs) appear and differentiate into osteoblasts. The PSCs are, in transcriptional signature, distinct from skeletal mesenchymal stem cells (MSCs) that mediates endochondral ossification. PSCs and MSCs represent discrete stem cell populations and are normally not interconverted into each other. However, in response to bone fracture, PSCs show plasticity to be interconverted into endochondral-competent MSCs, eventually contributing to chondrocytes in the fracture callus. This is a case in which new developmental studies unraveled a developmental basis behind the long-standing discrimination between intramembranous and endochondral ossifications. So far, PSCs and MSCs have been observed to be separate in the mouse and human, but such a separation has remained uncertain in other taxa. Considering the diversity in the modes of bone formation, it is possible that they are in a continuum and are interconverted to each other as a normal developmental process in other vertebrate species, such as the antlers of the Cervidae.

Endochondral ossification involves the invasion of blood vessels into a cartilage template (Fig. 2b). As development of the cartilage template proceeds, chondrocytes become hypertrophic, eventually accepting the intrusion of precursor cells of endochondral osteoblasts. These osteoblast precursor cells are differentiated from the perichondrium that ensheaths the cartilage, and they migrate into the cartilage along with blood vessels (Maes et al. 2010). The endochondral osteoblasts deposit bone tissues, or bony trabeculae, late in development,

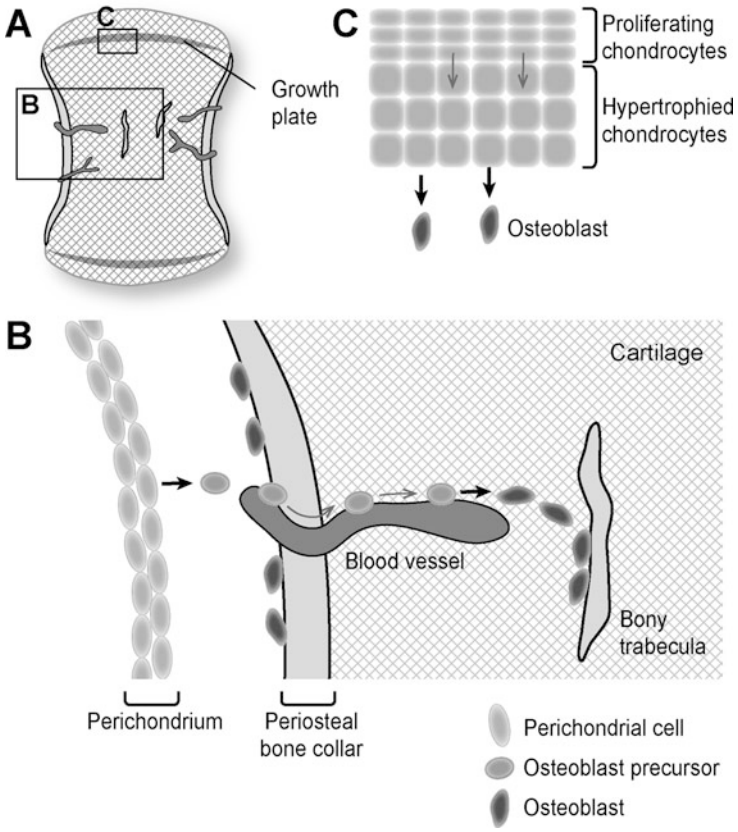


Fig. 2 Ossification process of endoskeleton. (**a**) Schematic drawing of perichondral and endochondral ossifications. (**b**) Perichondral ossification produces the periosteal bone collar. In endochondral ossification, osteoblastic precursors differentiate from perichondrial cells, and migrate into the cartilage along blood vessels. Then, the precursors differentiate into osteoblasts to produce bony trabeculae. (**c**) At the growth plate, osteoblasts differentiate from hypertrophied chondrocytes and proceed to endochondral ossification. (Modified from Hirasawa and Kuratani 2015)

typically in the postembryonic stages. In birds, cartilage is replaced by bone marrow prior to endochondral ossification (Hall 2015). Postembryonic growth of long bones, or limb bones, is mediated by growth plates of cartilaginous caps at both ends (Fig. 2a, c), where hypertrophied chondrocytes differentiate into endochondral osteoblasts (Wuelling and Vortkamp 2019).

Neurocranium and Viscerocranium

The cranial skeleton can be divided into the *neurocranium* and the *viscerocranium*, based on their relationship with pharyngeal arches (see chapter ▶ "Evolution and

Development of the Vertebrate Cranium"). The neurocranium refers to skeletal elements developing around the brain and sensory organs. On the other hand, the viscerocranium (or, splanchnocranium) refers to skeletal elements developing within pharyngeal arches, thereby surrounding the pharynx lined by the endoderm, namely a visceral component.

In most gnathostome vertebrates, dermal bones develop extensively to cover the endoskeletal elements of the neuro- and viscerocranium. In some medical textbooks, these dermal bones are classified into either neuro- or viscerocranium, according to the class of underlying endoskeletal element. On the other hand, the cranial dermal bones are often designated as another class, the *dermatocranium*.

Neural Crest Cell-derived Skeleton and Mesoderm-derived Skeleton

Two distinct cell lineages contribute to the formation of the vertebrate skeleton: the neural crest cells (NCCs) and the mesoderm (Hirasawa and Kuratani 2015; Kuratani et al. 2016; see chapter ▶ "Evolution and Development of the Vertebrate Cranium"). NCCs form the rostral part of the dermato- and neurocranium and the entire part of the viscerocranium, while mesodermal cells form the rest of the dermato- and neurocranium (i.e., the caudal parts) in addition to most of the postcranial skeleton. In mice, the contribution of NCCs to a fraction of the dermal bones of the shoulder girdle has been proposed, unlike in nonmammalian vertebrates.

In the postcranial skeleton, somite- and lateral plate-derived mesodermal cells generate dorsal and ventral skeletal elements, respectively, the latter of which include paired fin/limb bones. Focusing on this difference in developmental origin, the postcranial skeleton is classified into *primaxial* and *abaxial* skeletons (Burke and Nowicki 2003; see chapter ▶ "Shifting the Black Box: Approaches to the Development and Evolution of the Vertebrate Mesoderm").

Both NCC- and mesoderm-derived cells can contribute to both intramembranous and endochondral ossifications; thus, types of ossification do not correlate with the cell lineages. In addition, neither cell lineages nor ossification types are stable during evolution, and these criteria are not sufficient to prove homologies of skeletal elements (Hirasawa and Kuratani 2015).

Fossil Record

Extant vertebrates represent only a fraction of the various forms that have ever evolved in vertebrate history (Fig. 3). The extant forms, namely the cyclostomes (hagfishes and lampreys), chondrichthyans (sharks, rays, skates, and elephant sharks), actinopterygians (ray-finned fishes), and sarcopterygians (lobe-finned fishes and tetrapods), diverged deep in time – from the late Cambrian to the mid-Silurian (roughly 500–430 million years ago) – and diverse extinct groups branched off from their ancestors. Therefore, data from the fossil record are indispensable to understanding the evolutionary process of vertebrate skeletal tissues. Fossils preserve

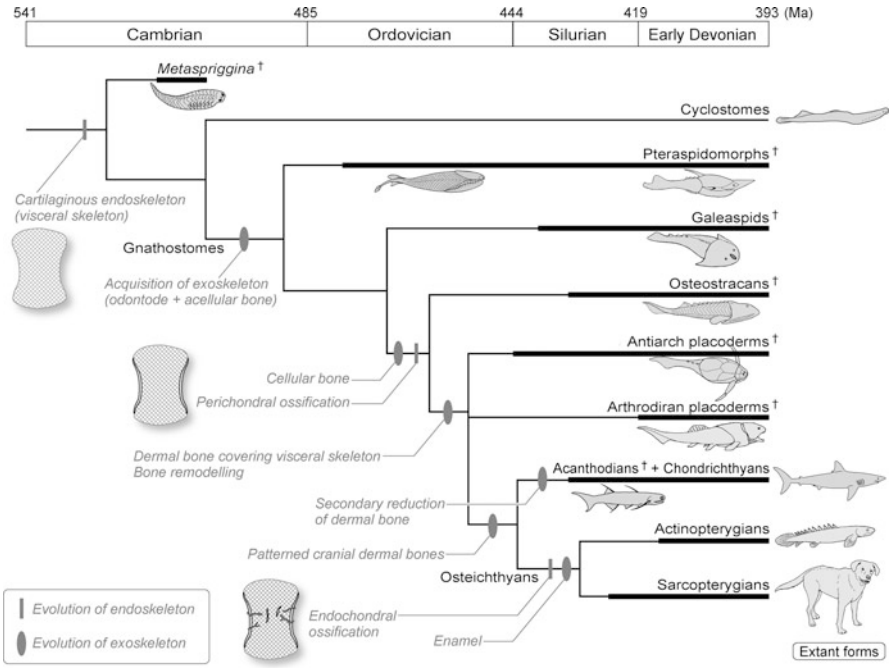


Fig. 3 Evolution of vertebrate skeletal tissues. Symbols (†) indicate extinct groups. Bold lines correspond to geological ages of fossil occurrences

minute histological structures of bones and associated hard tissues (dentines and enameloids), as well as, in favorable conditions, cartilage, from which we are able to infer modes of histogenesis. In this section, major transitions in the evolution of vertebrate skeletal tissues are explained in the phylogenetic order.

The common ancestor of vertebrates likely possessed cartilaginous endoskeletal elements supporting gills (Fig. 3). Such a visceral skeleton was present in the stem vertebrate *Metaspriggina* from the Cambrian, although its detailed histology remains unclear. Also, the skeletons of modern cyclostomes consist solely of endoskeletal elements, including branchial baskets and inconspicuous axial skeletons (Ota et al. 2011).

The exoskeleton was acquired in the gnathostome lineage after divergence from the cyclostomes (Fig. 3). Those stem gnathostome members, such as osteostracans, were jawless forms, unlike the living gnathostomes. According to the fossil record analyzed so far, exoskeletons of basal gnathostomes already exhibit three histological layers consisting of a superficial layer of dentine and enameloid (odontode), and middle and basal layers of bone (Donoghue and Sansom 2002; Sire et al. 2009; Keating et al. 2018). The bone of the basal gnathostomes was acellular, and the osteocytes were not encapsulated within mineralized tissue. On the other hand, tooth-shaped mineralized skeletal elements were also present in the mouths of conodonts from the age of the early evolution of vertebrates, namely

the late Cambrian and the Ordovician. However, these skeletal elements are not homologous with the teeth of crown jawed vertebrates (Witten et al. 2014). The latter structures evolved later from the exoskeleton (Chen et al. 2016).

Endoskeletal bones evolved in the lineage of the osteostracans and jawed vertebrates (Fig. 3). While the closest outgroup to the osteostracan/jawed vertebrate lineage, namely the galeaspids, possessed an endoskeleton consisting of cartilage (Wang et al. 2005), the endoskeleton of the osteostracans was covered by perichondral bone (Donoghue and Sansom 2002). In addition, both exoskeletal and endoskeletal bones of the osteostracans were cellular (Donoghue and Sansom 2002; Sire et al. 2009), unlike the exoskeletal bones of the galeaspids and other jawless gnathostomes; thus, the skeletal tissues of the osteostracans were similar in histology, and probably in histogenesis, to the extant gnathostomes. The stem jawed gnathostomes collectively called "placoderms" (paraphyletic groups) possessed skeletal tissues basically comparable to those of the osteostracans. However, the bone histology of placoderms also shows some features shared with extant members of osteichthyans: for example, in bones of arthrodiran placoderms, Haversian canals and traces of bone remodeling are identifiable (Giles et al. 2013).

As the name suggests, chondrichthyans have cartilaginous skeletons devoid of bones, but such a constitution is due to the secondary reduction of bone tissues in the evolution towards this lineage (Hirasawa and Kuratani 2015). In other words, chondrichthyans do not represent an ancestral condition in the aspect of skeletal evolution.

In another group of crown gnathostomes, namely the osteichthyans, endochondral ossification evolved (Fig. 3). Also, in this lineage, enamel tissue was acquired primarily on the surface of trunk exoskeletal elements and subsequently expanded onto the surfaces of head exoskeletal elements and teeth as well (Qu et al. 2015). In the tetrapod lineage, such enamel-coated tissue was lost on exoskeletal elements and has persisted only on teeth.

Evolutionary Genomics

The evolution of vertebrate skeletal tissues has been vigorously analyzed from the genomic point of view, too. In particular, studies on duplications of genes encoding fibrillar collagens and SPARC (secretory protein acidic and rich in cysteine) glycoproteins have revealed the evolutionary process of the protein repertoire involved in skeletogenesis (Wada 2010). By comparison with nonvertebrate chordates, it was shown that fibrillar collagen genes had been duplicated to bring about cartilage- and bone-specific collagens in vertebrate evolution (Wada 2010). SPARC genes also experienced duplications, from which SCPPs (secretory calcium-binding phosphoproteins) were evolved. SCPPs play a pivotal role in mineralization and regulation of calcium phosphate concentration during ossification and among extant vertebrates are present only in osteichthyans. Considering the secondary loss of bone in the chondrichthyan lineage (Fig. 3), ancestral SCPP genes likely evolved prior to the chondrichthyan–osteichthyan split and later might have been eliminated from chondrichthyan genomes (Ryll

et al. 2014). This example provides an important lesson for utilization of paleontological data in evolutionary genomics.

Also, transcriptional regulation in the differentiation of skeletogenic cell types and in the production of certain molecular components of skeletal tissues has received a lot of attention (Wada 2010). There is a similarity in transcriptional regulation during development between the vertebrate cartilage and invertebrates' cartilage-like tissues: in both tissues, Sox transcriptional factors activate transcription of the gene encoding fibrillar collagen (Tarazona et al. 2016). This fact implies the possibility that a part of the genetic basis for vertebrate skeletal tissue already existed in the bilaterian common ancestor. On the other hand, within vertebrates, there is some difference in transcriptional regulation between the cyclostome and gnathostome cartilages, suggesting that the genetic bases of vertebrate skeletal tissues had continued to evolve even after the cyclostome–gnathostome divergence (Cattell et al. 2011). This scenario is consistent with the difference in composition of ECM of cartilaginous tissue between cyclostomes and gnathostomes, mentioned above. These studies on the genetic bases provide important clues for an evolutionary history that is not determined only by histogenetic analyses and the fossil record.

Summary of Evolutionary History of Vertebrate Skeletal Tissues

Studies on the developmental genetic bases of skeletal tissues suggest that vertebrate skeletal tissues evolved through the elaboration of an ancestral developmental mechanism, which can be traced back to the bilaterian common ancestor, as evidenced by the commonality in transcriptional regulation (Sox transcriptional factors). During the vertebrate evolution, genes involved in skeletal tissue development (SPARC genes) experienced duplications, eventually enabling diversification of skeletal tissue types. The skeletons of early vertebrates consisted of the notochord and cartilage (endoskeleton), and a comparable condition is recognizable in extant cyclostomes (Fig. 3). In the evolution towards the gnathostome lineage, a new type of skeletal tissue, bone, was acquired to build the exoskeleton (Fig. 3). Bone appeared in the endoskeleton before the establishment of the jaw joint and was reduced secondarily in the lineage towards the chondrichthyans (Fig. 3). Ancestrally, the endoskeletal bone was formed by perichondral ossification (intramembranous ossification), and later, in the osteichthyan lineage, a new type of developmental process, endochondral ossification, evolved (Fig. 3).

Cross-References

- ► Convergence
- ► Developmental Homology
- ► Evolution and Development of the Vertebrate Cranium
- ► Shifting the Black Box: Approaches to the Development and Evolution of the Vertebrate Mesoderm

References

Arratia G, Schultze HP, Casciotta J (2001) Vertebral column and associated elements in dipnoans and comparison with other fishes: development and homology. J Morphol 250:101–172

Burke AC, Nowicki JL (2003) A new view of patterning domains in the vertebrate mesoderm. Dev Cell 4:159–165

Cattell M, Lai S, Cerny R, Medeiros DM (2011) A new mechanistic scenario for the origin and evolution of vertebrate cartilage. PLoS One 6:e22474

Chen D, Blom H, Sanchez S, Tafforeau P, Ahlberg PE (2016) The stem osteichthyan *Andreolepis* and the origin of tooth replacement. Nature 539:237–241

Clack JA (2012) Gaining ground: the origin and evolution of tetrapods, 2nd edn. Indiana University Press, Bloomington

Debnath S, Yallowitz AR, McCormick J, Lalani S, Zhang T, Xu R, Li N, Liu Y, Yang YS, Eiseman M, Shim JH, Hameed M, Healey JH, Bostrom MP, Landau DA, Greenblatt MB (2018) Discovery of a periosteal stem cell mediating intramembranous bone formation. Nature 562:133–139

Donoghue PCJ, Sansom IJ (2002) Origin and early evolution of vertebrate skeletonization. Microsc Res Tech 59:352–372

Giles S, Rücklin M, Donoghue PCJ (2013) Histology of "placoderm" dermal skeletons: implications for the nature of the ancestral gnathostome. J Morphol 274:627–644

Haines RW, Mohuiddin A (1968) Metaplastic bone. J Anat 103:527–538

Hall BK (2015) Bones and cartilage: developmental and evolutionary skeletal biology, 2nd edn. Academic, San Diego

Hirasawa T, Kuratani S (2015) Evolution of the vertebrate skeleton: morphology, embryology, and development. Zool Lett 1:2

Hirasawa T, Kuratani S (2018) Evolution of the muscular system in tetrapod limbs. Zool Lett 4:27

Hirasawa T, Nagashima H, Kuratani S (2013) The endoskeletal origin of the turtle carapace. Nat Commun 4:2107

Janvier P (2007) Homologies and evolutionary transitions in early vertebrate history. In: Anderson JS, Sues HD (eds) Major transitions in vertebrate evolution. Indiana University Press, Bloomington, pp 57–121

Keating JN, Marquart CL, Marone F, Donoghue PCJ (2018) The nature of aspidin and the evolutionary origin of bone. Nat Ecol Evol 2:1501–1506

Kuratani S, Oisi Y, Ota KG (2016) Evolution of the vertebrate cranium: viewed from the hagfish developmental studies. Zool Sci 33:229–238

Lauri A, Brunet T, Handberg-Thorsager M, Fischer AH, Simakov O, Steinmetz PR, Tomer R, Keller PJ, Arendt D (2014) Development of the annelid axochord: insights into notochord evolution. Science 345:1365–1368

Maes C, Kobayashi T, Selig MK, Torrekens S, Roth SI, Mackem S, Carmeliet G, Kronenberg HM (2010) Osteoblast precursors, but not mature osteoblasts, move into developing and fractured bones along with invading blood vessels. Dev Cell 19:329–344

Ota KG, Fujimoto S, Oisi Y, Kuratani S (2011) Identification of vertebra-like elements and their possible differentiation from sclerotomes in the hagfish. Nat Commun 2:373

Pattappa G, Li Z, Peroglio M, Wismer N, Alini M, Grad S (2012) Diversity of intervertebral disc cells: phenotype and function. J Anat 221:480–496

Patterson C (1977) Cartilage bones, dermal bones and membrane bones, or the exoskeleton versus the endoskeleton. In: Andrews SM, Miles RS, Walker AD (eds) Problems in vertebrate evolution. Academic Press, London, pp 77–121

Qu Q, Haitina T, Zhu M, Ahlberg PE (2015) New genomic and fossil data illuminate the origin of enamel. Nature 526:108–111

Ryll B, Sanchez S, Haitina T, Tafforeau P, Ahlberg PE (2014) The genome of Callorhinchus and the fossil record: a new perspective on SCPP gene evolution in gnathostomes. Evol Dev 16:123–124

Sire JY, Donoghue PCJ, Vickaryous MK (2009) Origin and evolution of the integumentary skeleton in non-tetrapod vertebrates. J Anat 214:409–440
Tarazona OA, Slota LA, Lopez DH, Zhang G, Cohn MJ (2016) The genetic program for cartilage development has deep homology within Bilateria. Nature 533:86–89
Trinajstic K, Sanchez S, Dupret V, Tafforeau P, Long J, Young G, Senden T, Boisvert C, Power N, Ahlberg PE (2013) Fossil musculature of the most primitive jawed vertebrates. Science 341:160–164
Wada H (2010) Origin and genetic evolution of the vertebrate skeleton. Zool Sci 27:119–123
Wang NZ, Donoghue PCJ, Smith MM, Sansom IJ (2005) Histology of the galeaspid dermoskeleton and endoskeleton, and the origin and early evolution of the vertebrate cranial endoskeleton. J Vertebr Paleontol 25:745–756
Witten PE, Sire JY, Huysseune A (2014) Old, new and new-old concepts about the evolution of teeth. J Appl Ichthyol 30:636–642
Wuelling M, Vortkamp A (2019) Stem cells: the fountain of bone growth. Nature 567:178–179

History and Current Theories of the Vertebrate Head Segmentation

Shigeru Kuratani

Contents

Abstract

Investigation of vertebrate head segmentation provided the origin of the fields of comparative morphology and embryology and has offered basic concepts that remain important for evolutionary developmental biology, or "evo-devo." This line of inquiry started as the vertebral theory of the skull, followed by the comparative embryological search for somite-like segments in the head mesoderm. Vertebrate-specific neuromeres were also investigated in pursuit of an integrated segmented body plan scheme, but to date no satisfactory scheme has been obtained. More recently, experimental embryological and molecular developmental biology techniques have been applied but the question of head segmentation has not been fully resolved. In a wider evolutionary context, questions

S. Kuratani (✉)
Laboratory for Evolutionary Morphology, RIKEN Center for Biosystems Dynamics Research (BDR), Kobe, Japan

Evolutionary Morphology Laboratory, RIKEN Cluster for Pioneering Research (CPR), Kobe, Japan
e-mail: shigeru.kuratani@riken.jp

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_186

on the origin and organization of the vertebrate head should be treated as part of the evolution of the coelomic mesoderm. Current evo-devo studies suggest that enterocoels have their origin in the gut pouch, which could explain the origin of the mesodermal compartments in bilaterians, including vertebrates.

Keywords

Vertebrates · Head segmentation · Development · Mesoderm

Glossary

Cranial nerves	Peripheral nerves that pass through the cranium. Includes several different types of nerves, (e.g., motor, sensory, and mixed). Those cranial nerves (e.g., V, VII, IX, and X) innervating visceral arches are collectively called branchiomeric nerves.
Head mesoderm	Unsegmented mesenchymal mesoderm located in the anterior part of the embryo.
Head somites	Classical concept in comparative embryology that refers to hypothetical somite-like segments of epithelial blocks of tissue in the vertebrate embryonic head.
Metameres	Repeating unit observed in metamerically organized animal bodies. Ideally, one metamere is expected to contain a full set of organs.
Neural crest cells	Ectodermally derived pluripotent cell lineage unique to vertebrates. In the head, cephalic crest cells form extensive ectomesenchyme that will differentiate into various skeletogenic and connective tissue cell types, in addition to pigment cells and the peripheral nervous system.
Neuromeres	Neurepithelial segments with clear boundaries arising in the neural tube.
Somites	Segmented paraxial mesoderm observed in the trunk of vertebrate embryos.
Somitomeres	Hypothetical segments in the paraxial mesoderm of the head.
Spinal nerves	Peripheral nerves that pass through the vertebral column or arise from the spinal cord. Autonomic elements are usually excluded from this category.

Introduction: Classical Views

The question of vertebrate head segmentation, or the "head problem," is one of the oldest topics of morphological inquiry (reviewed by Carus (1828), Gegenbaur (1887), Sewertzoff (1895), De Beer (1937), Neal and Rand (1946), Starck (1979),

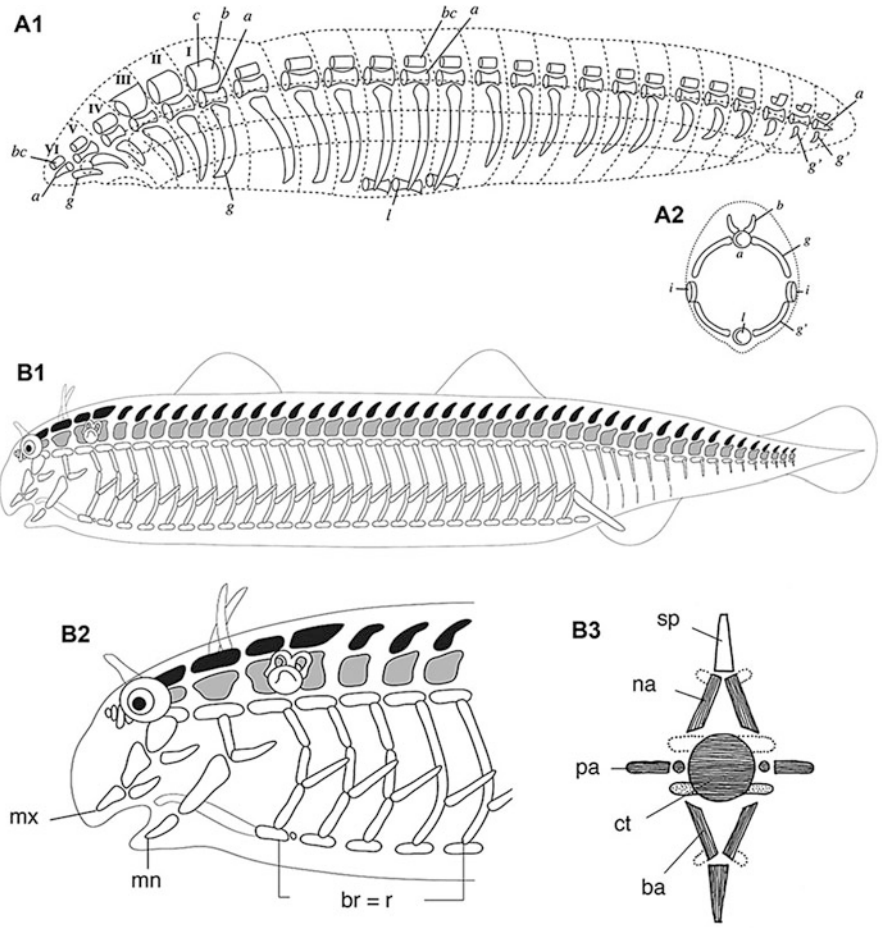


Fig. 1 Vertebral theories of vertebrate skeletons. (**A1**) Carus (1828) drew a schematic diagram of a generalized vertebrate skeleton consisting of a series of vertebral elements. (**B**) Inspired by German natural philosophy, Owen (1866) also proposed a segmental scheme of an anatomically more realistic archetypal vertebrate, composed of modified vertebrae. Abbreviations: *ba* hemapophysis, *br = r* branchial arches explained by Owen to represent ribs in the head, *ct* centrum, *mn* mandible, *mx* maxilla, *na* neural arch, *pa* pleuroapophysis, *sp* processus spinosus. ((**A**) Redrawn from Carus (1828). (**B**) Redrawn from Owen (1866))

Horder et al. (2010), Noden and Schneider (2006), and Kuratani (2008)). The idea of a segmented head dates back to the "vertebral theory" derived from German natural philosophy more than two centuries ago (reviewed by Gegenbaur (1887) and De Beer (1937)), which postulated that the vertebrate skull is simply an assemblage of modified vertebrae. This was an epistemological concept and did not consider the process of evolution. In the mid-nineteenth century, Richard Owen (1866) developed the idea into an archetype of vertebrates, a schematic and idealistic representation of a generalized vertebrate skeleton, which also explained

the vertebrate body as consisting of vertebrae, with visceral arches described as modified ribs as once Carus (1828) did (Fig. 1a and b). Before Darwin (1959) published *The Origin of Species*, the scheme did not refer to any vertebrate ancestors.

Owen's archetype, as well as vertebral theory, were refuted by Huxley (1858) who showed that vertebrate embryos never develop head vertebrae prior to craniogenesis. Huxley's argument relied on the concept of recapitulation (before Ernst Haeckel (1834–1919) famously introduced his theory of recapitulation): if the vertebrate ancestor was once segmented, that condition should appear in the development of modern vertebrates. However, Huxley could not identify a partially segmental nature of the parachordal cartilage (the primordium of the neurocranium of modern jawed vertebrates, which continues posteriorly into the occipital cartilage), which represents modified vertebral elements. Support for this concept was found in embryological analyses, and it was also shown to be correct by experimental embryology (Couly et al. 1993). Thus, the vertebral theory was only partially correct as far as the posterior part of the modern gnathostome (but not cyclostome) cranium is concerned.

Somites in the Head?

In the late nineteenth century, comparative embryologists discovered pairs of mesodermal epithelial coeloms rostral to the level of the otocyst (preotic level) in vertebrate embryos, which started a second round of debate regarding head segmentation. These coeloms do not represent enterocoels but secondarily arise in the mesenchyme at the early to mid-pharyngular developmental stage of elasmobranch embryos. They are now generally called head cavities (reviewed by Kuratani and Adachi (2016)).

They were first reported by Francis Balfour (1851–1882) (1878), who described three pairs of "head somites" (head cavities) in shark embryos. He stated that they grew ventrally into pharyngeal arches except for the most rostral cavity, which arises rostral to the mandibular arch. According to Balfour, head cavities comprise both paraxial and pharyngeal archmesoderm, showing a single metamerism in the head, one head cavity being equivalent to a single somite in the trunk. This view was employed recently by Holland et al. (2008 and references therein) (Fig. 2).

van Wijhe (1882) published a more detailed description of the development of head cavities in sharks and as a serial homologue of trunk somites, he defined the head cavity as the dorsal balloon-like part of the coelom alone; this became the standard definition. Work from Platt (1891) also supported this schema whereby the head mesoderm was thus divided dorsoventrally into paraxial somatic portions (head cavities) and visceral portions confined in pharyngeal arches, like somites and lateral plates in the trunk, respectively.

Based on the head cavities in elasmobranchs, Edwin Stephen Goodrich (1868–1946) (1930) posited a segmental scheme for the gnathostome head, reminiscent of the amphioxus head, which possesses a notochord and myotomes throughout its body axis, but no overt head. This scheme showed not only a morphological

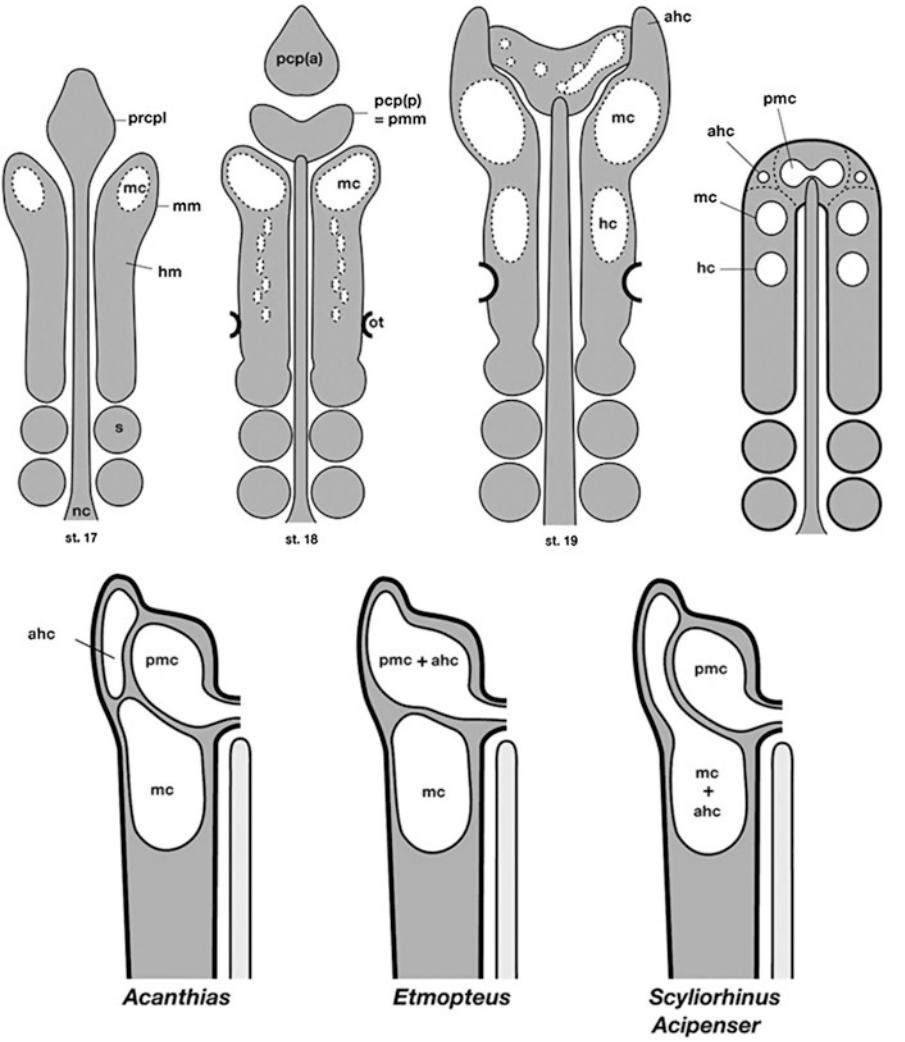


Fig. 2 Head cavities. Top. Developmental sequence of head cavities in *Scyliorhinus torazame* (st 17–19) and scheme of cavity-forming sites in the elasmobranch head mesoderm. Note that the anterior head cavity (ahc) arises between the premandibular (prm) and mandibular (mc) cavities. Bottom. Various types of cavity formation observed in various elasmobranch and *Acipenser* species. The head cavities fuse secondarily in various patterns in different species. Abbreviations: *ahc* anterior head cavity, *hc* hyoid cavity, *hm* hyoid mesoderm, *mc* mandibular cavity, *mm* mandibular mesoderm, *nc* notochord, *ot* otic vesicle, *pcp*(*a*) anterior part of the prechordal plate in elasmobranch embryos, *pcp*(*p*) = *pmm*, posterior part of the prechordal plate derivative that will form the premandibular mesoderm in elasmobranchs, *pmc* premandibular cavity, *prcpl* prechordal plate, *s* somites. (Modified from Adachi and Kuratani (2012))

pattern but also schematized the developmental fates of mesodermal anlage. For example, the head cavities differentiate into subsets of extrinsic eye muscles, each innervated by cranial nerves III, IV, and VI, respectively, showing a segmental

organization reminiscent of the relationship between spinal nerves and the myotome in the trunk.

However, Goodrich's segmental mapping appears correct only at the early pharyngula stage (Fig. 3). In neurula of many gnathostomes, the head mesoderm first appears as paraxial mesoderm, but it soon becomes secondarily dorsoventrally specified into definite paraxial and pharyngeal arch portions, as shown by examination of the expression patterns of the *Pitx2* and *Tbx1* genes (reviewed by Adachi and Kuratani (2012)); the question of head segmentation is primarily concerned with the morphological specification established at the phylotypic period. Typical segmentalists, like Goodrich, were more or less biased by the morphology of the elasmobranch pharyngula or basal osteichthyans, but those who studied other taxa like amniotes reported different numbers of head segments and emphasized the independence of somitomerism and branchiomerism, both evolutionarily and developmentally (reviewed by Kuratani (2008)).

Even within elasmobranchs, the number of head cavities varies. The head cavities have been suggested to represent secondary epithelialization of mesodermal mesenchyme, consistent with the process of head cavity development (Fig. 2). The number of head cavities (as defined by van Wijhe) decreases towards more derived gnathostomes, and there is no clear evidence for head cavities in cyclostomes (reviewed by Kuratani and Adachi (2016)). Head cavities are, therefore, more likely to represent a synapomorphy that defines gnathostomes, not to reflect a prototypical segmented head of vertebrate ancestors.

If head cavities represent the ancestral somite-like properties of the head mesoderm, elasmobranch head cavities should show gene expression profiles more similar to those of somites than to those of the head mesoderm of more derived vertebrates. However, head cavities and head mesoderm show highly conserved gene expression profiles, conspicuously different from those of somites. Moreover, a similar distinction is observed across vertebrates, whether or not head cavities arise. Curiously, amphioxus somites are not always somite-like, but they show both head- and trunk-like patterns of gene expression (reviewed by Adachi et al. (2012)).

Somitomeres

Additional evidence in support of head segments was obtained when somitomeres were observed (cephalic somitomeres, to be precise) in scanning electron microscopy analysis of early chicken embryos (reviewed by Jacobson (1988)). Somitomeres primarily refer to precursors of somites in the unsegmented paraxial mesoderm, but in the head, only segmental bulges were reported to appear in various vertebrate species. Interestingly, the suggested numbers of somitomeres (usually 7) were not consistent with those in traditional head segmental theories (see above). To date, no further analyses support the presence of such segments, nor are gene expression patterns suggestive of paraxial segmentation in the pre-otic head (reviewed by Kuratani (2008)).

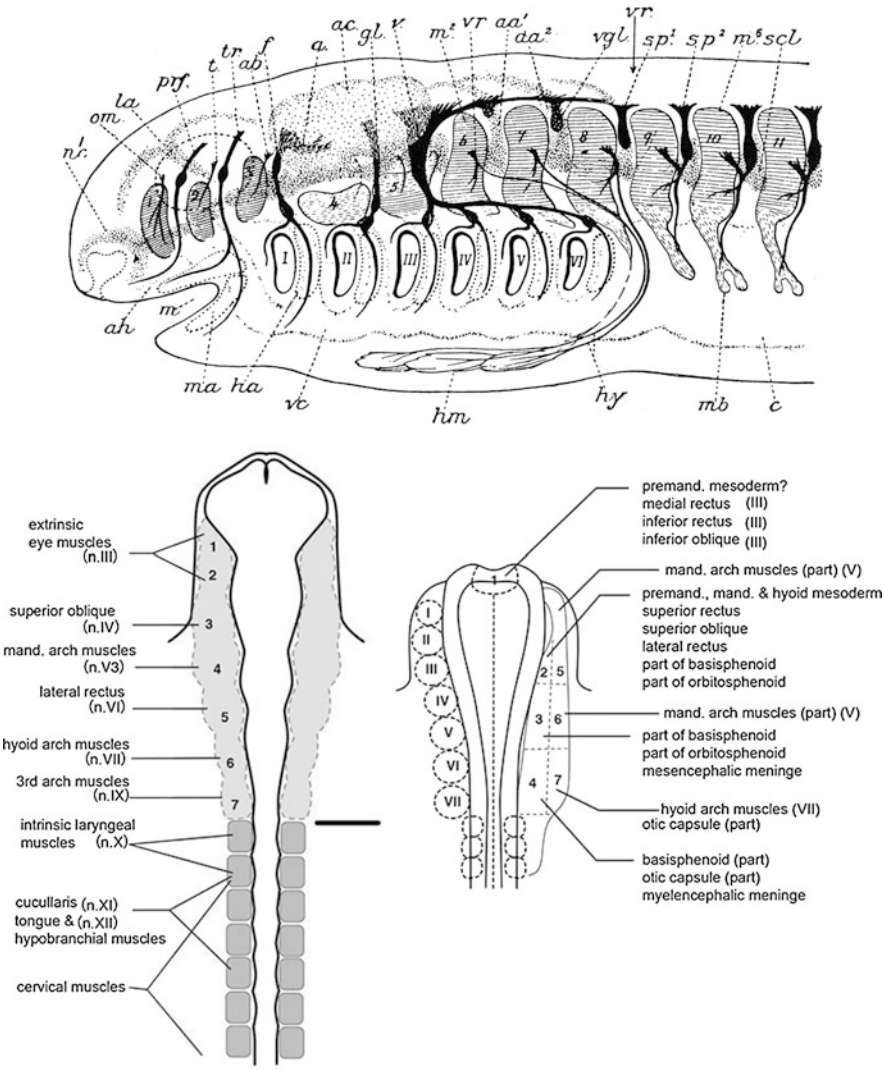


Fig. 3 Embryological theories of head segmentation. Top. The scheme proposed by Goodrich (1930) based on observation of the elasmobranch embryonic head. Three pairs of head cavities are counted as head somites (numbered 1–4), serial homologues of the true somites in the trunk (typically, 6–11). Each head somite was assumed to be associated with pharyngeal arches directly below it, as is typical in segmental theories. Bottom. Summaries of fate-mapping studies of head mesoderm performed at different developmental stages in chicken embryos. Note that the map obtained at stages 9–10 by Noden (1988) is more consistent with the Goodrich-like segmental scheme than that at stage 8 obtained by Couly et al. (1992). Abbreviations: n.III–XII and III–VII, cranial nerve innervations; 1–7 (bottom left) and I–VII (bottom right), hypothetical cephalic somitomeres not observed in these studies; 1 (bottom right), prechordal plate derivative; 2–7 (bottom right), head mesodermal domains defined by Couly et al. (1992). (Top. From Goodrich (1930). Bottom. Redrawn from Noden (1988) and Couly et al. (1992). Also see Evans and Noden (2006) for more detailed data)

Cyclostome embryos contain no indication of somitomeres or head somites as mentioned previously (reviewed by Kuratani and Adachi (2016)). As an exception, lampreys develop enterocoelic mesoderm in the mandibular arch, which, however, does not fall in the category of "head somites" or "somitomeres." Nevertheless, it must be noted that somitomere-like structures were repeatedly reported near the turn of nineteenth to twentieth centuries, but only in *Torpedo* embryos (reviewed by Kuratani and Adachi (2016)). Further analysis of these structures using more recently developed technologies would be worthwhile, to examine whether they show any somite-like properties.

Nervous System

Study of neuromeres forms another stream of head segmentation research. This line of inquiry began with the discovery of neuromeres, or neurepithelial compartments in the neural tube, and the suggestion that they were possible segmental units, and was strengthened by the discovery of the *Hox* code at the end of the twentieth century (reviewed by Lumsden and Keynes (1989) and Hunt and Krumlauf (1991)). The rhombomeres were shown to restrict cell lineages within developmental compartments (Fraser et al. 1990). This discovery suggested the existence of metameres comprising neuromeres, cephalic neural crest cells, cranial nerves, and visceral arches and their derivatives, which repeat along the anteroposterior body axis, established and specified by common molecular developmental mechanisms including *Hox* gene expression (Hunt and Krumlauf 1991). Of course, this is restricted to a single part of the head, and the metamerical organization is less clear in amphioxus. Furthermore, rhombomeres become more conspicuous in the embryos of more "advanced" species (Neal and Rand 1946).

Neuromeres have been recognized in other domains of the vertebrate central nervous system as well; in the spinal cord, myeromeres arise with boundaries corresponding to mid-somite levels. These neuromeres are, therefore, likely to be secondarily induced by the presence of somites (Lim et al. 1991). In the forebrain, another series of segments called "prosomeres" arise, also by cell lineage restriction, with compartment-restricted gene expression (Figdor and Stern 1993). These neuromeres are dorsoventrally compartmentalized into four domains, each polygon representing a center of cell proliferation, as once advocated by Swedish research groups such as Bergquist and Källen (1954), or anlage of specific anatomical units (Figdor and Stern 1993; Puelles and Rubenstein 1993).

Thus, from the viewpoint of head segmentation, the fundamental question is whether or not all the above-listed segments represent continuous serial homologues, as predicted by the theory. This question was first asked by Locy and Hill near the beginning of the twentieth century (Locy 1895; Hill 1900). They observed, in early embryos of chickens and salmon, a series of bulges along the entire neuraxis; however, this observation was not repeated by any other researchers. Neal, on the other hand, focused on the pharyngular morphology of vertebrate embryos and tried to relate all neuromeres to hypothetical mesodermal segments, including head

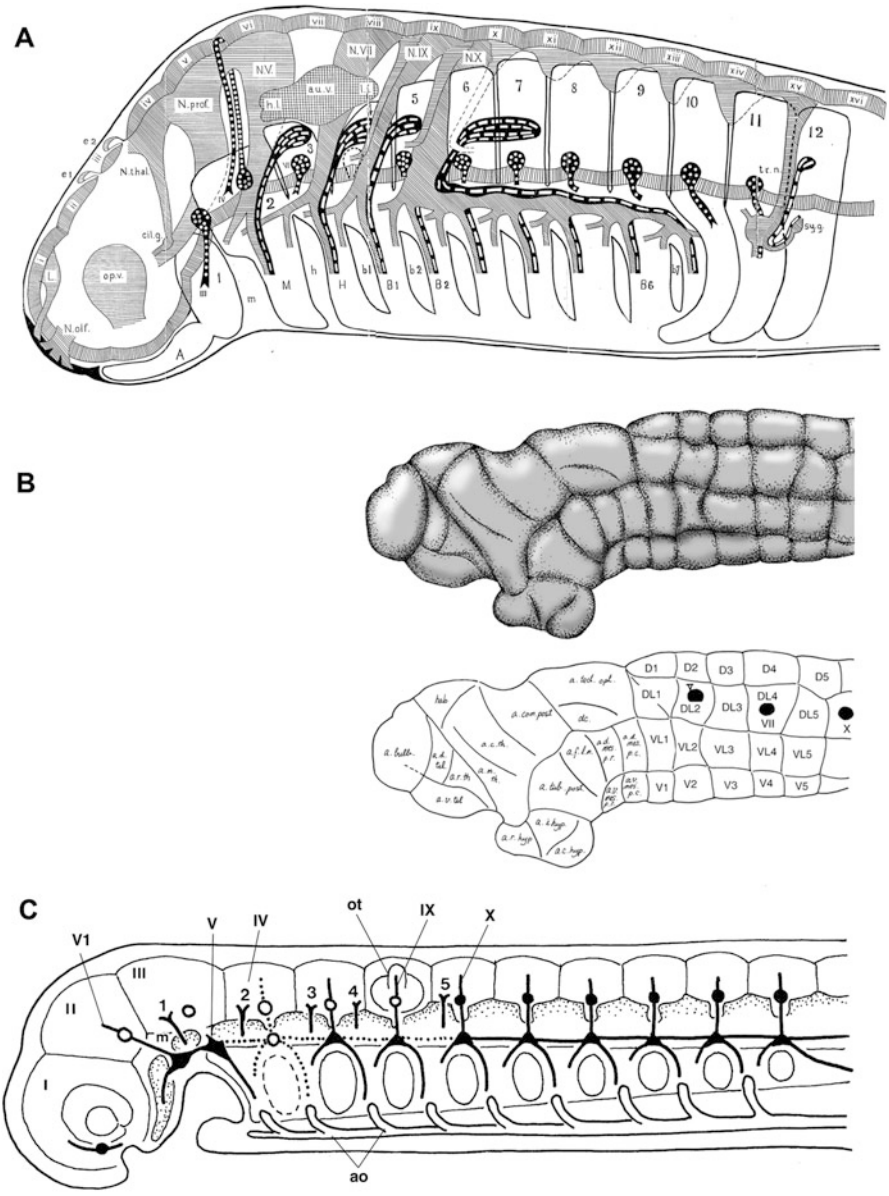


Fig. 4 Various neuromere theories. (**a**) The scheme proposed by Johnston (1905). One neuromere was assumed to contain primarily a full set of functional columns containing a full set of neurons. (**b**) Reconstruction of a lamprey larval brain by Bergquist and Källen, emphasizing cell proliferation centers (top), showing the polygonal architecture of the vertebrate neural tube dorsoventrally divided into four columns, corresponding to the neuronal functional columns. This scheme is very similar to the currently accepted polygonal model proposed by Puelles and others.

cavities, by means of peripheral nerves; this attempt was not successful, either (Fig. 4c; reviewed by Neal and Rand (1946)). Neal believed that one neuromere should primarily correspond to one metamere, with one mesodermal segment innervated by one segmental nerve.

Based on the model of so-called "functional columns," or longitudinal zones of particular groups of neurons, identified in the brainstem, Johnston (1905) put forth an idealized scheme, in which neuromeres, containing a set of neuronal cell types, are arranged one after another along the anteroposterior neuraxis (Fig. 4a). Typical neuromeres can be found in rhombomeres, but other neuromeres were thought to have lost many of the columns. A similar model was also put forth by Bergquist and Källen (1954) (Fig. 4b), but they did not try to expand their model to encompass head segmentation. In a similar concept, rhombomeres were shown to be serial homologues among themselves based on the observation that zebrafish rhombomeres possess an identical set of reticulospinal neurons, which, to some extent, is applicable to amniote and cyclostome rhombomeres (reviewed by Murakami et al. (2004)).

Neural Crest, Placodes, and Generative Constraints

Missing from the traditional conception of the head problem was the idea of neural crest cells; the classical hypotheses were too focused on the mesodermal architecture of embryos. The reason is clear: distinguishing crest-derived ectomesenchyme from mesodermally derived mesomesenchyme is very difficult using histological observations alone. In addition, mesenchyme and mesoderm were thought to be synonymous. Second, the head problem, especially for segmentalists, primarily involved viewing the head as a modified trunk, which is the default, but the ectomesenchyme is absent from the trunk. A further complication is that neural crest cells are absent from amphioxus, a favored comparison for segmentalists. As first pointed out by Northcutt and Gans (1983), however, the neural crest and placode cell lineages are vertebrate-specific and characterize the vertebrate head, as has been shown by experimental embryological studies (for discussion of the precursors of these cell lineages in nonvertebrate chordates, see chapter ▶ "Evolution and Development of the Vertebrate Cranium" for the skull).

Curiously, the developmental sequencing of placodes and ectomesenchyme clearly shows embryonic patterning. For example, epibranchial placodes, the source of the cranial sensory ganglia associated with branchiomeric nerves, are induced in the surface ectoderm by factors released by the endodermal pouches, generating

Fig. 4 (continued) (**c**) Neal proposed a metamerical scheme in which neuromeres were associated with peripheral nerves, pharyngeal arches, and mesodermal segments, involving all the organ systems in the vertebrate body. Abbreviations I–X cranial nerves, *ao* aortic arches, 1–5 in **c**, ventral roots of spinal nerves. ((**a**) From Johnston (1905). (**b**) Redrawn from Bergquist and Källen (1954). (**c**) Redrawn from Neal and Rand (1946)))

the repeated pattern of pharyngeal arches. Thus, the pharyngeal pouches, which are the initiator of the branchiomeric patterns in the head, are situated upstream. Similarly, in the trunk, the neural crest cells are not segmented, but due to the presence of somites, migrating crest cells are divided into segmental streams, leading to segmental development of the dorsal root ganglia (somites as the source of somitomerism). Thus, the head problem, in the context of developmental mechanism, should be considered in terms of the distribution of the source of the generative constraints that lead to the segmental patterning of the embryonic body, and the central question is whether the head mesoderm possesses trunk-like constraints that divide the crest cell stream (reviewed by Kuratani (2008)).

Based on previous experiments, it is not the paraxial mesoderm but the rhombomeres (r3 and r5) that divide the dorsal part of the cephalic crest cell stream into three cell populations, leading to formation of cranial nerve roots and sensory ganglia, and the ventral crest cells are divided into the pharyngeal arches by the pharyngeal pouches (reviewed by Kuratani (2008); also see Trainor and Krumlauf (2000) and reference therein for developmental interactions between cephalic crest cells and head mesoderm). Therefore, the vertebrate body is uniquely patterned segmentally by pharyngeal pouches in the head and by somites in the trunk, i.e., a known developmental mechanism now supports the nonsegmental view. Posterior to the otic vesicle, the somites and pharyngeal pouches overlap dorsoventrally, thereby forming a unique S-shaped interface, a feature which is not present in amphioxus, an entirely somitomeric organism.

Evolutionary Origin of Head Segmentation

From a wider evolutionary perspective, the question of head segments can be traced back to the origin of segments in ancestral bilaterians. Hejnol and Martindale (2008) postulated an image of a hypothetical ur-bilaterian, constructed by assemblage of symplesiomorphic molecular developmental features of major bilaterian model organisms (Fig. 5). In this scheme, body segments are assumed to be present, indirectly suggesting that the deuterostome and protostome segments are homologous. Although it is true that Notch-Delta signaling plays a central role in segmental patterning processes in different phyla, the question of its origin remains unsettled (reviewed by Couso (2009)).

Potentially relevant to this question would be the origin of the mesodermal coelom: the segments were traditionally regarded as coeloms, and in the late nineteenth century, possession of coeloms was regarded to represent a certain grade of evolution. Some British embryologists compared the “gut pouches” of cnidarians (jellyfish) with the coelomic cavities in bilaterians (Fig. 5; reviewed by Starck (1979) and Arendt (2018)). In particular, the model proposed by Masterman clearly explains the tricoelomic larval morphology of dipleurula larva of deuterostomes or actinotrocha of phoronids. From such a tricoelomic state, Masterman derived bilaterian adult segmental body plans.

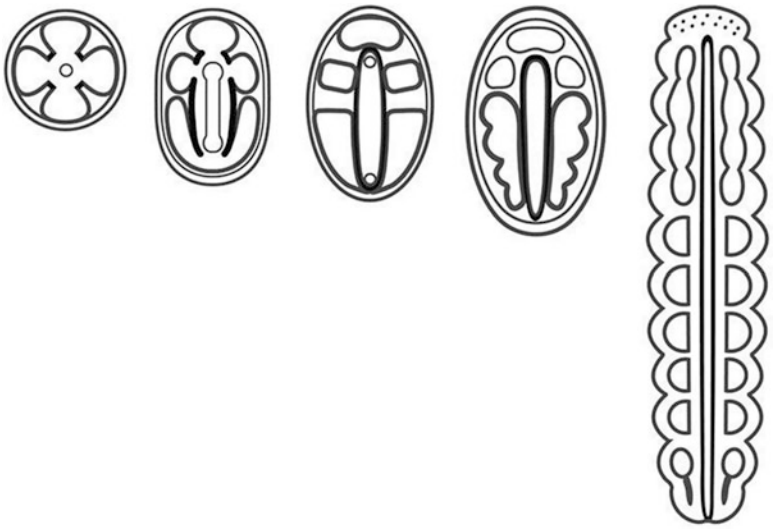


Fig. 5 Evolutionary origin of mesodermal segments. Gastric pouch theory identified the origin of enterocoels in a jellyfish (left). By obtaining an anteroposterior axis, the pouches became three pairs of coelomic cavities, as found in bilaterian larvae (middle three). Of those, the posterior pouch, the metacoel, became secondarily elongated and segmented to produce typical somites, for example, in the vertebrate trunk (right). According to this explanation, the trunk somites and head mesoderm of vertebrates may not represent serial homologues. (Redrawn from Starck (1979))

Curiously, cnidarians and bilaterians show striking similarity of genomes, gene expression profiles that define cell types, and central nervous system organization (Tosches and Arendt 2013). Although Haeckel's Gastrea theory (Haeckel 1874) has been discredited, it now appears more realistic to search for the origins of segmented coeloms in cnidarian development.

The data presented so far do not support the presence of paraxial mesodermal segments in the head. Nevertheless, this question gave rise to the field of comparative morphology and offered basic concepts such as serial homology and body plans, which remain valid today. It now seems most appropriate to view the question in terms of the origin of coelomic cavities including vertebrate somites – do the segments of vertebrates and invertebrates share a common origin in cnidarians?

Cross-References

- ▶ Developmental Homology
- ▶ Gavin de Beer (1899–1972)
- ▶ Shifting the Black Box: Approaches to the Development and Evolution of the Vertebrate Mesoderm

References

Adachi N, Kuratani S (2012) Development of head and trunk mesoderm in a dogfish, *Scyliorhinus torazame*. I. Embryology and morphology of the head cavities and related structures. Evol Dev 14:234–256

Adachi N, Takechi M, Hirai T, Kuratani S (2012) Development of the head and trunk mesoderm in the dogfish, *Scyliorhinus torazame*. II. Comparison of gene expressions between the head mesoderm and somites with reference to the origin of the vertebrate head. Evol Dev 14:257–276

Arendt D (2018) *Hox* genes and body segmentation. Science 361:1310–1311

Balfour FM (1878) The development of the elasmobranchial fishes. J Anat Physiol 11:405–706

Bergquist HB, Källen B (1954) Notes on the early histogenesis and morphogenesis of the central nervous system in vertebrates. J Comp Neurol 100:627–660

Carus CG (1828) Von den Ur-Theilen des Knochen- und Schalengerrüstes. G. Fleischer, Leipzig

Couly GF, Colty PM, Le Douarin NM (1992) The developmental fate of the cephalic mesoderm in quail-chick chimeras. Development 114:1–15

Couly GF, Coltey PM, Le Douarin NM (1993) The triple origin of the skull in higher vertebrates: a study in quail-chick chimeras. Development 117:409–429

Couso JP (2009) Segmentation, metamerism and the Cambrian explosion. Int J Dev Biol 53:8–10

Darwin C (1959) The Origin of Species by Means of Natural Selection. John Murray, London

De Beer GR (1937) The Development of the Vertebrate Skull. Oxford Univ. Press

Evans DJ, Noden DM (2006) Spatial relations between avian craniofacial neural crest and paraxial mesoderm cells. Dev Dyn 235:1310–1325

Figdor MC, Stern CD (1993) Segmental organization of embryonic diencephalon. Nature 363:630–634

Fraser S, Keynes R, Lumsden A (1990) Segmentation in the chick embryo hindbrain is defined by cell lineage restriction. Nature 344:431–435

Gegenbaur C (1887) Die Metamerie des Kopfes und die Wirbeltheorie des Kopfskelets. Morphol Jb 13:1–114

Goodrich ES (1930) Studies on the structure and development of vertebrates. McMillan, London

Hejnol A, Martindale MQ (2008) Acoel development indicates the independent evolution of the bilaterian mouth and anus. Nature 456:382–386

Hill C (1900) Developmental history of primary segments of the vertebrate head. Zool Jahrb Abt Anat Ontog Tiere 13:393–446

Holland LZ, Holland ND, Gilland E (2008) Amphioxus and the evolution of head segmentation. Integr Comp Biol 48:630–646

Horder TJ, Presley R, Slipka J (2010) The head problem. The organizational significance of segmentation in head development. Acta Univ Carol Med Monogr 158:1–165

Hunt P, Krumlauf R (1991) Deciphering the *Hox* code: clues to patterning branchial regions of the head. Cell 66:1075–1078

Huxley TH (1858) The Croonian lecture: on the theory of the vertebrate skull. Proc Zool Soc London 9:381–457

Jacobson AG (1988) Somitomeres: mesodermal segments of vertebrate embryos. Development 104(suppl):209–220

Johnston JB (1905) The morphology of the vertebrate head from the viewpoint of the functional division of the nervous system. J Comp Neurol 15:175–275

Kuratani S (2008) Is the vertebrate head segmented? – evolutionary and developmental considerations. Integr Comp Biol 48:647–657

Kuratani S, Adachi N (2016) What are head cavities? – history of studies on the vertebrate head segmentation. Zool Sci 33:213–228

Lim TM, Jaques KF, Stern CD, Keynes RJ (1991) An evaluation of myelomeres and segmentation of the chick embryo spinal cord. Development 113:227–238
Locy WA (1895) Contributions to the structure and development of the vertebrate head. J Morphol 11:497–594
Lumsden A, Keynes R (1989) Segmental patterns of neuronal development in the chick hindbrain. Nature 337:424–428
Murakami Y, Pasqualetti M, Takio Y, Hirano S, Rijli F, Kuratani S (2004) Segmental development of reticulospinal and branchiomotor neurons in the lamprey: insights into evolution of the vertebrate hindbrain. Development 131:983–995
Neal HV, Rand HW (1946) Comparative anatomy. Blakiston, Philadelphia
Noden DM (1988) Interactions and fates of avian craniofacial mesenchyme. Development 103(Suppl):121–140
Noden DM, Schneider RA (2006) Neural crest cells and the community of plan for craniofacial development: historical debates and current perspectives. In: Saint-Jeannet J-P (ed) Neural crest induction and differentiation. Landes Bioscience, Georgetown, pp 1–23
Northcutt RG, Gans C (1983) The genesis of neural crest and epidermal placodes: a reinterpretation of vertebrate origins. Q Rev Biol 58:1–28
Owen R (1866) On the anatomy of vertebrates, vol 1. Longmans, Green & Co, London
Platt JB (1891) A contribution to the morphology of the vertebrate head, based on a study of Acanthias vulgaris. J. Morphol 5:79–106
Puelles L, Rubenstein JLR (1993) Expression patterns of homeobox and other putative regulatory genes in the embryonic mouse forebrain suggest a neuromeric organization. Trends Neurosci 16:472–479
Sewertzoff AN (1895) Die Entwicklung der occipital Region der niederen Vertebraten im Zusammenhang mit der Frage über die Metamerie des Kopfes. Bull Soc imp Nat Moscou, Annee 1895:186–284
Starck D (1979) Vergleichende Anatomie der Wirbeltiere auf evolutionsbiologischer Grundlage. 2. Das Skeletsystem; Allgemeines, Skeletsubstanzen, Skelet der Wirbeltiere einschliesslich Locomotionstypen. Springer, Berlin, Heidelberg, New York
Tosches MA, Arendt D (2013) The bilaterian forebrain: an evolutionary chimaera. Curr Opin Neurobiol 23:1080–1089
Trainor P, Krumlauf R (2000) Plasticity in mouse neural crest cells reveals a new patterning role for cranial mesoderm. Nat Cell Biol 2:96–102
van Wijhe JW (1882) Über die Mesodermsegmente und die Entwicklung der Nerven des Selachierkopfes. Ver Akad Wiss Amsterdam, Groningen, pp 1–50

Evolution and Development of the Vertebrate Cranium

Shigeru Kuratani

Contents

Abstract

The cranium represents a derived trait that defines vertebrates; two different cell lineages, the NC and the mesoderm, are involved in its development. Functionally, the cranium can be divided into the neurocranium and the viscerocranium. However, this division does not reflect the developmental origins of the cells in jawed vertebrates. Developmental specification of the cranial primordium is mediated by coordinated expression of homeobox genes, established through tissue interactions. Comparison with cyclostome embryonic patterns shows that the cranium of jawed vertebrates appears to have undergone a series of changes in developmental program, making this structure highly complicated.

S. Kuratani (✉)
Laboratory for Evolutionary Morphology, RIKEN Center for Biosystems Dynamics Research (BDR), Kobe, Japan

Evolutionary Morphology Laboratory, RIKEN Cluster for Pioneering Research (CPR), Kobe, Japan
e-mail: shigeru.kuratani@riken.jp

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_187

Keywords

Cranium · Verterates · Evolution · Development

Glossary

Cavum epiptericum	Extracranial space located between the palatoquadrate and primary cranial wall at the level of the midbrain.
Chordal cranium	Mesodermally derived neurocranial portion arising on both sides of the notochord. It arises from paired cartilaginous rod-like structures called parachordals.
Dermatocranium	The part of the cranium that is formed from dermal bone.
Head mesoderm	Unsegmented mesoderm in the rostral part of vertebrate embryos. In the cranium, the posterior part of the neurocranium (including the sphenoid, otic capsule, ala hypochiasmatica in mammals, and the supratrabecula in sauropsids) is derived from this mesoderm.
Neural crest (NC) cells	Vertebrate-specific multipotent cell lineage, derived from the ectodermal ridge between the epidermis and neural plate of embryos. NC cells arising from the cephalic neural crest (CNC) form an extensive ectomesenchyme, and give rise to a large part of the cranium.
Neurocranium	The dorsal part of the cranium that encapsulates the brain and sensory organs.
Palatoquadrate	Dorsal half of the skeleton arising in the mandibular arch of jawed vertebrates.
Prechordal cranium	CNC cell-derived, rostral part of the neurocranium. Arises from a pair of cartilaginous bands called trabeculae.
Viscerocranium	The ventral part of the cranium that supports the pharynx.

Introduction

The vertebrate cranium represents the most conspicuous skeletal module associated with the head, and defines vertebrates including cyclostomes (modern jawless vertebrates comprising lampreys and hagfish). The morphological diversification of the cranium has attracted the interest of comparative morphologists and embryologists, providing traits useful in developing taxonomic keys (Gregory 1935; De Beer 1937; Portmann 1976; Hanken and Hall 1993). Its central component is the endoskeleton, but the exoskeletal portion, or dermatocranium, occupied an increasing portion of the cranium as its functional importance increased through evolutionary time and has become dominant especially in adult osteichthyans (including amniotes). The endoskeleton initially differentiates into cartilage that later ossifies to form bony tissues, whereas the exoskeleton directly ossifies in the

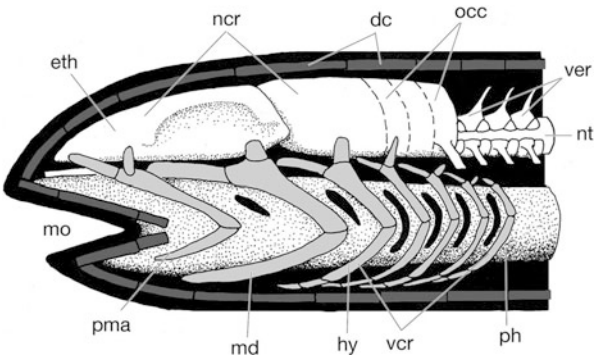


Fig. 1 Classical concept of the vertebrate cranium. Based on the morphology of basal jawed vertebrates such as sarcopterygians, as developed by Portmann (1976). The occipital (occ), the posterior part of the neurocranium (ncr), is considered to be modified rostral vertebrae secondarily assimilated into the cranium. Note the positions of the cranial components (neurocranium, viscerocranium, and dermatocranium). The premandibular arch (pma) is drawn in front of the mandibular arch (md), which does not match the modern understanding. Abbreviations: *dc* dermatocranium, *eth* ethmoidal region, *hy* hyoid arch skeleton, *md* mandibular arch skeleton, *mo* mouth, *ncr* neurocranium, *nt* notochord, *occ* occipital, *ph* pharynx, *pma* hypothetical premandibular arch skeleton, *vcr* branchial arch skeletons, *ver* vertebrae. (Redrawn from Portmann (1976))

dermis (for a discussion of types of vertebrate skeletal elements and types of histogenesis of bones, see Hirasawa and Kuratani 2015).

The entire cranium is dorsoventrally divided into the neurocranium, which consists of capsules that contain the brain and sensory organs, and the viscerocranium, which consists of the components of the visceral arch skeleton that supports the pharynx. Both cranial parts consist of exo- and endoskeletal elements. This division is primarily defined by functions that were secondarily modified through evolution (Fig. 1; reviewed by Kuratani and Ahlberg 2018).

Morphological Concepts and Development

Traditionally, the vertebrate cranium was described as a segmental structure composed of modified vertebrae and ribs (reviewed by De Beer 1937; Jollie 1977; see chapter ▶ History and Current Theories of the Vertebrate Head Segmentation). This idea was employed by a number of comparative morphologists and embryologists, and the positions of cranial nerves were used as a key to compare cartilaginous pillar structures with the neural arches of hypothetical cranial vertebrae (Jollie 1977). This scheme is no longer accepted, nor is the neurocranium understood to be a simple capsule.

In traditional comparative morphology, the neurocranium was thought to arise from mesenchyme directly covering the brain; the original position of the neurocranium was assumed to arise from mesoderm just outside the meninges (Gaupp 1902). The original (conceptual) neurocranium is called the "primary cranial

wall," a large part of which is secondarily reduced and functionally replaced by dermal elements or secondarily modified visceral elements, making it difficult to identify the original position of the neurocranium. The reduction is conspicuous at the level of the midbrain, or the "orbitotemporal region," where the neuraxis is strongly flexured to create an extensive extracranial space called the "cavum epiptericum" (the trigemino-facial chamber in fishes) that contains the cranial sensory ganglia and the proximal portions of the extrinsic eye muscles.

This cavum is laterally covered by the epipterygoid or ala temporalis and alispenoid in mammals (both derivatives of the palatoquadrate) that function as the secondary cranial wall, attached lateral to the original one. The mammalian dorsum sellae and associated cartilaginous nodules, and the pila antotica of monotremes and reptiles, are described as representing remnants of the original brain case. However, the more rostral part of the gnathostome neurocranium has a more complex history and developmental origin, i.e., the neurocranium in jawed vertebrates is known to develop from two types of mesenchyme, the mesodermal mesenchyme (meso-mesenchyme) and the CNC-derived mesenchyme (ectomesenchyme).

Mesoderm and Neural Crest

In jawed vertebrate embryos, CNC cells are primarily distributed in the ventral part of the head, subsequently differentiating into craniofacial and viscerocranial elements (reviewed by Le Douarin 1982; and Noden 1984; see McCauley and Bronner-Fraser 2003 for cyclostomes). Such a skeletogenic property is only associated with the CNC cells but not in trunk neural crest cells found in postotic region of the embryo. The CNC cells delaminate from the epithelial NC (de-epithelialization) and migrate along the pathway called, the "dorsolateral pathway" beneath the surface ectoderm and characteristically populate the ventral part of the embryonic head. This pathway is inhibited by the presence of dermomyotomes and found extensively only in the somite-free region, i.e., the preotic part of the embryonic head. Such a characteristic migration and distribution pattern stand in contrast to those of trunk NC cells that are segmentally divided by the presence of somites in more medial parts of the embryo, prefiguring the spinal nerve's anatomical patterns. The distribution of the CNC cells is, therefore, the key to understand basic anatomical patterns of cranial (branchiomeric) nerves (CnV, VII, IX and X) as well as oro-visceral part of the vertebrate cranium.

In cephalochordates (amphioxus) and hemichordates, type II collagen, a cartilage-specific component of extracellular matrices (ECM), and expression of *Sox9/10* homologues upstream of the type II collagen-encoding genes are seen in the pharyngeal epithelia, unlike in vertebrates, where these molecules are associated with CNC-derived ectomesenchyme (reviewed by Ota and Kuratani 2009). Whether the CNC cells or the mesoderm first differentiated into skeletal elements in vertebrate evolution has yet to be determined (Hall 1999).

In jawed vertebrates, the rostral part of the neurocranial base (trabecula) is of CNC origin (Fig. 2). The contribution of the CNC cells to the cyclostome

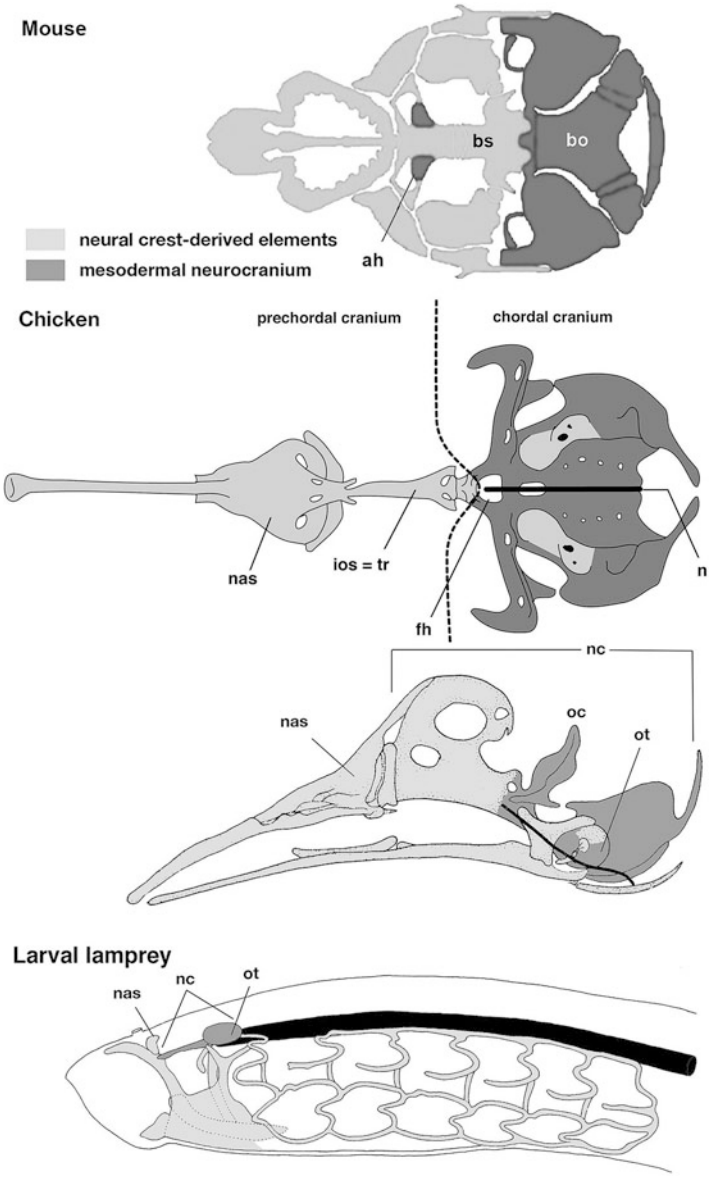


Fig. 2 Distribution of CNC- and mesoderm-derived cells in the vertebrate cranium. Top: contributions of the mesoderm (dark grey) and CNC (light grey) in the formation of the mouse cranium, based on transgenetic experiments. Middle: Fate mapping analyses in a chicken embryo (Couly et al. 1993). The mesodermally derived part of the cranium is medially accompanied by the notochord (n) and called the chordal cranium. The rostral, CNC-derived part is lacking the notochord and is called the prechordal cranium. Bottom: In the lamprey, the entire neurocranium is thought to be derived from head mesoderm, except for the nasal capsule. Abbreviations: *ah* ala hypochiasmatica, *bs* basisphenoid, *bo* basioccipital, *fh* hypophyseal foramen, *ios = tr* interorbital

neurocranium appears to be less than that in jawed vertebrates (Kuratani and Ahlberg 2018). The mesoderm, on the other hand, differentiates into the posterior part of the neurocranium, which develops as a pair of cartilaginous rod-like structures, the parachordals. The latter continues posteriorly into the occipital cartilage, which will differentiate into the basioccipital, exoccipital, and supraoccipital elements. This set of occipital elements is derived from rostral somites, revealing its identity as secondarily modified vertebral column. The occipital represents a synapomorphy that defines modern gnathostomes, and it is not found in cyclostomes.

In jawed vertebrates, the unsegmented part of the parachordal develops into the posterior half of the neurocranial base or the central stem, and it has been shown in avian embryos to be derived from unsegmented head mesoderm. This cartilage is associated with the notochord, which is the source of signaling molecules required for normal chondrification, and for this reason, the mesodermal neurocranium is called the chordal cranium (Couly et al. 1993). Its rostral end, the mesoderm/CNC cells boundary, is located near the rostral tip of the notochord, in the acrochordal, or the orbital cartilage situated below the cephalic flexure of the brain primordium. This position ultimately occupies the middle of the sphenoid, slightly caudal to the hypophyseal foramen. The CNC-derived rostral part of the neurocranium, on the other hand, is called the prechordal cranium (reviewed by Kuratani and Ahlberg 2018). Experiments using transgenic mice revealed that the boundary occupies a very similar position in the mouse cranium, also (McBratney-Owen et al. 2008).

Although the parachordal cartilage has also been described as being located in the cyclostome chondrocrania, it has been suggested that the more rostral part of the neurocranial precursor, or the trabecula of cyclostomes, corresponds to the rostral part of the parachordal (reviewed by Oisi et al. 2013). In cyclostomes, the distinction between neuro/viscerocranium corresponds to mesoderm/CNC contributions (reviewed by Kuratani and Ahlberg 2018). Involvement of the ectomesenchyme in the rostral neurocranium is thought to have adapted in response to the enlargement of the forebrain in jawed vertebrates, which is also associated with the acquisition of the jaw (Dupret et al. 2014).

Exoskeleton

The exoskeletal part of the cranium, or the dermatocranium, is evolutionarily derived from the dermal armor of ancestral vertebrates, which covered the entire head and the shoulder girdle region. The skull was secondarily dissociated from the shoulder

Fig. 2 (continued) septum derived from trabecula, *n* notochord, *nas* nasal capsule, *nc* neurocranium, *oc* orbital cartilage (rostralmost part of the mesodermal neurocranium), *ot* otic capsule. (Modified from: (**a**) McBratney-Owen et al. (2008); (**b**) Couly et al. (1993); (**c**) Oisi et al. (2013))

by establishment of the neck, and the shoulder girdle in modern vertebrates still contains dermally derived elements. These elements are very hard to homologize between different animal lineages. Some osteichthyan elements can be identified in the placoderm exoskeleton (Fig. 3b, c; Zhu et al. 2013). There is an overt tendency that the number of dermal elements decreases approaching the mammalian lineage (Williston's law; see Gregory 1935). However, whether this pattern is due to loss or fusion of dermal elements remains unknown (Koyabu et al. 2012).

The developmental patterning mechanism that underlies the dermatocranium is not well understood, either. The dermal elements are classified into several groups, corresponding to lateral lines. Obviously, they are developmentally coupled. As implied above, the assumption that dermal elements exhibit an ancestral segmental prepattern is not supported. Some researchers have hypothesized that dermal elements are induced either by underlying brain subdomains or venous sinus primordia (Jarvik 1980). The CNC cells/mesoderm boundary is also present in the dermatocranium, but its position is not consistent among species (Fig. 4; reviewed by Hirasawa and Kuratani 2015). The reason for this inconsistency has yet to be elucidated.

Viscerocranium

The viscerocranium of jawed vertebrates shows a typical metameric pattern, in which every visceral arch consists of a consistent set of elements, comprising supra- and infrapharyngeal, epal, ceratal, hypal, and basal elements. This prototype is highly conserved in basal Osteichthyes, and it has been shown that the common ancestor of Osteichthyes and Chondrichthyes, a lineage of placoderms, possessed this ancestral pattern (Fig. 5; Pradel et al. 2014). These viscerocranial elements are mostly derived from ectomesenchyme, but the basal elements are derived from the mesoderm (Davidian and Malashichev 2013). Cyclostomes lack the latter elements.

The evolution of visceral skeletal elements offers a good example of position-dependent metamorphosis, comparable to that seen in flowers and in the heads of insects. In jawed vertebrates, the mandibular arch, or the first visceral arch, has been transformed into the central part of the biting jaw (the premandibular ectomesenchyme also participates in jaw formation in most jawed vertebrates), and the second or hyoid arch has become a part of the hyoid apparatus (third arch elements are also involved) and the hyomandibular, which functions in suspension of the jaw to the neurocranium. The third and more posterior arches are called branchial arches in many aquatic species. One of the most conspicuous examples of visceral arch transformation is provided by mammalian ear ossicles.

Mammals characteristically possess three ossicles, the malleus, incus, and stapes in the middle ear, and the evolutionary origin of these ossicles was regarded as one of the most intriguing questions in comparative and evolutionary morphology. These ossicles are pharyngeal arch derivatives, derived via homeotic modification of the visceral arches based on positional values. Thus, the malleus has been homologized with the articular (a proximal lower jaw element), the incus with the quadrate

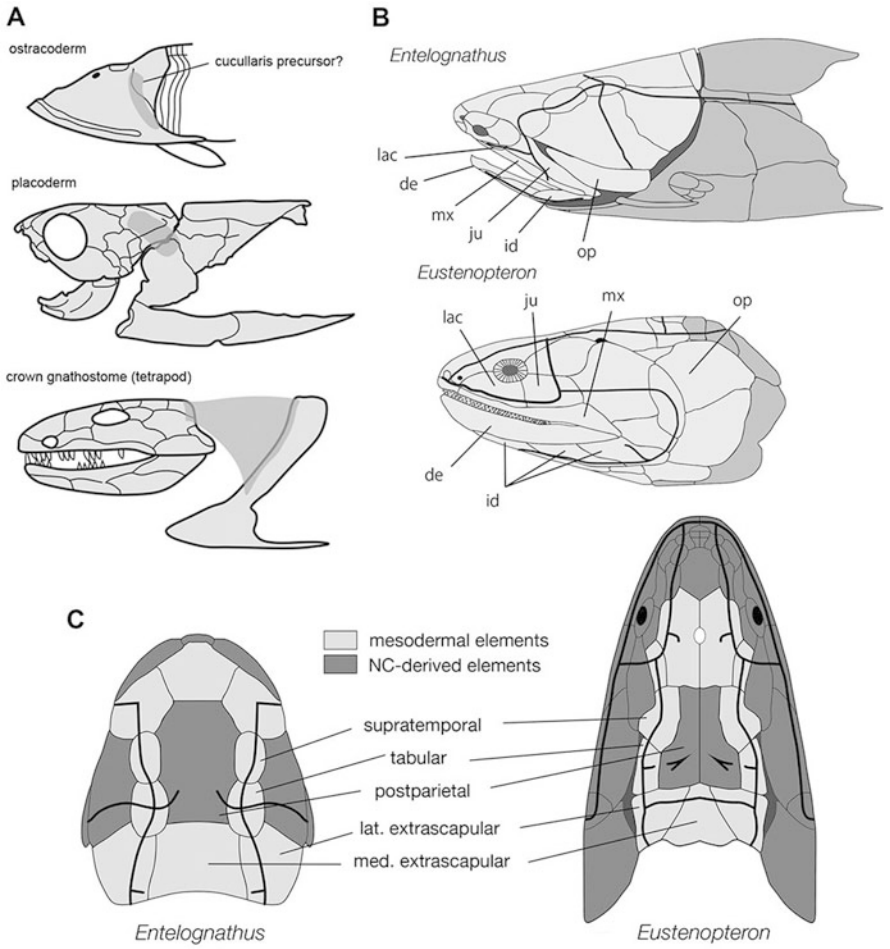


Fig. 3 Evolution of dermatocranial elements. (**a**) The dermatocranium in crown gnathostomes is thought to have been obtained by separation of the dermal shoulder girdle, which is derived from the posterior part of the dermal shield in ostracoderms (fossil jawless vertebrates belonging to gnathostomes). (**b** and **c**) Comparison of dermatocranium of *Eustenopteron* (fossil bony fish) and *Entelognathus* (a placoderm), lateral (**b**) and dorsal (**c**) views. The distribution of lateral lines corresponds to patterns of some dermal elements. In **c**, CNC- and mesoderm-derived elements are different colors based on the assumption that the CNC–mesoderm interface is located between the frontal and parietal bones and that postparietal homologues are consistently derived from the CNC in sarcopterygians (including mammals). (Modified from: (**a**) Kuratani 2013; (**b** and **c**) Hirasawa and Kuratani 2015 based on Zhu et al. (2013))

(a proximal upper jaw element), and the stapes with the hyomandibular, the dorsal element of the hyoid arch (Reichert 1837). Of these, the stapes is homologous to the single ear ossicle, called the columella auris, that is found in nonmammalian tetrapods.

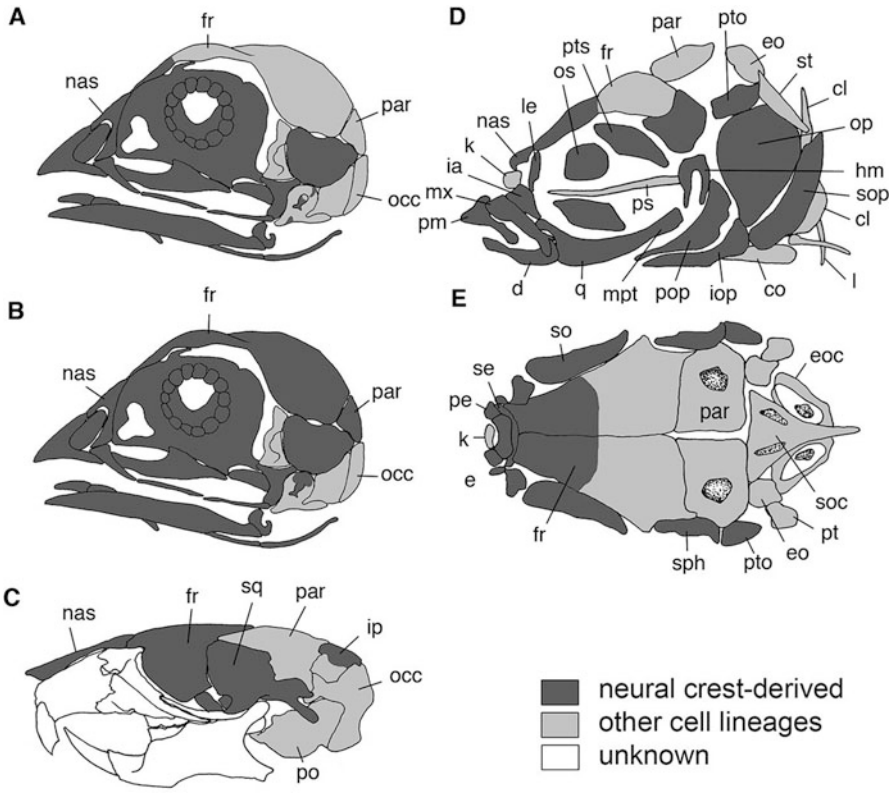


Fig. 4 CNC-derivatives in the dermatocranium. Bony elements colored light grey are of mesodermal origin. (**a** and **b**) Chicken. According to Noden (1984), the mesoderm/CNC cells boundary is present in the rostral part of the avian frontal (**a**), whereas according to Couly et al. (1993) it is located between the parietal and occipital bones. The occipital represents an endoskeletal element. (**c**) Mouse, based on transgenic approaches by several different research groups. The boundary is located between the frontal and parietal bones. (**d** and **e**) Mapping in zebrafish based on transgenic experiments. Left lateral (**d**) and dorsal (**e**) views of an adult zebrafish. The boundary is located in the frontal bone. Abbreviations: *boc* basioccipital, *bp* basal plate, *cl* cleithrum, *co* coracoid, *d* dentary, *e* ethmoid, *eoc* exoccipital, *fr* frontal, *hm* hyomandibula, *ia* intercalar, *iop* interopercle, *ip* interparietal, *k* kinethomoid, *le* lateral ethmoid, *mpt* metapterygoid, *mx* maxilla, *nas nasal, nc* notochord, *oc* otic capsule, *occ* occipital, *op* opercle, *os* orbitosphenoid, *par* parietal, *pe* preethmoid, *pm* premaxilla, *po* periotic, *pop* preopercle, *pp* postparietal, *pro* prootic, *ps* parasphenoid, *pto* pterotic, *pts* pterosphenoid, *q* quadrate, *se* supraethmoid, *soc* supraoccipital, *so* supraorbital, *soc* supraoccipital, *sop* subopercle, *sph* sphenotic, *sq* squamosal, *st* supratemporal, *tc* trabecula, *tma* taenia marginalis anterior, *tmp* taenia marginalis posterior. (Modified from Hirasawa and Kuratani (2015) based on Gross and Hanken (2008) and Kague et al. (2012))

The essence of middle ear evolution is the acquisition of a new function through modification of skeletal elements while maintaining homologous relationships, through transformation of the arches in a position-dependent manner. The identity of the hyoid arch is provided by *Hoxa-2*; in *Hoxa-2*-deficient mice, the hyoid arch is transformed

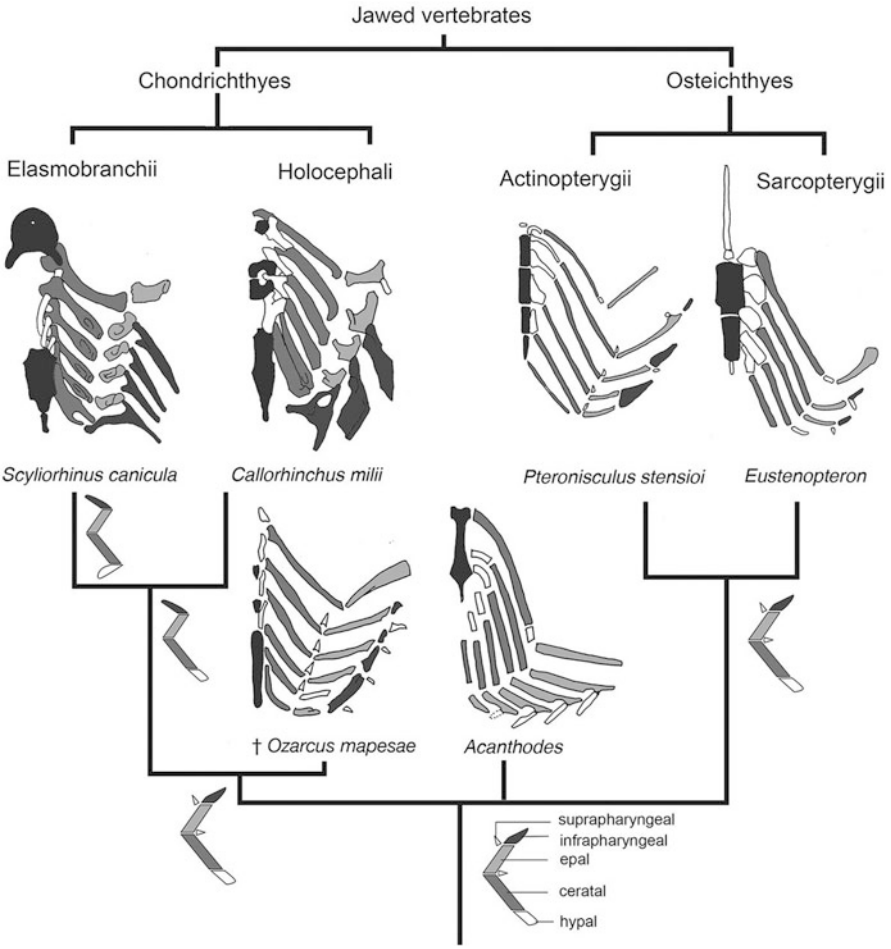


Fig. 5 Evolution of visceral arch skeleton in gnathostomes. Modern cartilaginous fishes lack suprapharyngeal elements, but basal lineages possess a pattern identical to that in bony fishes. (Redrawn from Pradel et al. (2014))

into the mandibular arch (Rijli et al. 1993), showing that Reichert's hypothesis was mostly correct. Recently, the novelty of the mammalian middle ear was redefined as a shift of the position of the tympanic membrane; in mammals, the tympanic membrane is located in the lower jaw domain, whereas in amphibians and sauropsids, the tympanic membrane arises in association with the upper jaw (Kitazawa et al. 2015).

Hox Code and Dlx Code

The morphological identities of the various elements of the visceral arch skeleton depend on developmental specification of the CNC-derived ectomesenchyme in each of the arches, the source of the visceral arch skeleton (reviewed by Noden

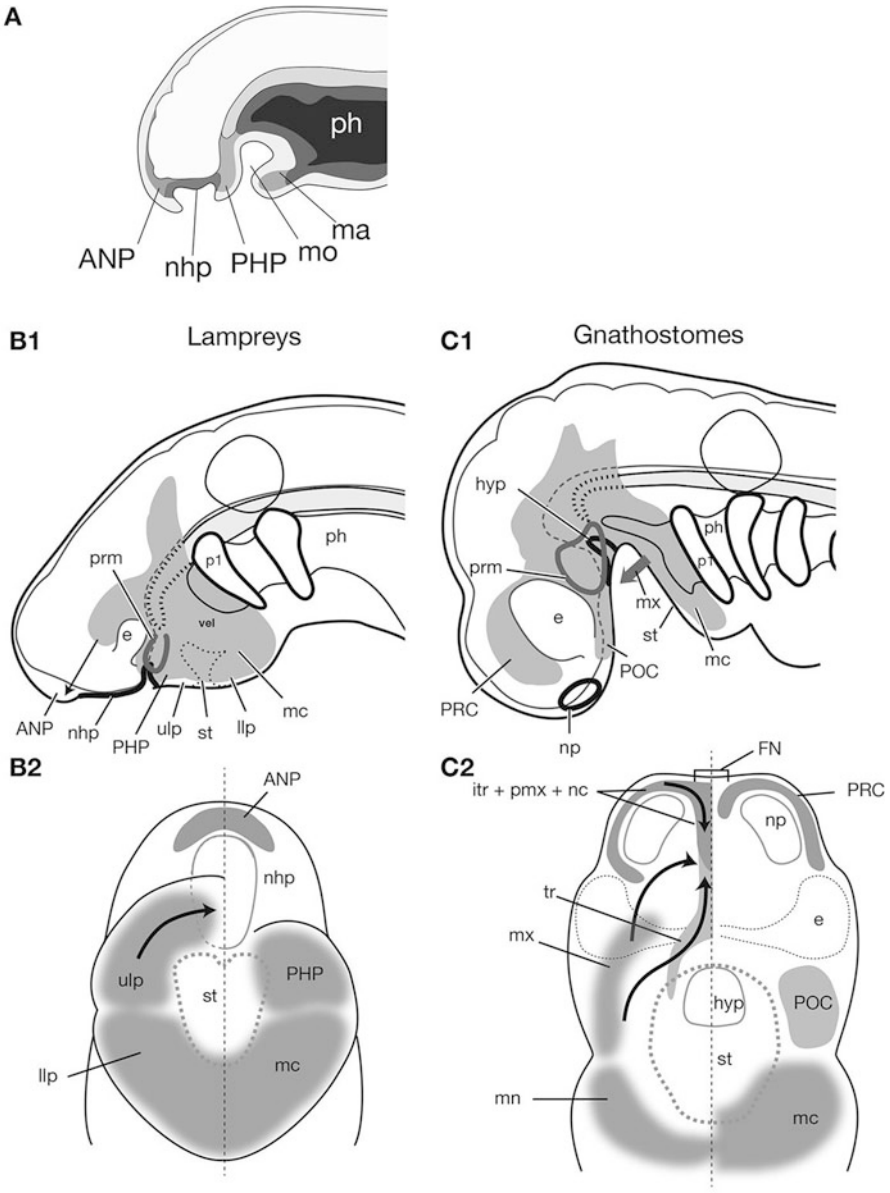


Fig. 6 Cyclostome developmental pattern compared with that of jawed vertebrates. (**a**) Embryonic pattern common to lamprey and hagfish embryo. (**b**) Lateral (B1) and ventral (B2) views of a lamprey embryo, showing the distribution of CNC-derived ectomesenchyme (grey). (**c**) Lateral (C1) and ventral (C2) views of a shark-like gnathostome embryo showing the ectomesenchymal distribution. Note that in lampreys, the oral apparatus (upper and lower lips) is formed by the mandibular arch and the PHP ectomesenchyme, whereas in gnathostomes, the jaw is mainly derived from the mandibular arch. The so-called trabecula in gnathostomes is derived from POC, which is near the PHP ectomesenchyme in lampreys. Abbreviations: *ANP* anterior nasal process, *e* eye, *hyp* adenohypophysis, *ma* mandibular arch, *llp* lower lip, *mc* mandibular arch CNC cells,

1984; also see Schneider and Helms 2003). The latter specification is mediated by cell-autonomous functions of *Hox* genes in the ectomesenchyme: *Hox* genes are expressed in a nested pattern, with 5′ genes expressed in more posterior arches (Hunt and Krumlauf 1991). In this scheme of Hox expression, also known as the Hox code, no *Hox* genes are expressed in the mandibular arch, which is thus specified as the Hox code-default state (see Rijli et al. 1993 and Couly et al. 1998). The function of *Hox* genes as homeotic selector genes for visceral arch specification has been demonstrated most clearly in *Hoxa-2* expression in the hyoid arch, as noted above (Rijli et al. 1993).

Dorsoventral specification of the visceral arch skeleton is mediated by the nested expression pattern of *Dlx* genes (the Dlx code): *Dlx1* and *−2* are expressed across the entire arch ectomesenchyme, *Dlx5* and *−6* in the ventral halves, and *Dlx3* and *−7* in the distal tips of the arches. Double knock-out of *Dlx5* and *−6* leads to loss of ventral identity, and the upper morphology is seen in the lower jaw domain in the mouse model (Depew et al. 2002).

The Hox and Dlx codes together form a Cartesian grid of coordinates on the ectomesenchyme of jawed vertebrate embryos. This homeobox gene-dependent scheme of morphological specification, however, is not very clearly established in cyclostomes: the Hox code is very similar in lampreys, but the dorsoventrally nested expression pattern of *Dlx* genes is lacking, although many *Dlx* genes are expressed in the cyclostome ectomesenchyme. Therefore, the ectomesenchymal Hox code is a vertebrate-defining synapomorphy, and the dorsoventrally nested Dlx code defines jawed vertebrates.

Upstream of the homeobox gene-dependent positional values are local tissue interactions between the ectomesenchyme and the embryonic environment, and the resultant gene regulatory networks. One candidate interaction is the endoderm-derived factors that specify the polarity and morphological identities of skeletal elements (Couly et al. 2002). In this context, endothelin signaling has been shown to upregulate *Dlx* genes (reviewed by Takechi et al. 2013). Another type of specification is species-specific craniofacial morphology, which is also specified in CNC-derived ectomesenchyme by semi-cell-autonomous patterns (Schneider and Helms 2003). Species-specific craniofacial traits reside in CNC cells, and interspecific grafting of NC gives rise to chimeras with the morphology of the species of NC origin (donor species). The developmental polarities and morphological homologies of skeletal elements are thus decoupled from the animal-specific "shape" of the elements, through both development and evolution.

Fig. 6 (continued) mn mandibular process, *mo* mouth, *nhp* nasohypophyseal plate, *np* nostril, *p1* first pharyngeal pouch, *ph* pharynx, *POC* postoptic CNC cells, *PRC* preoptic CNC cells, *prm* premandibular mesoderm, *st* stomodaeum, *ulp* upper lip, *vel* velum. (Redrawn from Kuratani (2012))

Cyclostome Cranium and the Evolution of the Skull

Being a sister group of gnathostomes, cyclostomes (modern lampreys and hagfish) potentially exhibit the ancestral condition of vertebrate craniogenesis. Their cranium arises from embryonic primordia that are distinct from those in jawed vertebrates. Due to the development of nasohypophyseal plate in the midline, they possess both anterior nasal (ANP) and posterior hypophyseal processes (PHP), as well as a mandibular arch, the dorsoventral division of which is unclear (Fig. 6a). The premandibular ectomesenchyme resides in the ANP and PHP, and the so-called trabecula of cyclostomes seems likely to correspond to the parachordals in jawed vertebrates, as shown by vital dye labeling experiments (Fig. 6b, c). The PHP differentiates into the upper lip and associated cartilage in lampreys, and into tentacle-supporting cartilage and cartilage in the oro-nasal septum in hagfish (Oisi et al. 2013). Therefore, the cyclostome neurocranium is mostly derived from mesoderm, whereas the ectomesenchymal part is seen in the nasal capsule, oral apparatus, and visceral arches (Fig. 2). Lampreys and hagfish are highly diversified, and their ancestral cranial morphology is difficult to define.

Curiously, the stem lineages of gnathostomes, especially jawless fossil animals called ostracoderms, possessed a single nostril close to the eye, which also leads to the dorsally located hypophysis, reminiscent of lampreys, and hagfish, showing that these traits are plesiomorphic for all vertebrates. Thus, the majority of cyclostome-specific traits can be regarded as ancestral features, and research on cyclostomes is expected to reveal the primitive developmental program of the vertebrate cranium.

Cross-References

- ► History and Current Theories of the Vertebrate Head Segmentation
- ► Shifting the Black Box: Approaches to the Development and Evolution of the Vertebrate Mesoderm
- ► The Evolution and Development of Segmented Body Plans

References

Couly GF, Coltey PM, Le Douarin NM (1993) The triple origin of the skull in higher vertebrates: a study in quail-chick chimeras. Development 117:409–429

Couly G, Grapin-Botton A, Coltey P, Ruhin B, Le Douarin NM (1998) Determination of the identity of the derivatives of the cephalic neural crest: incompatibility between *Hox* gene expression and lower jaw development. Development 125:3445–3459

Couly G, Creuzet S, Bennaceur S, Vincent C, Le Douarin NM (2002) Interactions between *Hox*-negative cephalic neural crest cells and the foregut endoderm in patterning facial skeleton in the vertebrate head. Development 129:1061–1073

Davidian A, Malashichev Y (2013) Dual embryonic origin of the hyobranchial apparatus in the Mexican axolotl (*Ambystoma mexicanum*). Int J Dev Biol 57:821–828

De Beer GR (1937) The development of the vertebrate skull. Oxford University Press, Oxford

Depew MJ, Lufkin T, Rubenstein JL (2002) Specification of jaw subdivisions by *Dlx* genes. Science 298:371–373

Dupret V, Sanchez S, Goujet D, Tafforeau P, Ahlberg PE (2014) A primitive placoderm sheds light on the origin of the jawed vertebrate face. Nature 507:500–503

Gaupp E (1902) Über die Ala temporalis des Säugerschädels und die Regio orbitalis einiger anderer Wirbeltierschädels. Anat Heft 15:433–595

Gregory WK (1935) 'Williston's law' relating to the evolution of skull bones in the vertebrates. Am J Phys Anthropol 20:123–152

Gross JB, Hanken J (2008) Review of fate-mapping studies of osteogenic cranial neural crest in vertebrates. Dev Biol 317:389–400

Hall BK (1999) The neural crest in development and evolution. Springer, New York

Hanken J, Hall BK (eds) (1993) The skull, vol 1–3. University of Chicago Press, Chicago

Hirasawa T, Kuratani S (2015) Evolution of the vertebrate skeleton – morphology, embryology and development. Zool Lett 1:2

Hunt P, Krumlauf R (1991) Deciphering the *Hox* code: clues to patterning branchial regions of the head. Cell 66:1075–1078

Jarvik E (1980) Basic structure and evolution of vertebrates, vol 2. Academic, New York

Jollie MT (1977) Segmentation of the vertebrate head. Am Zool 17:323–333

Kague E, Gallagher M, Burke S, Parsons M, Franz-Odendaal T, Fisher S (2012) Skeletogenic fate of zebrafish cranial and trunk neural crest. PLoS One 7:e47394

Kitazawa T, Takechi M, Hirasawa T, Hirai T, Narboux-Nême N, Kume H, Oikawa S, Maeda K, Miyagawa-Tomita S, Kurihara Y, Hitomi J, Levi G, Kuratani S, Kurihara H (2015) Developmental genetic bases behind the independent origin of the tympanic membrane in mammals and diapsids. Nat Commun 6:6853

Koyabu D, Maier W, Sánchez-Villagra MR (2012) Paleontological and developmental evidence resolve the homology and dual embryonic origin of a mammalian skull bone, the interparietal. Proc Natl Acad Sci 109:14075–14080

Kuratani S (2012) Evolution of the vertebrate jaw from developmental perspectives. Evol Dev 14:76–92

Kuratani S (2013) Evolution. A muscular perspective on vertebrate evolution. Science 341:139–140

Kuratani S, Ahlberg PE (2018) Evolution of the vertebrate neurocranium: problems of the pre-mandibular domain and trabecula. Zool Lett 4:1

Le Douarin NM (1982) The neural crest. Cambridge University Press, Cambridge

McBratney-Owen B, Iseki S, Bamforth SD, Olsen BR, Morriss-Kay GM (2008) Development and tissue origins of the mammalian cranial base. Dev Biol 322:121–132

McCauley DW, Bronner-Fraser M (2003) Neural crest contributions to the lamprey head. Development 130:2317–2327

Noden DM (1984) The use of chimeras in analyses of craniofacial development. In: Chimeras in developmental biology. Academic, London, pp 241–280

Oisi Y, Ota KG, Fujimoto S, Kuratani S (2013) Development of the chondrocranium in hagfishes, with special reference to the early evolution of vertebrates. Zool Sci 30:944–961

Ota GK, Kuratani S (2009) Evolutionary origin of bone and cartilage in vertebrates. In: Pourquié O (ed) The skeletal system Cold Spring Harbor monograph series, vol 53. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 1–18

Portmann A (1976) Einführung in die vergleichende Morphologie der Wirbeltiere, 5th edn. Schwabe & Co, Basel

Pradel A, Maisey JG, Tafforeau P, Mapes RH, Mallatt J (2014) A Palaeozoic shark with osteichthyan-like branchial arches. Nature 509:608–611

Reichert KB (1837) Über die Visceralbogen der Wirbelthiere im Allgemeinen und deren Metamorphosen bei den Vögeln und Säugethieren. Arch Anat Physiol Wiss Med 1837:120–220

Rijli FM, Mark M, Lakkaraju S, Dierich A, Dollé P, Chambon P (1993) Homeotic transformation is generated in the rostral branchial region of the head by disruption of *Hoxa-2*, which acts as a selector gene. Cell 75:1333–1349

Schneider RA, Helms JA (2003) The cellular and molecular origins of beak morphology. Science 299:55–58

Takechi M, Adachi N, Hirai T, Kuratani S, Kuraku K (2013) The *Dlx* genes as clues for vertebrate genomics and craniofacial evolution. Semin Cell Dev Biol 24:110–118

Zhu M, Yu XB, Ahlberg PE, Choo B, Lu J, Qiao T, Qu QM, Zhao WJ, Jia LT, Blom H, Zhu YA (2013) A Silurian placoderm with osteichthyan-like marginal jaw bones. Nature 502:188–193

Evo-Devo of the Fin-to-Limb Transition

Yoshitaka Tanaka, Hidehiro Kudoh, Gembu Abe,
Sayuri Yonei-Tamura, and Koji Tamura

Contents

Abstract

Tetrapod limbs evolved from paired fins. Although the limbs and fins share similar sets of tissue components, structure, and developmental processes, some characteristics of the skeletal morphology of fins are distinct from those of limbs. Fin rays, the distal-most components of the fin, consist of several types of tissues not seen in tetrapods. The fin ray skeletons of teleosts have the same developmental origin (lateral plate mesoderm, LPM) as that of the basal endoskeleton that also develops in the fin bud. The ectodermal jacket of the fin bud has a ridge along the dorsoventral border called the apical ectodermal ridge (AER), which elongates into the apical fold (AF) during development. Tetrapod limb buds never undergo this transformation, suggesting that epithelial changes might be the key to understanding the evolutionary developmental mechanism behind the fin-to-

Yoshitaka Tanaka, Hidehiro Kudoh and Gembu Abe contributed equally with all other contributors.

Y. Tanaka · H. Kudoh · G. Abe · S. Yonei-Tamura · K. Tamura (✉)
Laboratory of Organ Morphogenesis, Department of Ecological Developmental Adaptability Life Sciences, Graduate School of Life Sciences, Tohoku University, Sendai, Japan
e-mail: yoshitaka.tanaka.q1@dc.tohoku.ac.jp; hidehiro.kudoh.q3@dc.tohoku.ac.jp; gembu.abe.b5@tohoku.ac.jp; yone@tohoku.ac.jp; tam@tohoku.ac.jp

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_193

limb transition. The epithelial transformation involves a change in cell shape, which may have played a role in the fin-to-limb evolution.

Keywords

Fin-to-limb transition · Tetrapod · ZPA · AER · Cell shape

Introduction

Paired appendages in vertebrates, the limbs and fins, are locomotory organs that exhibit species-specific and highly specialized morphology. From a morphological point of view, the limb skeleton can be divided into three domains: stylopod (humerus/femur), zeugopod (radius/tibia and ulna/fibula), and autopod (carpals/tarsals, metacarpals/metatarsals, and phalanges). The tetrapod limbs evolutionarily originated from paired fins. Comparative anatomy, embryology, and molecular evidence have long suggested that the paired fins and limbs are homologous to each other. Fossils of sarcopterygians (e.g., *Eusthenopteron* and *Tiktaalik*) and stem tetrapods (e.g., *Ichthyostega* and *Acanthostega*) have provided clues for the developmental background of the fin-to-limb transition, which has offered an interesting model of evolutionary developmental biology. In this chapter, we review the differences in morphogenetic processes between fin and limb with a focus on alterations in morphogenesis at the cellular level that must drive the morphological changes involved in the fin-to-limb transition.

Fin-to-Limb Transition from the Viewpoint of Morphology

Paired fins are composed of two types of bones formed through different developmental pathways (Hirasawa and Kuratani 2015; Nakamura et al. 2016): endochondral bones and dermal bones. Endochondral bones develop through endochondral ossification, in which cartilage is formed first from the condensed mesenchyme and then replaced by mineralized bone. Dermal bones develop in the dermis and directly mineralized without any cartilaginous primordial stage. The paired fin has both types of bones, the proximal endochondral bones (radials), and distal dermal bones (fin rays).

Based on skeletal patterns and components, endochondral skeletons in the pectoral fins can be classified into three categories: type I, fins retaining a primitive pattern in the endochondral bone region; type II, fins with a small endochondral region and relatively large dermal bone region; and type III, fins with a long endochondral region that expands along the proximal-distal axis. Basal actinopterygians such as *Acipenser* and *Polyodon* represent type I (Fig. 1b) (Davis et al. 2004). Three endochondral bones forming joints of the pectoral girdle are called the tribasal form (propterygium, mesopterygium, and metapterygium: Fig. 1a, b), and the joint between the appendage and the girdle shows ancestral features, as has been supported by paleontological evidence and extant chondrichthyans

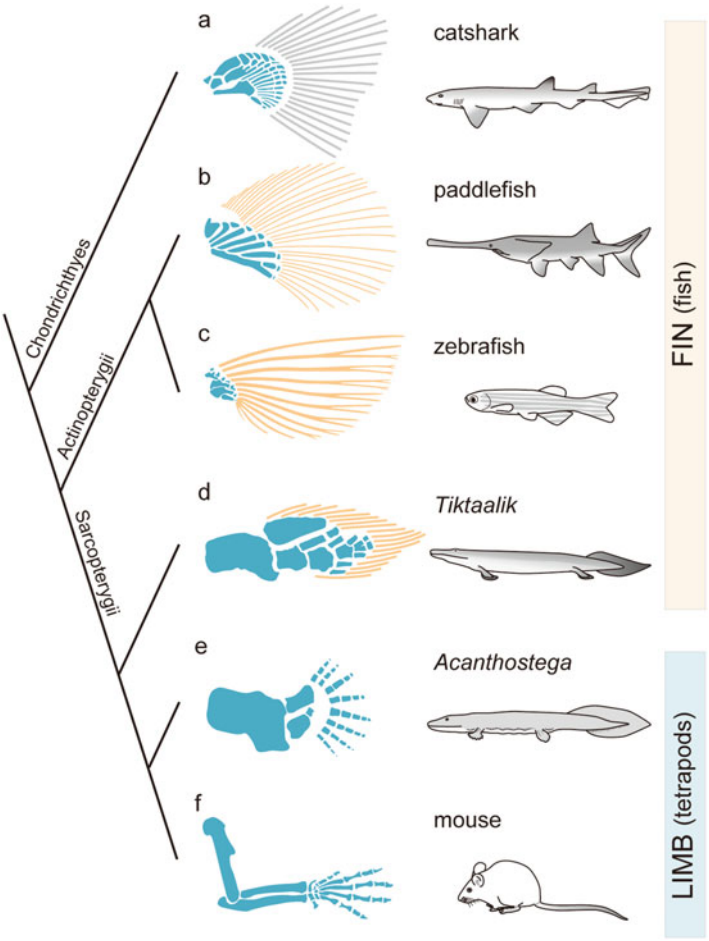


Fig. 1 Comparative anatomy of pectoral fins and forelimbs. Skeleton of pectoral fins (**a**-**d**) and forelimbs (**e**, **f**). The blue region shows endochondral bones, and the red region shows dermal bones (fin ray). (**a**) Chondrichthyan, catshark (*Scyliorhinus canicula*). The gray region shows ceratotrichia. (**b**) Basal actinopterygian, paddlefish (*Polyodon spathula*), (**c**) teleost, zebrafish (*Danio rerio*). (**d**) Sarcopterygian, *Tiktaalik*. (**e**) Stem tetrapod, *Acanthostega*. (**f**) tetrapod, mouse (*Mus musculus*). (Figures were adapted from Davis et al. (2007) and Shubin et al. (2006))

(Fig. 1a) (Compagno 1973; Coates 2003). This finding suggests that basal actinopterygians have retained ancestral patterns in the pectoral fin. Teleosts such as zebrafish constitute type II (Fig. 1c) (Grandel and Schulte-Merker 1998); their pectoral fins have lost metapterygium together with the ancestral tribasal joint. Instead, their pectoral girdle-proximal radial joints are generated from a large endochondral disk (a presumptive mesopterygial primordium) during development. Sarcopterygians such as lungfish and coelacanth are type III (Fig. 1d) (Shubin et al. 2006; Johanson et al. 2007). Here, only one large endochondral bone articulates with

the pectoral girdle, and many endochondral bones are lined up distally on the basal bone. Together with expansion of the endochondral bone, the architecture of muscles and nerves also changes in the type III pectoral fin. The neural and muscular pattern of type III resembles that of limbs (Hirasawa and Kuratani 2018), such that the sarcopterygian fin resembles the hypothetical precursor for the tetrapod limb.

Fin rays consist of several types of tissues, including blood vessels, connective tissues, and dermal bones (lepidotrichia). In the fin ray development, the supporting structures of the expanding fin fold, or the actinotrichia, are formed before the lepidotrichia (Dane and Tucker 1985). The former are composed of non-mineralized tissue with extracellular matrix proteins like collagen and actinodin (Zhang et al. 2010). Chondrichthyans, including sharks and skates, do not form mineralized bones (Fig. 1a), and instead of lepidotrichia, they form ceratotrichia, collagenous fibers similar to actinotrichia (Francillon-Vieillot et al. 1990). Lepidotrichia in osteichthyans are cylindrical dermal bones that are formed in the region supported by actinotrichia. The actinotrichia are thin, soft, and stuffed, whereas the lepidotrichia are thick and hard with a space between two semicircular tiles of dermal bones (similar to bamboo). After the formation of lepidotrichia, actinotrichia are replaced by lepidotrichia, except at the distal tip. The fin ray is a characteristic structure specific to non-tetrapod osteichthyans, and tetrapods never develop these independent dermal elements in a limb.

During the fin-to-limb transition, tetrapod ancestors seem to have experienced two major changes: expansion of the endochondral region and loss of fin rays. The idea of endochondral expansion is implied by much paleontological evidence such as *Eusthenopteron* and *Tiktaalik* (Fig. 1d) (Shubin et al. 2006) as well as the Australian lungfish (Johanson et al. 2007). In *Eusthenopteron* and *Tiktaalik*, one large endochondral bone articulates the girdle, as mentioned above. Distally, two endochondral bones are jointed. The latter two bones are thought to be homologous to the zeugopod elements in tetrapods, and the large proximal bones are homologous to the stylopod (Johanson et al. 2007). Therefore, enlargement of the endochondral bones appears to have already been achieved in sarcopterygians. Alternatively, the loss of fin rays would have occurred somewhere between sarcopterygians (*Tiktaalik*) and stem tetrapods such as *Acanthostega* and *Ichthyostega* (Fig. 1e) (Coates 1996; Shubin et al. 2006). Instead of losing fin rays, the stem tetrapods obtained digits, thus acquiring terrestrial mobility at the expense of aquatic mobility. However, *Acanthostega* and *Ichthyostega* retained fin rays in their caudal appendage, the tail (Zhang et al. 2010). These gradual morphological changes might have enabled graded terrestrialization.

The tetrapod limb skeleton is exclusively composed of endochondral bones, with no independent dermal element. The fin skeleton in sarcopterygians contains bones comparable to stylopod and zeugopod, but an autopod is not clearly recognizable. Therefore, digits are tetrapod-specific morphological features, whereas the fin rays are distinctive of non-tetrapod osteichthyans. Morphology of the fin and that of the limb are very different from each other, making it difficult to nail down what happened in the process of fin-to-limb transition. Other approaches for elucidating

the evolutionary processes of these complicated structures, including developmental biology, molecular genetics, and cell biology, are needed.

Similarities and Differences Between Developmental Mechanisms of Fins and Limbs

Long cell-lineage tracing experiments showed that not only endochondral bones but also dermal bones (fin rays) in paired fins are derived from the lateral plate mesoderm (LPM) (Shimada et al. 2013). Potentially, in the process of losing fin rays during the fin-to-limb transition, a certain population of cell types neither disappeared nor lost the ability of osteogenesis. Rather, developmental changes in skeletal morphogenesis might have played important roles in the loss of fin rays for the fin-to-limb transition.

During embryonic development, the primordium of paired appendages, both the fin bud and limb bud, appears bilaterally on the trunk as a simple protrusion containing mesenchymal cells derived from the LPM and ectodermal epithelial cells surrounding the mesenchyme (Fig. 2a, b) (Gilbert and Barresi 2016; see also references therein). The fin and limb buds have an ectodermal ridge structure running along the entire dorsoventral border on the bud ectoderm called the apical ectodermal ridge (AER) (Fig. 2a, b). In tetrapods, the AER is maintained throughout limb bud outgrowth, whereas in teleosts, the AER is retained for a relatively short period after the fin bud appears; the AER then changes its shape and elongates into a fold-like structure, the apical fold (AF) (Fig. 2b, c) (Grandel and Schulte-Merker 1998; Yano et al. 2012; Yano and Tamura 2013). After AF formation, LPM-derived mesenchymal cells migrate into the space of the fold and give rise to the fin rays (Fig. 2c) (Yano and Tamura 2013; Shimada et al. 2013).

The formation of a three-dimensional pattern from a simple protrusion depends on the signaling centers that direct morphogenesis along the axes. There are two well-known signaling centers in limb/fin bud development: the AER and the zone of polarizing activity (ZPA), a population of mesenchymal cells located in the posterior region of the bud (Fig. 2a). The AER plays an important role in pattern formation along the proximo-distal (PD) axis in the limb/fin bud (Fig. 2a). During limb/fin development, the AER maintains the underlying mesenchymal cells in a premature and proliferative state, resulting in the elongation of the bud along the PD axis. A zebrafish mutant lacking the AER shows a swelling of the pectoral fin bud but forms only the basal-most pectoral girdle structure without radials and fin rays, suggesting that the AER is essential for distal elongation as well as PD morphogenesis in the fin (Grandel et al. 2000). Fgf family genes are expressed throughout or in a portion of the AER, and FGFs are well-known molecular effectors of AER function (Fig. 2a).

The other signaling center, the ZPA, is an area of the posterior limb mesenchyme and has an organizer (inductive) activity of the anteroposterior (AP) appendage skeleton (Fig. 2a). Shh is the protein responsible for the ZPA activity (Fig. 2a). *Shh* expression in the ZPA is induced by retinoic acid (RA) signaling from the trunk region (Helms et al. 1994). ZPA and Shh activities are also found in fin bud

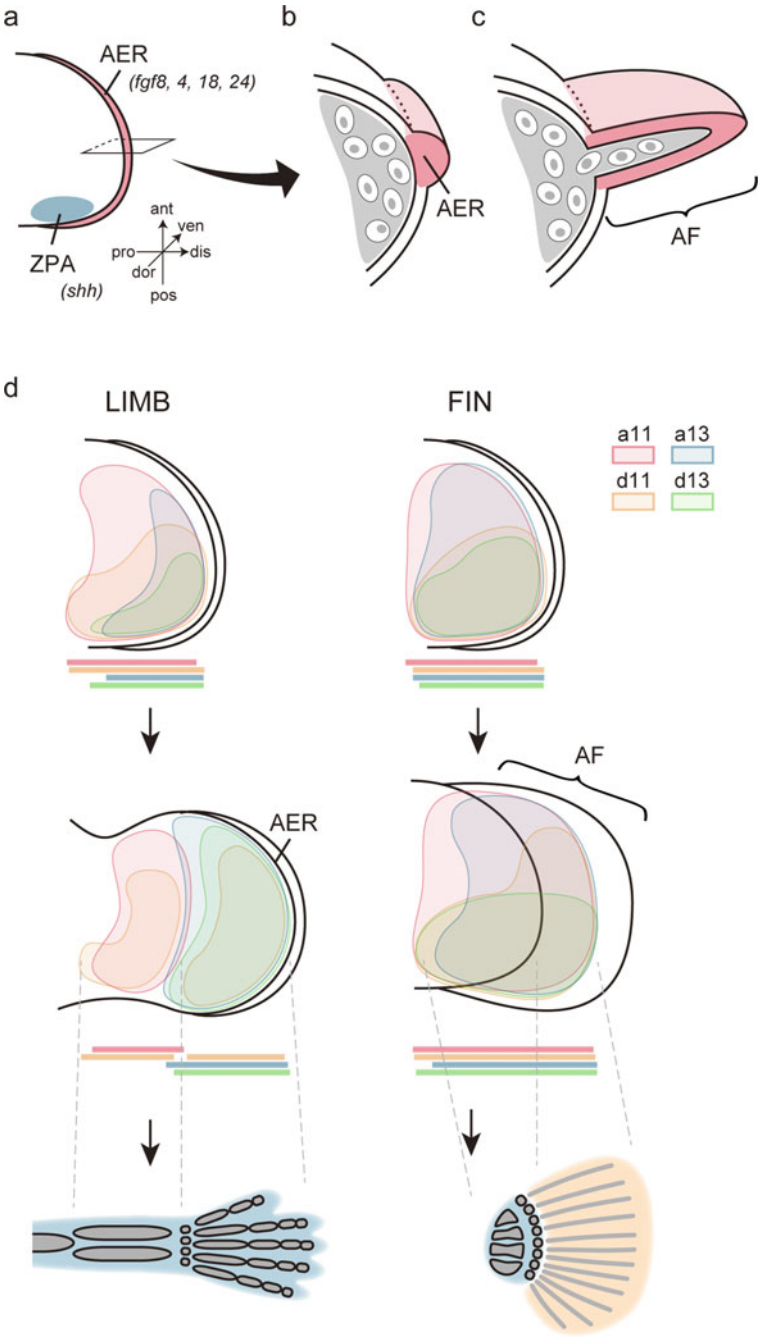


Fig. 2 Schematic representation of the development of the limb/fin bud. (**a**) Schematic drawing of the limb/fin bud at an early time point. Red and blue colors indicate the AER and ZPA,

development (Krauss et al. 1993). The expression of *shh* is localized to the posterior fin bud, and a zebrafish *shh* mutant (*syu*) has fin bud defects such that the fin bud can be formed but stops elongating soon after budding (Neumann et al. 1999). Moreover, the application of RA can induce ectopic *shh* expression at the anterior margin of the fin bud, resulting in abnormal thickening of the fin bud at the base (Akimenko and Ekker 1995).

As mentioned above, the two signaling centers have basically the same functions in both fin and limb buds, and the difference in the skeletal structure between them appears to be mediated by changes in the downstream activity of the signaling centers. The signaling centers direct polarities along the AP and PD axes and accordingly regulate the expression of patterning genes in the fin/limb bud. Well-known downstream patterning genes are 5′ *HoxA* and *HoxD* cluster genes. During the endoskeletal patterning of the limb bud, the expression of *Hox* clusters involves two sequential phases corresponding to the skeletal pattern (Fig. 2d). At an early stage, *HoxA11* and *HoxA13* are expressed in the mesenchyme of the limb bud with overlap (phase I) (Fig. 2d, left upper panel). Moreover, *HoxD* cluster genes show a nested pattern of expression since the expression of more 5′ *Hox* genes is restricted to the narrow posterior region (Fig. 2d, left upper panel). At later stages of limb bud development, the expression domains of *HoxA11* and *HoxA13* separate into the zeugopod and autopod regions, forming a clear border between them (phase II) (Fig. 2d, left middle and lower panels). In phase II, *HoxD* cluster genes show another nested pattern in the autopod region; *HoxD13* is expressed in a broader domain than *HoxD11*, which contrasts to its expression in phase I (Fig. 2d, left middle and lower panels). Functional assays of these genes revealed that their depletion results in a defect in the skeletal pattern corresponding to the region of gene expression. Considering the correspondence between the expression pattern and skeletal pattern, 5′ *Hox* genes are thought to be a series of patterning genes for limb morphogenesis.

The expression patterns and functions of 5′ *hox* genes in the developing fins were also investigated. Sordino et al. (1995) found the expression of both 5′ *hox* cluster genes in phase I, but not in phase II, in the fin bud mesenchyme of zebrafish (Fig. 2d, right upper panels). Phase I expression of 5′ *hox* cluster genes was also found in the medaka fin bud (Takamatsu et al. 2007). From these results, the authors suggested that the phase I expression of 5′ *hox* genes in the fin bud results in a relatively shorter

Fig. 2 (continued) respectively. Arrows at the right bottom indicate the orientation of the bud; ant, anterior; pos, posterior; ven, ventral; dor, dorsal; pro, proximal; dis, distal. (**b**) Sectional drawing of the early limb/fin bud at the site indicated by a parallelogram in A. (**c**) Sectional drawing of the late fin bud. AER changes to the AF between B and C. White circles with a gray dot in the gray area indicate mesenchymal cells. (**d**) Expression pattern of 5′ *hox-a* and *d* cluster genes in limb bud (left) and fin bud (right) development. Red, blue, orange, and green areas in the upper and middle drawings indicate the expression of *hoxa11*, *hoxa13*, *hoxd11*, and *hoxd13*, respectively. A combination of expression domains of 5′ *hox* genes determines a compartment in the limb/fin buds (middle), which gives rise to a region of endoskeleton and exoskeleton indicated by gray ovals in the blue area and gray bars in the orange area in the lower drawings, respectively

endochondral region in the fin skeleton and that the elaboration of phase II expression of 5′ *hoxA* and *D* genes facilitated the expansion of endochondral bones in fin-to-limb evolution.

Mesenchymal cells in the AF region also show the phase I expression pattern of *hoxa* and *hoxd* cluster genes in the zebrafish pectoral fin (Ahn and Ho 2008) (Fig. 2d, right middle and lower panels). Furthermore, depletion of both *hoxa13* and *hoxd13* genes leads to skeletal defects not only in the radials but also in the fin rays (Nakamura et al. 2016). Therefore, based on the expression patterns and functions of these genes, it was suggested that the radials and fin rays might share some developmental mechanisms involving these 5′ *hox* cluster genes. Interestingly, the pectoral fin bud in paddlefish, a basal actinopterygian, shows an anterior expansion of *hoxd13* expression domain after the phase I, producing a pattern that resembles that of phase II in the limb bud (Davis et al. 2007). Furthermore, analysis of the enhancer elements of 5′ *hoxd* clusters revealed that gar, another basal actinopterygian, has at least partially enhanced phase II expression of 5′ *hoxd* genes that have been found in tetrapods (Gehrke et al. 2015). These experimental findings suggest that the regulation of phase II expression pattern of *hoxd* cluster genes was already partially elaborated in the common ancestor of teleosts and tetrapods.

The distal skeletal components in fins and limbs show different morphologies that are produced by different osteogenic pathways, but their developmental mechanisms do not appear to differ greatly. Interestingly, the *HoxA11* enhancer that directs the segregation of *HoxA11* and *HoxA13* in the phase II expression pattern is regulated by *HoxA13* and *HoxD13* (Kherdjemil et al. 2016). The enhancer was not found in the zebrafish genome; however, the ectopic introduction of the enhancer into the zebrafish genome could lead to reporter EGFP expression in mesenchymal cells in the AF. In addition, overexpression of *hoxd13* in mesenchymal cells in the AF resulted in excessive cell proliferation and ectopic chondrogenesis in the AF region (Freitas et al. 2012). Thus, the evolution of the regulatory mechanisms of 5′ *hox* genes may be involved in the acquisition of the autopod region and loss of fin rays (Lalonde and Akimenko 2018).

Developmental Mechanisms of the Loss of Fin Rays

Although the loss of fin rays and acquisition of the autopod are drastic morphological changes in the fin-to-limb transition, changes in developmental mechanisms that drove these changes might not have been huge but rather relatively minor modifications in the developmental mechanisms for the distal part of fins into those for the autopod. As has been described previously, all osteichthyans before *Acanthostega* possessed fin rays, and all tetrapods after *Tiktaalik* did not have them; the loss of fin rays must thus be crucial for the fin-to-limb transition. This section discusses the developmental changes that might have been involved in the evolutionary loss of fin rays.

There is an interesting developmental model, the clock model, which describes the loss of fin rays (Fig. 3). According to this model, proposed by Thorogood (1991), the timing of the transition from AER to AF determines the ratio of endochondral bones and fin rays in paired appendages. As described in the previous section, a thickened epidermal layer (the AER) deforms into an elongated epithelium similar to a fold (the AF) in fin development, and mesenchymal cells derived from the LPM move into a space of two layers of epithelium (Fig. 2c) subsequently forming fin rays. The AER-to-AF transition never occurs in the developing tetrapod limb bud. Instead, the AER structure is retained throughout morphogenesis of the limb skeleton and then disappears. Thorogood (1991) assumed that the timing of the AER-to-AF transition differs among species. According to this hypothesis, the transition occurs relatively early in the paired fins of teleosts, which have a small region for endochondral bones and a large region for fin rays. The transition occurs later in sarcopterygians with a relatively larger endoskeletal region. Tetrapod limb buds never undergo transition and do not form dermal skeletons.

AF has been observed in developing pectoral fin buds of lungfish (reviewed by Yano and Tamura 2013), but whether the timing of the transition is shifted to late stages in sarcopterygians remains unresolved. In zebrafish, a heterochronic shift in the timing by artificial manipulation gives rise to elongation of the endochondral region and results in extra elements of endochondral bones (Yano et al. 2012; Yano and Tamura 2013). The authors suggested that the AER-AF transition represses the mechanisms for endochondral morphogenesis, such as phase I to phase II shift of

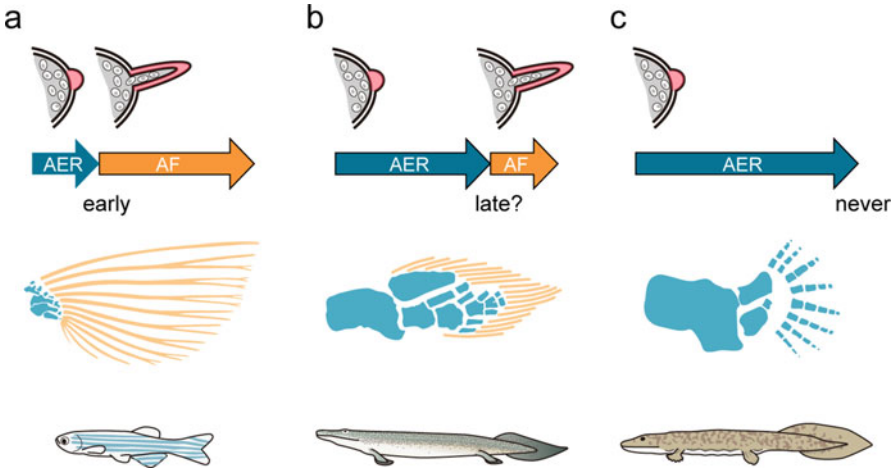


Fig. 3 Clock model for the fin-to-limb transition. Drawings schematically express the clock model by Thorogood (1991) (see text for details). (**a**) In the teleost (zebrafish) pectoral fin, the transition from the AER to the AF occurs at a relatively early stage of fin development, giving rise to a large fin ray region (orange) and a small endoskeletal region (blue). (**b**) In sarcopterygian, *Tiktaalik*, the AER-to-AF transition occurs at a later stage than that in teleosts, resulting in a large endoskeleton and small fin rays. (**c**) In stem tetrapod, *Acanthostega*, the AER-AF transition never occurs, and as a result, the limb does not have fin rays

Hox gene expression (repression mode). Since the transition to AF, elongation of the AF, and formation of actinotrichia within the AF are necessary steps for normal fin ray formation, it is reasonable that loss of the AER-to-AF transition played a critical role in the loss of fin rays during the fin-to-limb transition.

Cellular Mechanisms of the AER-to-AF Transition

This section discusses how the AER-to-AF transition occurs at the cellular level and how this transition failed to occur in stem tetrapods, resulting in the loss of fin rays.

Both the AER and AF are epidermal tissues arranged in a monolayer composed of epithelial cells. Thus, cellular mechanisms underlying the transition should involve alterations in morphogenesis of the epithelial sheet. Morphological changes in epithelial cells and molecular mechanisms in epithelial morphogenesis have been investigated in detail in many model systems. The neural tube and lens are formed by invagination of an epithelial sheet along the apical and basal axes, and cell-shape changes play important roles in this process. Before invagination, cells in the center of the invaginating layer have a columnar shape, which is altered to a wedge-like shape by constriction of the apical side during invagination (reviewed by Martin and Goldstein 2014). The force required for this cell-shape change (apical constriction) is generated by actomyosin filaments.

Similar to apical constriction, basal constriction contributes to epithelial morphogenesis, such as in the formation of the midbrain-hindbrain boundary and the optic cup. The epithelial sheet is deformed toward the apical side by basal constriction. Constrictive force by actomyosin filaments causes the basal side of cells to shrink in a manner similar to that in apical constriction (Gutzman et al. 2008; Bogdanović et al. 2012). Interestingly, during basal invagination in lens morphogenesis, cells at the boundary between the invaginating region and the non-invaginating region have a wedge-like shape with a smaller cell surface at the basal side and a larger cell surface at the apical side. Because of this cell shape, Breau and Schneider-Maunoury (2015) proposed that while cells at the center of the basal invagination undergo apical constriction, cells in the boundary region undergo basal constriction, driving closure of the lens placode.

The AER also seems to be formed by the deformation of cell shape (Fig. 4b). Cells forming the AER have a wedge-like shape at the constricted basal side (Ede et al. 1974), and actomyosin accumulates on the basal side (Lau et al. 2015). These findings suggest that basal constriction is involved in AER formation. In fin buds, cells forming the AER also have a wedge-like shape at the constricted basal side (Dane and Tucker 1985). In the AF, cells at the apex of the AF maintain a wedge-like shape similar to that of the AER cells, and cells at the sidewall of the AF have a tile-like flat shape (Fig. 4a). In the transition process from the AER to the AF, there should be epithelial morphogenesis with certain cellular mechanisms to restrict extending/raising cell layers of the epithelium toward the apical side.

Since the presence or absence of the AER-to-AF transition is crucial for fin ray formation, identifying alterations in the underlying cellular mechanisms must be

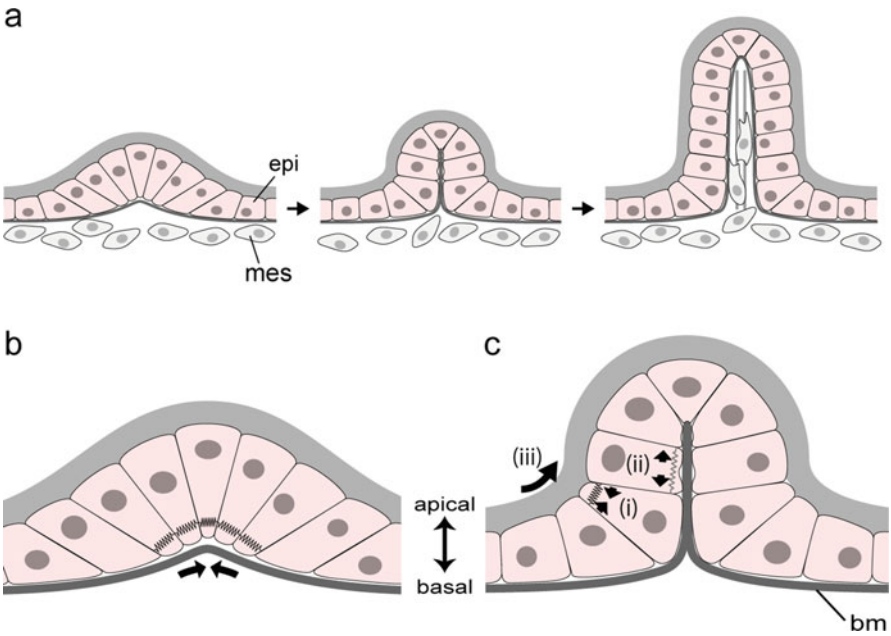


Fig. 4 Schematic representation of three possible cellular mechanisms in the AER-AF transition. (**a**) Cross-sections of the distal pectoral fin bud showing the transition from the AER (left) to the AF (right) (see also Fig. 2b, c). (**b**) AER cells with basal constriction due to the contraction force of localized actomyosin. Thin and wavy lines indicate contracting actomyosin filaments. (**c**) Three possible mechanisms for launching AF. (i) Apical constriction of cells at the boundary region between the AER and non-AER. (ii) Wall cells, which once had a wedge-like shape, return to a columnar shape. (iii) Addition of cells from the non-AER region to the AER region epi, epidermis; mes, mesenchyme; bm, basement membrane

vital for understanding the fin-to-limb transition. However, the mechanisms at the cellular level for the AER-to-AF transition remain largely unknown. Here, we propose three distinct mechanisms that might have been involved in the AER-to-AF transition. (i) Cells at the boundary between the AER and non-AER region undergo apical constriction (Fig. 4c-i). By taking the opposite shape to the AER cells, the epithelial sheet folds and protrudes to the apical side. (ii) Cells in the middle region (wall cells) between the apex AER and the boundary, which undergo basal constriction once before the transition, return to columnar cells by loosening the constriction and growing further to become flat (Fig. 4c-ii). In the caudal fin, wall cells in the AF have a cuboidal shape (Dane and Tucker 1985). (iii) Non-AER epidermal cells also join the AER region (Fig. 4c-iii). A comparison of cell numbers in the AF and the AER showed that cell number increases during/after AF formation (Dane and Tucker 1985). In addition, epidermal cells in the fin bud proliferate at this stage, but they do it in a random, not AER-specific manner (Yano et al. 2012).

The three mechanisms described here are not mutually exclusive (rather, it is likely that they combined) and do not exclude other mechanisms. Cell behaviors are

spatially and temporally dynamic in the epithelial field. For example, when non-AER cells participate in the AER, the cell shape changes continuously from a columnar to a wedge-like shape and then back to a columnar shape, and these cells are added to the folding AF along the AP axis. In any case, a three-dimensional approach for experiments on the epithelial sheet will be necessary. The process and mechanisms must be complicated but should be verifiable at the cellular and molecular levels because changes in cell behaviors are thought to be regulated in a manner similar to that in other organ model systems such as the neural tube and lens. If these possibilities are verified, evolution from the fin to limb could be understood at the cellular level of the AER-to-AF transition.

Conclusions

Morphological changes among animals can be described as differences in the process of morphogenesis (see chapter ▶ "Mechanisms of Pattern Formation, Morphogenesis, and Evolution"). The fin-to-limb transition can also be explained by developmental changes at the cellular level. Considering morphological evolution at the cellular level provides many new insights, since developmental changes during evolution must have been driven by modifications in cell behaviors.

Cross-References

▶ Developmental Homology
▶ Developmental Innovation and Phenotypic Novelty
▶ Devo-Evo of Cell Types
▶ Evo-Devo and Phylogenetics
▶ Evolution of Skeletal Tissues
▶ Mechanisms of Pattern Formation, Morphogenesis, and Evolution

Acknowledgments We thank all members of Tamura laboratory for helpful discussions on the manuscript. We would also like to thank Editage (www.editage.com) for English language editing. This work was supported by JSPS KAKENHI [grant numbers 18H04756, 18H04811, 18H02446, and 19H03289].

References

Ahn D, Ho RK (2008) Tri-phasic expression of posterior Hox genes during development of pectoral fins in zebrafish: implications for the evolution of vertebrate paired appendages. Dev Biol 322:220–233

Akimenko MA, Ekker M (1995) Anterior duplication of the sonic hedgehog expression pattern in the pectoral fin buds of zebrafish treated with retinoic acid. Dev Biol 170:243–247

Bogdanović O, Delfino-Machín M, Nicolás-Pérez M et al (2012) Numb/Numbl-Opo antagonism controls retinal epithelium morphogenesis by regulating integrin endocytosis. Dev Cell 23:782–795

Breau MA, Schneider-Maunoury S (2015) Cranial placodes: models for exploring the multi-facets of cell adhesion in epithelial rearrangement, collective migration and neuronal movements. Dev Biol 401:25–36

Coates MI (1996) The Devonian tetrapod Acanthostega gunnari Jarvik: postcranial anatomy, basal tetrapod interrelationships and patterns of skeletal evolution. Trans R Soc Edinb Earth Sci 87:363–421

Coates MI (2003) The evolution of paired fins. Theory Biosci 122:266–287

Compagno LJ (1973) Interrelationships of living elasmobranchs. Zool J Linnean Soc 53:15–61

Dane PJ, Tucker JB (1985) Modulation of epidermal cell shaping and extracellular matrix during caudal fin morphogenesis in the zebra fish Brachydanio rerio. J Embryol Exp Morphol 87:145–161

Davis MC, Shubin NH, Force A (2004) Pectoral fin and girdle development in the basal actinopterygians Polyodon spathula and Acipenser transmontanus. J Morphol 262:608–628

Davis MC, Dahn RD, Shubin NH (2007) An autopodial-like pattern of Hox expression in the fins of a basal actinopterygian fish. Nature 447:473–476

Ede BDA, Bellairs R, Bancroft M (1974) A scanning electron microscope study of the early limb-bud in normal and talpid 3 mutant chick embryos. J Embryol Exp Morphol 31:761–785

Francillon-Vieillot H, de Buffrénil V, Castanet J et al (1990) Microstructure and mineralization of vertebrate skeletal tissues. In: Skeletal biomineralization: patterns, processes and evolutionary trends. Van Nostrand Reinhold, New York, pp 103–124

Freitas R, Gomez-Marin C, Wilson JM et al (2012) Hoxd13 contribution to the evolution of vertebrate appendages. Dev Cell 23:1219–1229

Gehrke AR, Schneider I, de la Calle-Mustienes E et al (2015) Deep conservation of wrist and digit enhancers in fish. Proc Natl Acad Sci U S A 112:803–808

Gilbert S, Barresi M (2016) Developmental biology 11th. Sinauer Associates, Sunderland

Grandel H, Schulte-Merker S (1998) The development of the paired fins in the zebrafish (Danio rerio). Mech Dev 79:99–120

Grandel H, Draper BW, Schulte-Merker S (2000) Dackel acts in the ectoderm of the zebrafish pectoral fin bud to maintain AER signaling. Development 127:4169–4178

Gutzman JH, Graeden EG, Lowery LA et al (2008) Formation of the zebrafish midbrain-hindbrain boundary constriction requires laminin-dependent basal constriction. Mech Dev 125:974–983

Helms J, Thaller C, Eichele G (1994) Relationship between retinoic acid and sonic hedgehog, two polarizing signals in the chick wing bud. Development 120:3267–3274

Hirasawa T, Kuratani S (2015) Evolution of the vertebrate skeleton: morphology, embryology, and development. Zool Lett 1:2

Hirasawa T, Kuratani S (2018) Evolution of the muscular system in tetrapod limbs. Zool Lett 4:27

Johanson Z, Joss J, Boisvert CA et al (2007) Fish fingers: digit homologues in sarcopterygian fish fins. J Exp Zool Part B Mol Dev Evol 308B:757–768

Kherdjemil Y, Lalonde RL, Sheth R et al (2016) Evolution of Hoxa11 regulation in vertebrates is linked to the pentadactyl state. Nature 539:89–92

Krauss S, Concordet JP, Ingham PW (1993) A functionally conserved homolog of the Drosophila segment polarity gene hh is expressed in tissues with polarizing activity in zebrafish embryos. Cell 75:1431–1444

Lalonde RL, Akimenko MA (2018) Contributions of 5'HoxA/D regulation to actinodin evolution and the fin-to-limb transition. Int J Dev Biol 62:705–716

Lau K, Tao H, Liu H et al (2015) Anisotropic stress orients remodelling of mammalian limb bud ectoderm. Nat Cell Biol 17:569–579

Martin AC, Goldstein B (2014) Apical constriction: themes and variations on a cellular mechanism driving morphogenesis. Development 141:1987–1998

Nakamura T, Gehrke AR, Lemberg J et al (2016) Digits and fin rays share common developmental histories. Nature 537:225–228

Neumann CJ, Grandel H, Gaffield W et al (1999) Transient establishment of anteroposterior polarity in the zebrafish pectoral fin bud in the absence of sonic hedgehog activity. Development 126:4817–4826

Shimada A, Kawanishi T, Kaneko T et al (2013) Trunk exoskeleton in teleosts is mesodermal in origin. Nat Commun 4:1639

Shubin NH, Daeschler EB, Jenkins FA (2006) The pectoral fin of Tiktaalik roseae and the origin of the tetrapod limb. Nature 440:764–771

Sordino P, van der Hoeven F, Duboule D (1995) Hox gene expression in teleost fins and the origin of vertebrate digits. Nature 375:678–681

Takamatsu N, Kurosawa G, Takahashi M et al (2007) Duplicated Abd-B class genes in medaka hoxAa and hoxAb clusters exhibit differential expression patterns in pectoral fin buds. Dev Genes Evol 217:263–273

Thorogood P (1991) The development of the teleost fin and implications for our understanding of tetrapod evolution. In: Hinchliffe J, Hurle J, Summerbell D (eds) Developmental patterning of the vertebrate limb. Springer, Boston, pp 347–354

Yano T, Tamura K (2013) The making of differences between fins and limbs. J Anat 222:100–113

Yano T, Abe G, Yokoyama H et al (2012) Mechanism of pectoral fin outgrowth in zebrafish development. Development 139:2916–2925

Zhang J, Wagh P, Guay D et al (2010) Loss of fish actinotrichia proteins and the fin-to-limb transition. Nature 466:234–237

Evo-Devo of Scales, Feathers, and Hairs

Wei-Ling Chang, Mingxing Lei, Ping Wu, and Cheng-Ming Chuong

Contents

Abstract

Integument forms the interface between the organism and its environment. Because of the need for adaptation, it has evolved a robust ability to regenerate under physiological conditions (age, sex, and season) and sometimes in different

W.-L. Chang · M. Lei
Integrative Stem Cell Center, China Medical University Hospital, China Medical University Taichung, Taichung, Taiwan
e-mail: smolljade@gmail.com; lmxgoodluck@163.com

P. Wu · C.-M. Chuong (✉)
Department of Pathology, Keck School of Medicine of University of Southern California, Los Angeles, CA, USA
e-mail: Ping.Wu@med.usc.edu; cmchuong@usc.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_192

forms which we call organ-level metamorphosis. Here we identify the organization principles of skin appendage organs: periodic patterning, stem cell renewal, temporo-regional specification, the ability to undergo regenerative healing, and the robust ability to evolve. We identify "core-morphoregulatory modules" for basic appendage formation, and "morphoregulatory modules" which sense environmental changes and mediate the signals to modify core morphogenesis processes. Here, we highlight key knowledge and recent development in the evolution and development of feather, scales, and hairs.

Keywords

Skin appendages · Stem cells · Regeneration · Ectodermal organ · Evo-devo

Introduction

Amniotes have various types of integumentary appendages, such as scales, feathers, and hairs (Fig. 1) (Wu et al. 2018b). Integuments form the boundary between an organism and the environment. The evolution of novel developmental mechanisms in integuments and appendages enables organisms to adapt to terrestrial conditions. For example, the reptilian integument acquired water-impermeability, avian species developed filamentous integumentary appendages that allow them to fly, the mammalian ectoderm produced hairs for heat preservation, and mammary glands provide an effective way to nurture babies.

It is instructive to consider nature's way of integumentary organ design – the "Tao" of this process (Lai and Chuong 2016) (Fig. 1a). Some diverse forms of integumentary appendages can be selected by the expression of morphogens at specific skin regions or during specific developmental stages (Lu et al. 2016). Adaptable integument forms undergo three steps (1) First, the ectodermal organs form through *dermal-epithelial interactions.* Classical tissue recombination experiments have demonstrated that these interactions within a developmental time window can specify integument organ phenotypes. Later, *periodic patterning* plays an effective role as an integument organizer to build complex and adaptable integuments (Chuong et al. 2013). Periodic patterning evolved to compartmentalize the skin into multiple elements, each with its own stem cells that can undergo cyclic renewal (2) Second, regional specificity is also evolved by enabling each element to be independently controlled by regulating its stem cells to form different appendage types under different biological conditions. For example, different physiological or hormone status can promote the formation of sexually dimorphic rooster or hen feathers, and different body regions can display different shapes and sizes of downy or contour feathers. (3) Then, *morphogen signaling* acts at different developmental time points and positions to modulate the integumentary element forms. For example, transcriptome profiling shows distinctly different amounts of several bone morphogenetic proteins (BMP), WNT proteins, and FGF proteins in regions producing hair follicles compared to those producing sweat glands. Hair follicle and

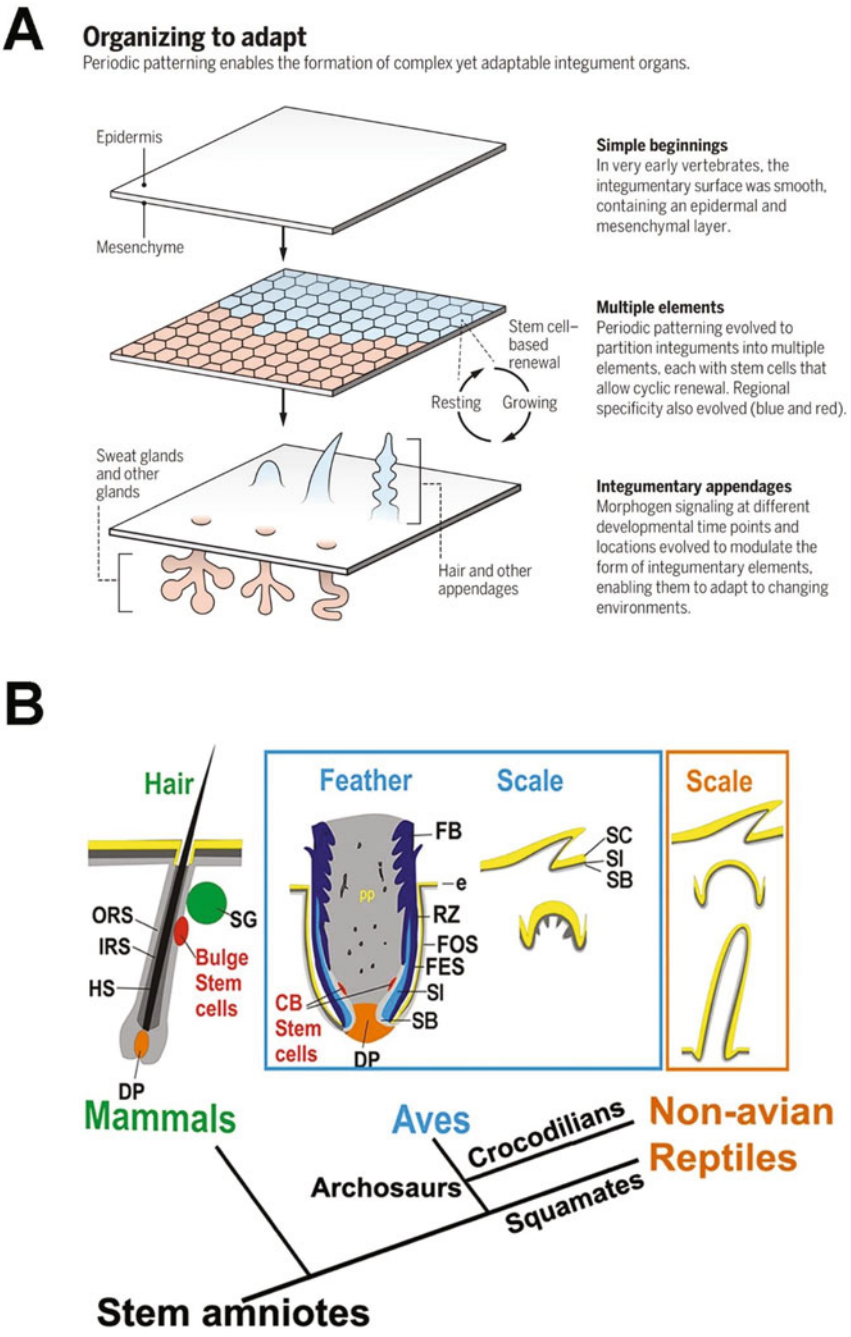


Fig. 1 **Evo-devo of amniote skin appendages**. (**a**) Organization principles of integuments show what is unique in feather and hair formation that allows the complex pattern to form and adapt on

sweat gland fates can be switched by the modulating of BMP, in which suppressing BMP signaling could convert sweat glands into hairs (Lai and Chuong 2016). Although vertebrate skin appendages appear to be quite different, they share common developmental pathways. In a molecular cell biology laboratory, molecular misexpression experiments can alter the size, number, and phenotypes of integument organs and provide insight on their development and evolutionary origin (Yu et al. 2002).

Collectively, modification of the integument helps animals adapt to their changing environment. Modulation of integument phenotypes can occur at the genomic level with consequences at the evolutionary scale, or at the epigenetic level with metamorphic changes in the same individual, or in the same species but under different physiological conditions. Variation and innovation in developmental processes are thought to be a key mechanism of organ novelty.

Integument appendages evolve and new forms can be afforded to be "tested" in an evolutionary context, since an animal can have tens of thousands of appendages on its body. Here we show the phylogenetic relationships of scales, hairs, and feathers (Fig. 1b). Their fate determination (for example, feathers versus scales) is guided by molecular signals exchanged during epidermal-dermal interactions. Heterochronic recombination showed that basic skin formation can be appreciated across different classes of animals, such as between mouse and chicken, or reptile and chicken (Dhouailly 1975), which in today's context, may represent the ability to activate β-catenin. But the information to make feather forms is missing in mice or reptiles, and they will not go on to that stage. Through evolution, core-morphoregulatory modules for feathers are established (Wu et al. 2018b). On this basis, more diverse feather forms are generated through modulation of the core-morphoregulatory modules to generate special feathers for flight (flight, tail feathers), communication (contour, tail feathers, etc.), or temperature regulation (downy feathers). Even within the flight-feather category, diverse bio-architectures of the feather rachis (main shaft) and vane modules are produced which allow birds to evolve different flight modes and adaptation to various ecospaces (Chang et al. 2019).

In this chapter, we highlight some recent progress in the evolution and development of feathers, scales, and hairs.

Fig. 1 (continued) the integument. (It is the one with multilayered. Tao of integuments). (Reproduced with permission from Lai and Chuong 2016). (**b**) Schematic drawing showing amniote skin appendages and their evolutionary relationships. Mammals have hairs. Chickens have feathers, scutate scales, and reticulate scales. The stem cell niche in hairs and feathers are marked in red. Other structures are also indicated. Alligators (crocodilians) have overlapping scales and tuberculate scales. Iguanas (squamates) have elongated scales. (Reproduced with permission from Wu et al. 2018b) *CB* collar bulge, *DP* dermal papilla, *e* epidermis, *FB* feather barb ridge, *FES* feather sheath, *FOS* feather follicle sheath, *HS* hair shaft, *IRS* inner root sheath, *ORS* outer root sheath, *RZ* ramogenic zone, *SG* sebaceous gland, *SB* stratum basal, *SC* stratum corneum, *SI* stratum intermedium

Feathers

During feather evolution, the integument has undergone a series of topological transformations to form fluffy plumages for thermoregulation, colorful contour feathers for display, and vanes for effective air-current maneuvers to flight. The discovery of fossils with feathered dinosaurs highlights the diverse paths that were undertaken during the early evolution of avian flight (Xu et al. 2014). Here we introduce how the feather shapes and colors are formed, and we describe how specific keratins interweave to form different skin appendages in birds and reptiles.

Shape

Diverse feather forms (Fig. 2a) are generated via the combination of core branching modules and morphoregulatory modules (Widelitz et al. 2019). The feathers are generated from a cylindrical feather follicle which is composed of a dermal papilla and an epidermal collar (Fig. 1a). The collar houses the feather epithelial stem cells where transient amplifying cell proliferation occurs. Above the collar, in the ramogenic zone, barb branching starts to form. Periodic branching is modulated by filapodial interactions among basal cells and involves FGF and Notch signaling (Cheng et al. 2018). Along the proximal-distal axis, branching is regulated by the relative activities between activators and inhibitors, including Sprouty/FGFs and BMP/Noggin. The basic structural unit in feather branches is the barb ridge, which is formed by the invagination of basal layer cylindrical epithelia which then segregates into cellular zones destined for proliferation or death (Chuong et al. 2014). Variation in the number, arrangement, and spacing of barb ridges contributes to the diversity of feather branch patterns and ultimately controls feather form and function. The transformation of fluffy barbs arranged in a three-dimensional configuration to a two-dimensional organized vane allows for flapping-based flight and for displaying color patterns. The formation of vane depends on the change of barbule cell shapes. Overlapping plate barbules allows fluffy 3D plumulaceous branches, which are made up of filamentous barbules, to be organized into a 2D vane plane. Later the barbule cells form hooklets in a Wnt2b-dependent mechanism (Chang et al. 2019). This allows the much stable vane for powered flight and marks a major event in feather evolution.

The central structure backbone of a feather, the rachis, is positioned along the anterior-posterior axis by a Wnt3a gradient (Fig. 2b), followed by cell arrangement changes involving planar cell polarity (Lin and Yue 2018). The size of the rachis is controlled by BMP and GDF10 (Li et al. 2017). These molecular signals modulate rachis formation and convert radially to bilaterally symmetric feather forms. The asymmetric feather vane morphology which is necessary for aerodynamic flight is controlled by a retinoic acid gradient (Li et al. 2017). Recent work shows the remarkable bioarchitecture of the rachis, from round to ovoid and elongated shape, the formation of composite material with medulla and cortex, and the formation of cortical ridges and the arrangement of vacuole cells within the medulla

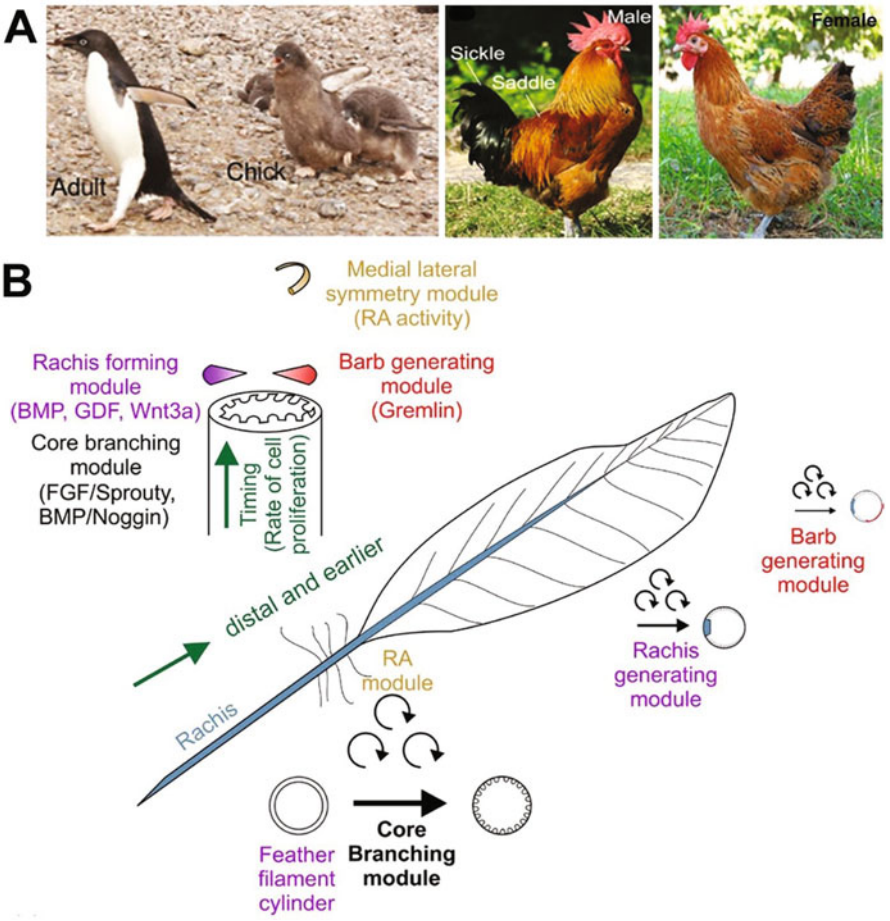


Fig. 2 Feather morphogenesis: The core branching modules and morphoregulatory modules. (**a**) Stem cells in the same feather follicles can be guided by the microenvironment (within feather follicle) and macroenvironment (age, sex, hormonal in the whole animal, temperature, season, etc.) to form different feather forms, in the context of "organ level metamorphosis" (Chuong et al. 2013). Therefore, the environment can affect feather morphology in terms of shapes, colors, and textures. (**b**) Feather morphogenesis results from epithelial-mesenchymal interactions. Keratinocytes are stem cells and adaptable. Mesenchyme forms the niches and can be modified by the environment. (Reproduced with permission from Widelitz et al. 2019)

(Chang et al. 2019). These bioarchitectural changes mark the differences of rachises in feathers from different feather types of the same bird, from flight feathers of extant birds with different flight modes, and from evolving feathers in feathered dinosaurs, mesozoic birds, and extant birds (Chang et al. 2019).

Each step here, such as generation of barbs, rachis, and barbules, represents a qualitative evolutionary novelty (see chapter ▶ "Developmental Innovation and Phenotypic Novelty"). Through the quantitative control of these different

"morphoregulatory modules," feathers adjust their length, feather vane width, ratio of pennaceous versus plumulaceous regions, etc. The developmental switch regulating these modules is differentially regulated in different body regions or at different life stages of a bird.

Color

Birds display great ranges of color wavelengths. Avian colors are based on melanin system (melanocyte), chemical color, and structure colors (Roy et al. 2020). The color patterns are based on the combination of the pigment and structure compartments and selected by natural or artificial selection.

Feathers color patterns can change to suit physiological needs through the cyclic regeneration of feathers that resets pigment patterns. Melanocyte progenitors are distributed as a horizontal ring in the follicle, sending melanocytes vertically up into the epithelial cylinder when feathers grow. By modulating the presence, arrangement, or differentiation of melanocytes, different pigment patterns form to respond. In the spatial dimension, the cylindrical follicle configuration creates a novel medial-lateral dimension once the feather vane opens. Interactions with peripheral pulp provide a third dimension of regulation. In the temporal dimension, pigmentation can be patterned along the proximal-distal feather axis, which is also a timing axis. Systemic factors, such as hormonal status or seasonal changes, add to regulate melanocyte behaviors. Thus, the evolution of stem cell niche topology increases the diversity of feather patterning by virtue of combinatorial regulatory mechanisms which offer greater temporospatial freedom in the new morphogenetic space.

Animal skin displays periodic pigment patterns, such as spots and stripes. Recent studies revealed that periodic stripe formation occurs through the sequential organization of space, combining early developmental landmarks and local refining mechanisms (Haupaix et al. 2018). The somitic mesoderm first provides positional information to the development of dermis, which controls the stripe position of *agouti* expression. Their width is then refined by locally modulating pigment production in a dose-dependent manner. Using melanocyte transplantation, Inaba and co-workers found that melanocytes have an autonomous periodic patterning role during body pigment stripe formation (Inaba et al. 2019). Developing melanocytes directly connect with each other via filopodia to form a network. Genetic modulations, either by misexpression of dominant or by overexpression of gap junction channel *connexin40* in melanocytes, alter the periodic pattern. Furthermore, the melanocytes interact with dermal cells and instruct them to express Agouti signaling protein to switch pigment, which at the end confer stable and distinct pigment stripe patterns.

On the chemical color, recent genetic mapping and gene-expression analysis have identified that combinations of biochemical activity of polyketide synthase provide a novel evolutionary use for colorful feather pigmentation (Cooke et al. 2017). Thus, the enriched avian pigment patterns result from co-opting mechanisms regulating melanocyte behavior.

Keratin

Feathers are mainly composed of α- and β-keratins. The former is found in all vertebrates, whereas the latter is present only in birds and reptiles. Comparative genomic studies have shown that the number of α- and β-keratin genes and the diversity of β-keratin genes are important for feather evolution that adapts birds to diverse ecological niches. α-keratin molecules show a helical arrangement and form polymers. The scale- and claw-β-keratins form interweaving filament bundles. A new feather β-keratin subfamily has evolved to form a fiber-like structure built using more composite isoforms which increase intra-feather architectural complexity. Feather-β-keratin diversification is crucial for the evolution of various feather types, providing improved thermoregulation and aerodynamic flight.

Scientists have wondered how keratins are interwoven to form different skin appendages. The topographic map of keratins on developing chicken skin integuments showed that temporal and spatial α- and β-keratin expression is involved in establishing skin appendage phenotype diversity (Wu et al. 2015). α- and β-Keratins show mutual dependence and mutations in either keratin type result in disrupted keratin networks and failure to form proper feather branches. For example, a mutation in KRT 75 (KRT 75A), an α-keratin, causes the formation of a curved rachis in domestic chickens producing a frizzled phenotype (Ng et al. 2012). The distribution of α-keratin expression in the medulla of the rachis and ramus suggests that α-keratin contributes to barb orientation and feather shape. On the other hand, expressing a mutant form of KRT5, a β-keratin, in developing feather follicles can affect embryonic feather filament morphogenesis and increase the size of the ramus while decreasing the size of feather branches (Wu et al. 2015). Suppressing β-keratin gene expression also alters α-keratin expression at both mRNA and protein levels, suggesting that β-keratins may have nonstructural and mechanical functions (Wu et al. 2015). Collectively, α- and β-keratin gene combinations contribute to the morphological and structural diversity of different avian skin appendages forms.

Scales

Avian Scales

Birds have scutate and reticulate scales on their lower legs and feet and have feathers on most of their remaining body parts. The feather is a novel organ that evolved from dinosaur integuments. Developmental biology studies and recent fossil findings have revealed that feathers evolve from a series of novel morphogenetic events (Brusatte et al. 2015; Chuong et al. 2000; Xu et al. 2014).

The origin of avian scales is controversial. One viewpoint is that avian scales are homologues of non-avian reptile scales. However, it also has been hypothesized that bird scutate scales appeared later in evolution and are secondary structures derived from feathers (Dhouailly 2009). It is interesting that an ancient hatchling bird preserved in amber has both feathers and scales on its feet (Xing et al. 2017).

Chicken scales changing to a feather morphology have been observed in domestic chickens (such as silkie). Molecular biology experiments demonstrate that experimental treatment with retinoic acid, Wnt/β-catenin, Notch/Delta pathway activation, or BMP pathway suppression can convert scales to feathers (summarize in Wu et al. 2018b).

Recently, Wu et. al. used transcriptome analyses and functional genomics to identify novel molecular circuits involved in scale-feather conversion (Wu et al. 2018b). We identified five novel scale-feather converters, including three transcription factors (TFs) and two growth factor antagonists, which generate intermediate phenotypes. Examples are shown in Fig. 3a. Intriguingly, some of these phenotypes are similar to the filamentous appendages found in the fossils of feathered dinosaurs (Xu et al. 2010). We now have a potential molecular explanation for these hypothesized "missing links." Our analyses led to the identification of five morphoregulatory modules that are essential for modern feather formation (Fig. 3b).

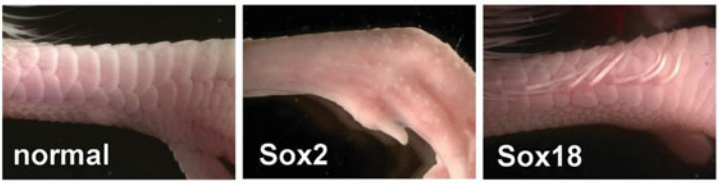
The phenotypes are clustered into different morphotypes, representing the activation of different morpho-regulatory modules and the expression of different feather specific keratins. The five key feather morphogenetic events are: (1) a localized growth zone (LoGZ) that leads to elongated appendages, (2) invagination that leads to the formation of follicles, (3) barb ridge formation that leads to feather branching, (4) the expression of feather specific keratins, and (5) dermal papilla formation (Fig. 3c). Scales can be induced to elongate without folliculogenesis (Sox2, Spry2). Phenotypes produced in Sox18, β-catenin, and RA treated samples meet all the five criteria necessary to be considered real feathers. The spectrum of these "intermediate morphotypes" suggests that the five key morphogenetic events can be uncoupled, and specific criteria can be induced by specific molecular perturbations. Thus, the scientists propose that the evolution of feathers requires the integrative combination of five morphoregulatory modules. Our work provides molecular clues to these modules.

Reptilian Scales

Crocodilians, such as American alligators, have different types of scales on their body surface. Some squamates, such as green iguanas, have elongated scales (Chang et al. 2009). Wu et al. found that reptiles (such as anoles, iguanas, and alligators) have limited regenerative ability when wounds were created on their skin (Wu et al. 2014, 2018a, b). In alligators, putative stem cells in overlapping scalen hinge regions participate in skin repair (Wu et al. 2018a). Intriguingly, iguana frills fail to regenerate following injury (Wu et al. 2018b). The authors speculate that this is because they do not proceed to form a real follicle structure containing clustered stem cells and dermal papillae.

More intriguingly, they found that these molecules also can induce a LoGZ on the alligator scale to produce a new outgrowth. Ectopic expression of Spry2 induces the formation of bud-like structures whereas ectopic expression of β-catenin induces elongated bud-like structures with invagination, suggesting that these two scale-feather converters identified from birds can also induce ectopic LoGZ and

A



B

	LoGZ	Invagination (follicle)	Branching	Feather β-keratin	Dermal papilla
Avian scutate scale	-	-	-	-	-
Sox2	+	-	-	-	-
Zic1ΔC	-	±	-	-	-
Grem1	-	-	?	-	-
Spry2	+	-	+	±	-
Sox18 (β-catenin, RA)	+	+	+	+	+
Avian feather	+	+	+	+	+

C

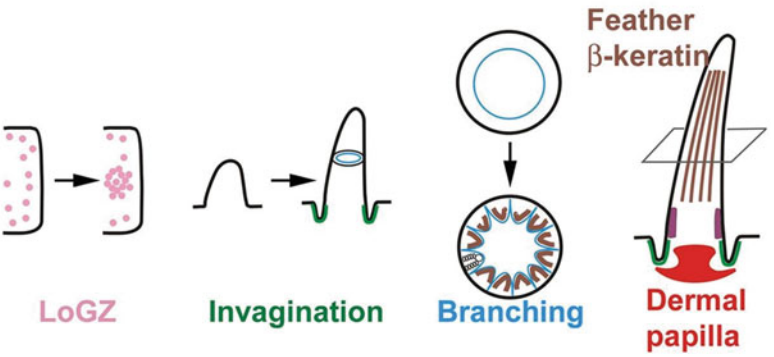


Fig. 3 Scale/feather conversion: Perturbation in chicken scales leads to different levels of hybrid scale/feather appendages. (**a**) Using RNA-seq, we have identified a group of molecules differentially expressed in scale and feather formation (Wu et al. 2018b). Several are evaluated and show phenotypes. Left panel, control. Middle panel, Sox2 misexpression shows appendages without trace of scale formation. Right panel, Sox18 misexpression leads to feather formation on scales. (**b**) A spectrum in intermediate phenotypes of scale to feather conversion as assessed by morphological alterations. – and + represent absence or presence of these feather characteristics? Represents ridged scales without mature barb ridge formation. Shh is used as a marker for placode (localized growth zone) and feather branching formation. Tenascin-C is used as a marker for follicle and dermal papilla formation. Feather β-keratin is used as a marker for feather differentiation. (**c**) Schematic drawing shows the five major events toward feathers formation LoGZ, invagination, branching, feather *β*-keratin, and dermal papilla. They are combinatorial, not sequential. (Reproduced with permission from Wu et al. 2018b)

grow feather bud-like elongated appendages from non-avian reptile skin. β-Catenin can even induce formation of some invaginations and elongated appendages, which share some characteristics similar to frills in iguanas. However, these morphologies are not sufficient to be called follicles.

A more recent work compared the molecular and cellular profiles in chicken feathers, chicken scales, and alligator scales, and found that chicken scutate scales are similar to chicken feathers in morphology at the early placode stage (Wu et al. 2018a). Comparing the expression of the recently identified feather-specific and scale-specific genes, the authors found that alligator scales are significantly different from both chicken feathers and chicken scales at the molecular level. These results suggest that avian scutate scales are more distant from reptilian scales. Chicken and alligator scales might have formed independently through convergent evolution (see chapter ▶ "Convergence").

Hair

The hair follicle is a complex mini-organ consisting of over 20 cell populations. As the major skin appendage of mammals, the hair follicle evolved various functions including thermoregulation, sensory perception, sexual attraction, protecting the body from external insults, and camouflage. During development, periodic patterning and epidermal-dermal interactions induce invagination of epidermal cells into the dermis of the skin to form a follicle which produces the hair (Fig. 4). During

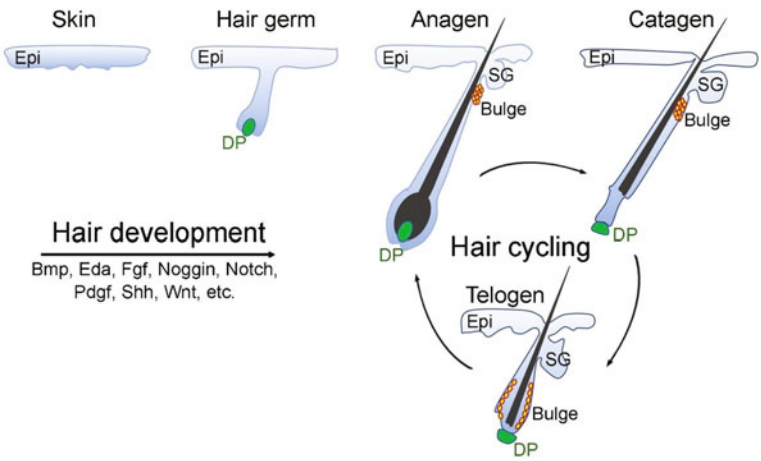


Fig. 4 Hair morphogenesis: development and cycling renewal in the adult. The skin initially forms a hair germ through developmental processes. Through epidermal-dermal interactions, periodically arranged hair primordia are laid out. They develop into hair follicles in the adult, with stem cell (bulge) and dermal papilla (DP) as inducers. Hair follicles are able to cycle through repeated phases of anagen (growth), catagen (regression), and telogen (rest). *Epi* epithelium, *SG* sebaceous glade

postnatal life, hair follicles undergo self-renewal with continuous growth cycles throughout the entire life of an individual. Thus, they represent a uniquely dynamic system to study tissue regeneration. Regional specificity implies that different skin areas have different characteristics of hair size, length, density, and types. Abnormalities in hair follicles will cause diseases including alopecia and hirsutism.

Evolutionary View of the Hair Follicle

The major driving force driving mammalian hair formation was likely thermoregulation, as mammals emerged and lived in cold climates millions of years ago. To protect against the cold, the mammoth, derived from the glabrous African elephant, evolved hairs during the glacial age. Animals such as the Manchurian tiger and musk-ox living at high latitudes grow longer and thicker hairs than animals such as the Bengal tiger and buffalo that live at low latitudes. By one means to regulate body temperature, the hair follicle is associated with the apocrine sweat gland which controls perspiration. It is unknown whether the increased distribution of sweat glands evolved before, during, or after the emergence of the hair follicle, but the dual presence of hair follicles and eccrine sweat glands in the skin is a recent primate acquisition. Hairs also evolved to provide other functions. Examples include sexual attraction, since the hairs can spread the scent of the hormone to attract the opposite sex; sensory perception, as the hair follicles are connected to nerves and arrector pili muscles; protection, as the hairs are colored by the melanocytes which protect the skin from UV radiation, etc.

Human beings are the only primates that lack abundant hairs on the skin. Particularly the glabrous or less hairy skin is observed on the ventral body surface which closely interacts with the environment. At least three theories are proposed to explain why humans gradually lost their hairs (Bergman 2007). The aquatic ape hypothesis suggests that the apelike ancestors of modern humans foraged for food in the shallow water. Hair is not an insulator in water thus apelike ancestors gradually lost their fur. Another interesting hypothesis for humans losing their hair is to reduce the prevalence of external parasites that routinely infest fur and cause chronic medical problems and even death. The third but most important theory is the driving force of thermoregulation. The apelike ancestors living in the cool forest required heavy fur to keep warm whereas the upright hominid living in the hot Africa savannah would have overheated if they had too many hairs. In addition, losing hairs benefits heat dissipation in the upright hominid during their long runs needed for foraging. Though hominids may need more heat at night, they learned to keep warm by manipulating their environment by making a fire and clothing.

Hair Development

Hair follicle development involves complex interactions between the epidermal cells and the underlying dermal cells. The hierarchical morphogenetic processes are

classically divided into placode induction, morphogenesis, and differentiation stages. The placode induction of diverse ectodermal organs shares common features at the cellular and molecular level. Epithelial appendages including sweat, mammary, sebaceous, and salivary glands, hairs, feathers, and tooth follicles form morphologically similar placodes when WNT signaling instructs the epithelial cells to become polarized and then invaginate into the dermis. Regionally specific signals determine which epithelial appendages will develop from the similar epithelial placodes. For example, the epithelial placodes develop into eccrine sweat gland in the foot skin when exposed to high mesenchymal bone morphogenetic proteins (BMPs) and fibroblast growth factors (FGFs), whereas they develop into hair follicle when BMP signaling is blocked and sonic hedgehog (SHH) is upregulated in the murine dorsal back skin (Lu et al. 2016). Likewise, lowering the BMP signal in the epidermal-dermal interface converts the nipple to hair-bearing epithelia. Further hair development requires many molecular pathways including Bmp, Eda, Fgf, Noggin, Notch, Pdgf, Shh, Wnt, etc., which have largely been reviewed (Chueh et al. 2013). In principle, this process involves a reaction-diffusion mechanism and continuous epithelial-mesenchymal interactions that allow well-arranged spacing and a hair follicle morphology to appear. As a result, the epithelial cells differentiate into multiple substructures in the hair follicle mainly including the outer root sheath, inner root sheath, matrix, cortex, medullae, and hair fiber, with multiple cell types in each of the substructures. The dermal cells that interact with the epithelial cells are engulfed by the hair bulb to become the dermal papilla (DP), which is the dermal signaling center of the hair follicle.

Sebaceous gland, arrector pili muscle (APM), and hair follicle stem cells (HFSCs) form accompanied with hair follicle development. Both Sox9 and Lrig1 are required for sebaceous gland morphogenesis (Nowak et al. 2008; Frances and Niemann 2012). The asymmetric distribution of Sox9- and Lrig1-positive cells in the developing hair follicle results in sebaceous gland lineage specification. Blimp1 marks a committed cell population within or adjacent to sebaceous glands that has the unipotency to give rise to the sebocytes. The APM which is responsible for piloerection anchors to the basement membrane in the hair follicle bulge region, where HFSCs reside. HFSC fate determination occurs during hair peg formation, through a WNT-SHH antagonism (Ouspenskaia et al. 2016). A group of suprabasal cells that express low Wnt in the developing hair follicle becomes HFSCs. This cell population responds to paracrine SHH and expands symmetrically, whereas the basal cells that express higher Wnt and have no response to SHH become short-lived progenitors that generate differentiated daughters of the hair follicle.

Formation of Specialized Hair Types

There are multiple hair types, such as terminal hairs and vellus hairs. Terminal hairs such as scalp hairs, whiskers, pubes, axillary hairs, chest hairs, etc., are long and stiff with heavy melanin deposition. Vellus hairs such as eyebrows, eyelashes, tragi, etc., are short with light pigmentation. Vellus hairs turn into terminal hairs in the chest,

abdomen, legs, arms, pubic area, and feet due to the influence of androgen during puberty in humans; whereas some of the terminal hairs become vellus hairs during aging. There are two major types of pelage hairs called primary hairs (guard) and secondary hairs (auchene, zigzag, and awl) which develop at embryonic day 14.5 and during embryonic days 16.5–18.5, respectively, in mice. Eda/Edar, FGF20, NF-κB, Wnt10b, Lgr4, etc. control primary hair formation, whereas Dkk4, Noggin/Lef1, Edar/Troy, Sox18, etc., regulate secondary hair follicle development. The secondary hairs gradually become primary hairs due to increased DP cell numbers during progressive hair cycling in adult mice. However, this is reversible as the primary hair follicle can form secondary hairs when the number of DP cells is decreased, indicating that the DP cell number modulates the formation of different hair types (Chi et al. 2013).

Hair Regeneration

The formation of HFSCs which migrate to the bulge niche allows the hair follicle to undergo cyclic renewal during postnatal life, including recurring cycles of growth phase (anagen), regression phase (catagen), and resting phase (telogen). In response to activation signals from the DP, the hair germ cells first proliferate to generate transient amplifying cells. These then undergo rapid and continuous proliferation and differentiation to form the differentiated layers of the hair follicle. Then bulge cells start to proliferate to generate the outer root sheath cells when stimulated by SHH secreted from the transient amplifying cells. HFSCs display a high regenerative ability. The bulge stem cells can be compensated by the hair germ cells when injured, and vice versa. In addition, transient amplifying cells at the DP and matrix interface have the unipotency to generate the seven different hair follicle differentiation layers. Transient amplifying cells also can compensate for the loss of neighboring matrix cells that are subject to injury. Both intrinsic factors (epigenetic and genetic) and extrinsic factors (microenvironment and macroenvironment) influence hair regeneration (Chueh et al. 2013). Among them, intrinsic factors such as Dlx3, Foxc1, Jak/Stat, Lhx2, Nfatc1, Sox9, Tbx1, and Tcf3/4, and extrinsic factors such as Bmps, Dkks, Fgf18, and Sfrps maintain HFSCs quiescence. Other intrinsic factors such as β-catenin, H3 K4/K9/K27me3, Ldha, and Runx1, and extrinsic factors such as Collagen XVII, Fgf7 & 10, Follistatin, Noggin, Pdgf, Tgfb2, and Wnt activate HFSCs and promote hair regeneration (Lei and Chuong 2016).

Melanocyte stem cells originate from the neural crest and share the same bulge niche with HFSCs. During hair regeneration, transcription factor NFIB coordinates melanocyte stem cell activation in synchrony with HFSCs. This generates melanocytes which produce and transfer melanin into the hair fiber (Chang et al. 2013). Hair follicles have two major types of melanin-based pigments that color hairs, including eumelanin which is the dominant pigment in brown and black hairs, and pheomelanin which is dominant in red hairs. Blond or gray hair results from little or no melanocyte differentiation in the hair follicle.

Conclusions

Scientists are attracted to understand the evo-devo regulation for the diverse types of integumentary appendages in amniotes. The major achievement of the scale as an integument type is the barrier function that allows reptiles to live on land. The feather is a relatively recent product in evolution and has brought in the novel functions of insulation, display, and flight. Avian ectoderm is primarily programmed toward forming feather buds that protrude out, and mammalian ectoderm toward forming hairs germs that invaginate into the dermis during development (Wu et al. 2018a, b). Several interesting arguments are arising from classical experimental embryology, from modern molecular biology, and from the recent discovery of new lithic and amber fossils which provide 3D configurations (Chang et al. 2019). As we learn more about how molecular cascades contribute to different morphogenetic processes and how developmental pathways regulate to establish novel and complex forms (Li et al. 2017), as well as the new information from the latest epigenetic genomics studies and powerful real-time imaging technology, we can appreciate the pressure of adaptation may act on the mechanics of signaling and development during evolution (Widelitz et al. 2019; Chang et al. 2019).

Cross-References

► Convergence
► Developmental Innovation and Phenotypic Novelty

Acknowledgments WLC and ML are supported by the Integrative Stem Cell Center, China Medical University in Taiwan (CMU) and Hospital. The work is also supported by the Drug Development Center, CMU, from the Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan, and grants from the Ministry of Science and Technology in Taiwan. PW and CMC are supported by NIH AR 047364 and AR 060306 and a research contract between CMU/Hospital and USC (USC grant number 5351285884), and CMC is a paid scientific advisor of CMU.

References

Brusatte SL, O'Connor JK, Jarvis ED (2015) The origin and diversification of birds. Curr Biol 25(19):R888–R898

Chang C, Wu P, Baker RE et al (2009) Reptile scale paradigm: Evo-Devo, pattern formation and regeneration. Int J Dev Biol 53(5–6):813–826

Chang CY, Pasolli HA, Giannopoulou EG et al (2013) NFIB is a governor of epithelial-melanocyte stem cell behaviour in a shared niche. Nature 495(7439):98–102

Chang WL, Wu H, Chiu YK et al (2019) The making of a flight feather. Archit Adapt Cell 179(6):1409–1423

Cheng D, Yan X, Qiu G et al (2018) Contraction of basal filopodia controls periodic feather branching via Notch and FGF signaling. Nat Commun 9(1):1345

Chi W, Wu E, Morgan BA (2013) Dermal papilla cell number specifies hair size, shape and cycling and its reduction causes follicular decline. Development 140(8):1676–1683

Chueh SC, Lin SJ, Chen CC et al (2013) Therapeutic strategy for hair regeneration: hair cycle activation, niche environment modulation, wound-induced follicle neogenesis, and stem cell engineering. Expert Opin Biol Ther 13(3):377–391
Chuong CM, Chodankar R, Widelitz RB et al (2000) Evo-devo of feathers and scales: building complex epithelial appendages. Curr Opin Genet Dev 10(4):449–456
Chuong CM, Yeh CY, Jiang TX et al (2013) Module-based complexity formation: periodic patterning in feathers and hairs. Wiley Interdiscip Rev Dev Biol 2(1):97–112
Chuong CM, Bhat R, Widelitz RB et al (2014) SnapShot: branching morphogenesis. Cell 158(5):1212–1212. e1
Cooke TF, Fischer CR, Wu P et al (2017) Genetic mapping and biochemical basis of yellow feather pigmentation in budgerigars. Cell 171(2):427.e21–439.e21
Dhouailly D (1975) Formation of cutaneous appendages in dermo-epidermal recombinations between reptiles, birds and mammals. Wilehm Roux Arch Dev Biol 177(4):323–340
Dhouailly D (2009) A new scenario for the evolutionary origin of hair, feather, and avian scales. J Anat 214(4):587–606
Frances D, Niemann C (2012) Stem cell dynamics in sebaceous gland morphogenesis in mouse skin. Dev Biol 363(1):138–146
Haupaix N, Curantz C, Bailleul R et al (2018) The periodic coloration in birds forms through a prepattern of somite origin. Science 361(6408):1202
Inaba M, Jiang TX, Liang YC et al (2019) Instructive role of melanocytes during pigment pattern formation of the avian skin. Proc Natl Acad Sci U S A 116(14):6884–6890
Bergman J (2007) The aquatic ape theory: challenge to the orthodox theory of human evolution. J Creat 21(1):111–118
Lai YC, Chuong CM (2016) The “tao” of integuments. Science 354(6319):1533–1534
Lei M, Chuong CM (2016) STEM CELLS. Aging, alopecia, and stem cells. Science 351(6273):559–560
Li A, Figueroa S, Jiang TX et al (2017) Diverse feather shape evolution enabled by coupling anisotropic signalling modules with self-organizing branching programme. Nat Commun 8: ncomms14139
Lin J, Yue Z (2018) Coupling of apical-basal polarity and planar cell polarity to interpret the Wnt signaling gradient in feather development. Development 145(17):dev162792
Lu CP, Polak L, Keyes BE et al (2016) Spatiotemporal antagonism in mesenchymal-epithelial signaling in sweat versus hair fate decision. Science 354(6319):aah6102
Ng CS, Wu P, Foley J et al (2012) The chicken frizzle feather is due to an alpha-keratin (KRT75) mutation that causes a defective rachis. PLoS Genet 8(7):e1002748
Nowak JA, Polak L, Pasolli HA et al (2008) Hair follicle stem cells are specified and function in early skin morphogenesis. Cell Stem Cell 3(1):33–43
Ouspenskaia T, Matos I, Mertz AF et al (2016) WNT-SHH antagonism specifies and expands stem cells prior to niche formation. Cell 164(1–2):156–169
Roy A, Pittman M, Saitta ET, Kaye TG, Xu X (2020) Recent advances in amniote palaeocolour reconstruction and a framework for future research. Biol Rev Camb Philos Soc 95:22–50
Widelitz RB, Lin GW, Lai YC et al (2019) Morpho-regulation in diverse chicken feather formation: integrating branching modules and sex hormone-dependent morpho-regulatory modules. Develop Growth Differ 61(1):124–138
Wu P, Alibardi L, Chuong CM (2014) Regeneration of reptilian scales after wounding: neogenesis, regional difference, and molecular modules. Regeneration (Oxf) 1(1):15–26
Wu P, Ng CS, Yan J et al (2015) Topographical mapping of alpha- and beta-keratins on developing chicken skin integuments: functional interaction and evolutionary perspectives. Proc Natl Acad Sci U S A 112(49):E6770–E6779
Wu P, Lai YC, Widelitz R et al (2018a) Comprehensive molecular and cellular studies suggest avian scutate scales are secondarily derived from feathers, and more distant from reptilian scales. Sci Rep 8(1):16766
Wu P, Yan J, Lai YC et al (2018b) Multiple regulatory modules are required for scale-to-feather conversion. Mol Biol Evol 35(2):417–430

Xing L, McKellar RC, Xu X et al (2017) Response to: phylogenetic placement, developmental trajectories and evolutionary implications of a feathered dinosaur tail in Mid-Cretaceous amber. Curr Biol 27(6):R216–R217
Xu X, Zheng X, You H (2010) Exceptional dinosaur fossils show ontogenetic development of early feathers. Nature 464(7293):1338–1341
Xu X, Zhou Z, Dudley R et al (2014) An integrative approach to understanding bird origins. Science 346(6215):1253293
Yu M, Wu P, Widelitz RB et al (2002) The morphogenesis of feathers. Nature 420(6913):308–312

Part IX

Modeling Approaches to Evo-Devo

Morphometrics in Evolutionary Developmental Biology

Philipp Mitteröcker

Contents

Abstract

Morphometrics, the measurement and statistical analysis of organismal form, has always been a core tool in evolutionary biology. With the advancement of 3D imaging technology and geometric morphometric methodology, it is also increasingly applied in developmental biology. At the interface of these two disciplines, evolutionary developmental biology (evo-devo) seeks to understand how organismal development affects evolutionary change by shaping the phenotypic variation that is subject to selection. Quantification of morphological variation thus is an important part of evo-devo research and bridges the field to quantitative evolutionary theory. Likewise, modern morphometrics enables the quantitative comparison of developmental trajectories across individuals, populations, or environments. Here, I review the current state of the art in morphometrics and provide examples from vertebrate development. I discuss advantages and limitations of current methods and outline directions for future developments.

P. Mitteröcker (✉)
Department of Evolutionary Biology, University of Vienna, Vienna, Austria
e-mail: philipp.mitteroecker@univie.ac.at

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_119

Keywords

Geometric morphometrics · Ontogenetic trajectories · Procrustes · Shape analysis · Sliding landmarks

Introduction

The term "morphometrics" originates from the Greek words for "form" and "measurement" (*morphē*, *métron*) and refers to a sub-discipline of statistics concerned with the measurement and analysis of organismal form. The first modern statistical approaches in the early twentieth century – such as those of Francis Galton, Ronald Fisher, and Sewall Wright – were applied to morphological data already. The foundations of multivariate statistics – the joint analysis of multiple variables – were established only a few decades later by Harold Hotelling, Samuel Wilks, P. C. Mahalanobis, and others. While mainly originating in psychology, multivariate statistics quickly found applications in biology and gave rise to a field that is meanwhile referred to as multivariate or traditional morphometrics. It is characterized by the application of standard multivariate statistical methods to a wide array of morphological measurements, including length, area, and volume of body structures such as bones, organs, or appendages, as well as counts of repeated elements such as vertebrae, digits, and fin rays. In today's biology, such approaches are still common, especially when concerned with traits such as organ weight and the length of elongated structures, such as long bones.

A new branch of morphometrics – *geometric morphometrics* – was developed in the 1980s by Fred Bookstein, James Rohlf, Ian Dryden, and others and has become very popular in biology, anthropology, and paleontology for analyzing complex organismal structures, such as vertebrate skulls and pelves, fly wings and heads, fish body shape, and human faces, to name just a few. Geometric morphometrics has also been applied to measure embryonic form and gene expression patterns (e.g., Hallgrimsson and Lieberman 2008, Mayer et al. 2014, Martínez-Abadías et al. 2016, and Green et al. 2017).

Geometric Morphometrics

All geometric morphometric approaches are based on two- or three-dimensional measurement points, so-called landmarks; the approaches differ in the way how the shape and overall size of these landmark configurations are parameterized. The term "shape" refers to the geometric information of an object that is invariant (i.e., unchanged) when scaling, translating, or rotating the object. The "form" of an object is only invariant to translation and rotation; hence, it comprises information on both size and shape. In most biological applications, the location and orientation of an organism are arbitrary and biologically meaningless. The size of an organism is biologically meaningful but often differs in its functional, developmental, or

evolutionary role from the organism's shape. Therefore, size and shape are often parameterized separately, and the statistical analysis may focus either on shape only or comprise both aspects of form, i.e., shape and size (Mitteroecker et al. 2004). Geometric morphometric approaches in biology include Euclidean distance matrix analysis (Lele and Richtsmeier 1991), Fourier analysis (Lestrel 1982), eigenshape analysis (MacLeod 1999), and so-called non-label-based methods that are usually based on large sets of automatically collected 3D points (Polly and McLeod 2008; Rolfe et al. 2011; Pomidor et al. 2016) or volumetric 3D images (Ashburner and Friston 2000). By far most common in today's biology and evo-devo, however, is the Procrustes method (Bookstein 1991; Dryden and Mardia 1998; Mitteroecker and Gunz 2009; Klingenberg 2010).

For a sample of measured landmark coordinates, Procrustes analysis separates shape information from variation in the overall size, location, and orientation of the configurations (Rohlf and Slice 1990; Mitteroecker and Gunz 2009; Klingenberg 2010). First, variation in the location of the configurations is removed by translating all configurations to the same *centroid* (center of gravity, computed as the average x, y, and z coordinates of all landmarks of a configuration). Second, size variation is removed by scaling all configurations to the same *centroid size* (square root of the summed squared distances between the landmarks and their centroid). Third, variation in orientation is removed by iteratively rotating all configurations so as to minimize the summed squared distances between corresponding landmarks across the sample (Fig. 1a). After this standardization for position, scale, and orientation, the resulting landmark coordinates are referred to as *Procrustes shape coordinates* as they contain only information on the shape of the landmark configurations.

The *Procrustes distance*, often approximated by the Euclidean distance (square root of summed squared differences) between two vectors of shape coordinates, is a measure of shape difference between two landmark configurations (Dryden and Mardia 1998); it is zero only if they have exactly the same shape (see Fig. 2b for an example). Procrustes distance can also be interpreted as the distance between two shapes when considered as points in a high-dimensional *shape space* (Dryden and Mardia 1998). For p landmarks in two or three dimensions, Procrustes shape space is a Riemannian manifold with $2p - 4$ or $3p - 7$ dimensions, respectively; it can be considered a special case of a "morphospace" (Mitteroecker and Huttegger 2009). Because Procrustes distance pools the differences for all the shape coordinates, without accounting for the spatial distribution of landmarks, it is not always guaranteed to be biologically interpretable, especially when comparing magnitudes of shape differences along divergent shape *patterns*.

Like other variables at an interval scale, shape coordinates can be averaged (*consensus shape*) or regressed on other variables (*shape regression*). Typical examples in evo-devo include the computation of stage- or group-specific mean shapes (Fig. 2b) as well as regressions of organismal shape or form on environmental variables, age, or genotype. Using either longitudinal or cross-sectional samples, the regression of shape on age allows for estimating individual or group-specific *ontogenetic trajectories* (Alberch et al. 1979; Mitteroecker et al. 2004). In order to reflect nonlinearities in the developmental program, such shape regressions can comprise

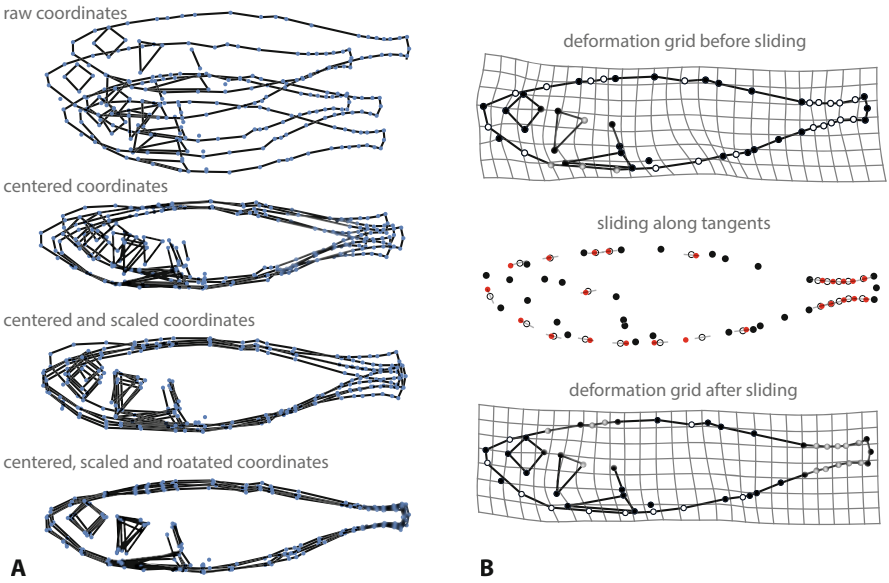


Fig. 1 Illustration of two core techniques of geometric morphometrics, shown in an example of three-spine stickleback body shape as captured by 30 anatomical landmarks (black points) and 23 semilandmarks (open circles) (Data from Ramler et al. 2014). (**a**) Generalized Procrustes analysis. The raw coordinates of every landmark configuration are translated to the same *centroid*, scaled to the same *centroid size*, and iteratively rotated to minimize the summed squared distances between corresponding landmarks across the sample. After this standardization for position, scale, and orientation, the landmark coordinates are called *Procrustes shape coordinates*. (**b**) Sliding landmark algorithm. Semilandmarks slide along tangents to the body outline in order to minimize the bending energy of the thin-plate spline (TPS) function – a measure of local shape difference – between each configuration and the mean shape. Top and bottom figures show the TPS deformation grids between one configuration and the sample average before and after sliding the semilandmarks, respectively. Note how the deformation of the grid is reduced through the sliding algorithm, thus removing shape differences due to arbitrary placement of semilandmarks. The middle panel shows how the semilandmarks slid away from their initial position

nonlinear terms or, more commonly, consist of local linear regressions (Fig. 2c). Individual age may be substituted by developmental stage or the size of the organism, which reduces the influence of variation in developmental timing on the regression.

Ordination techniques – low-dimensional representations of multivariate differences between individuals and groups – are central to many morphometric analyses. Most common is principal component analysis (PCA), which derives linear combinations of the shape coordinates with maximal sample variance. Two- or three-dimensional scatter plots of these linear combinations, the principal components (PCs), can be used to identify clusters of individuals, developmental and evolutionary trajectories, or outliers (Figs. 2c and 3b). Because most geometric morphometric studies are characterized by an excess of variables over cases, variable reduction techniques, such as PCA, have to precede many other statistical methods, including

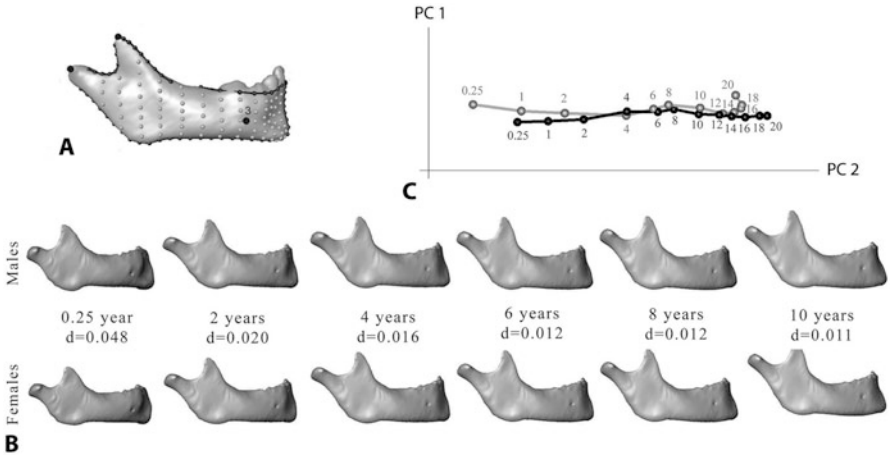


Fig. 2 Geometric morphometrics of postnatal development of the human mandible (From Coquerelle et al. (2011), reproduced with permission from RightsLink). (**a**) In a sample of 159 modern humans, three-dimensional shape of the mandible was measured by 4 anatomical landmarks (large black dots), 128 curve semilandmarks (small black dots), and 273 surface semilandmarks (gray dots). (**b**) Age-specific mean shapes were estimated by local linear regressions separately for males and females and visualized in a lateral view. The total magnitude of shape difference between male and female mean shapes (as a measure of age-specific sexual dimorphism) was estimated by the Procrustes distance. (**c**) Plot of the first two principal components (PCs) of the age-specific mean shapes for females (gray) and males (black), labelled by their age. The line connecting the mean shapes represents the female and male average ontogenetic trajectories of postnatal mandibular development. Note the perinatal sex difference in developmental timing and the divergence of trajectories during puberty

canonical variate analysis (CVA), between-group PCA, linear and quadratic classification, as well as multivariate analysis of variance (MANOVA). Magnitudes of separation or overlap between groups, as inferred from ordination analyses, should generally be verified by cross-validation methods. Bookstein (2015) suggested a valuable modification of PCA that accounts for the spatial relationships among landmarks.

Visualization of statistical results is a particular strength of geometric morphometrics: mean shapes, regressions, principal components, and other multivariate statistics can be displayed as actual shapes or shape deformations, rather than mere vectors of numbers. This facilitates biological interpretation of results and enables powerful exploratory analyses. Two-dimensional shape deformations are usually represented by deformation grids based on thin-plate spline interpolation (TPS, Bookstein 1991), as illustrated in Figs. 1b and 4c, d. Three-dimensional shape differences or shape changes are more effectively represented by surface deformations (Figs. 2b and 3b).

Procrustes shape coordinates allow for the statistical analysis and visualization of organismal shape, separately from that of the organism's size, which is commonly represented by the centroid size of a landmark configuration. However, when size

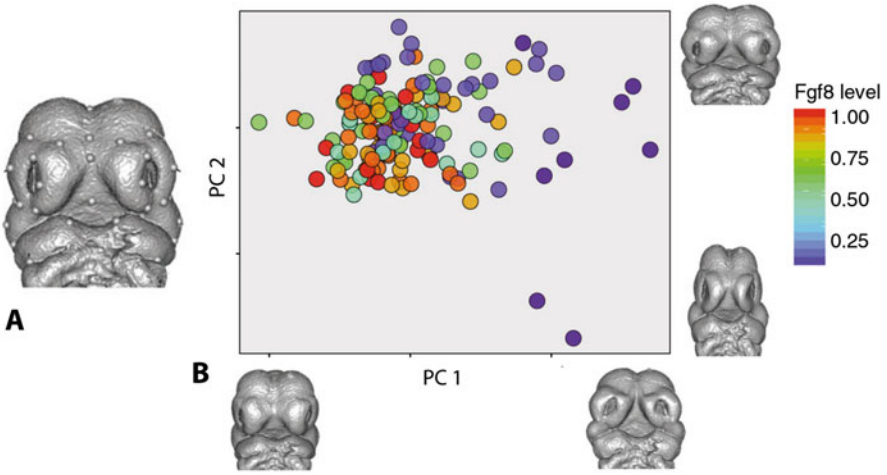


Fig. 3 Geometric morphometric analysis of mouse embryos (day 10.5) with different *Fgf8* expression patterns (Modified from Green et al. (2017), Creative Commons Attribution License). (**a**) On surface representations derived from microCT scans, 37 anatomical landmarks were placed manually on every specimen. (**b**) The first two principal components (PCs) of the Procrustes shape coordinates show that both mean shape and shape variation are affected by *Fgf8* expression. The shape patterns associated with PC1 and PC2 are illustrated by deformed embryo surfaces along the corresponding axes

and shape should be assessed jointly, the abovementioned methods can also be applied to the *form* of the configurations. This can be achieved by standardizing the landmark configurations only for position and orientation – not for size – or by augmenting the shape coordinates with the natural logarithm of centroid size as additional variable (Mitteroecker et al. 2004).

From Points to Surfaces and Volumes

Landmarks represent anatomical or geometric point homology (Type I and Type II landmarks sensu Bookstein 1991). With the advancement of 3D imaging technology, such as surface scanning, photogrammetry, and CT and microCT scanning, morphometric analyses of curves, surfaces, and even volumetric data have become more frequent. Two- and three-dimensional curves as well as surfaces that show biological homology within a sample can be included into geometric morphometric analysis by the use of so-called semilandmarks. These are landmarks placed on curves or surfaces, but their exact location along these curves or surfaces cannot be determined. Typical applications are points on smooth segments of 2D body outlines (such as in Figs. 1b and 4a) or continuous edges or smooth surfaces of 3D structures (as in Fig. 2). Semilandmarks are initially placed manually or semiautomatically in approximate positions; to remove shape differences resulting from their ambiguous

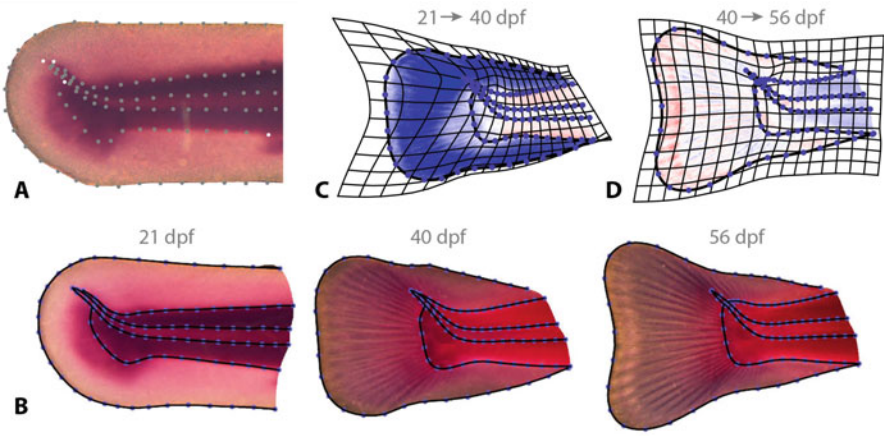


Fig. 4 Geometric morphometric image analysis of tail fin development in rainbow trout (Modified from Figs. 3 and 4 of Mayer et al. (2014), Creative Commons Attribution License). (**a**) Four anatomical landmarks (white points) and 95 semilandmarks (gray points) were digitized on each of 20 microscopic images of *Oncorhynchus mykiss*. The semilandmarks delineate the outlines of the fin fold, the musculature, and the notochord. (**b**) Average shapes and average color values (representing cell density) for 21, 40, and 56 days postfertilization (dpf). Note how the fin rays emerge at 40 dpf; they are not represented by landmarks but are still registered well by the image unwarping. (**c**, **d**) Visualization of average shape change together with average change in cell density between age groups. Shape changes are represented by TPS deformation grids and changes in cell density by color maps (increase in blue, decrease in red)

placement, their final locations are computed by the *sliding landmark algorithm* (Bookstein 1991; Gunz and Mitteroecker 2013): semilandmarks iteratively slide along their curve or surface (linearly approximated by tangents or tangent planes) until shape differences between every configuration and the sample mean shape are minimal. These shape differences are quantified either by the *bending energy*, a measure of local shape difference based on the thin-plate spline formalism, or by Procrustes distance (Fig. 1b).

Other approaches of surface analysis, commonly used in medical image analysis, are based on the iterative closest point algorithm (e.g., Pomidor et al. 2016). Thereby, Procrustes-based surface registration is iteratively combined with the projection of each individual's surface points on the surface of a reference specimen to achieve geometric correspondence. Further methods of outline or surface quantification include Fourier analysis and its three-dimensional extension, spherical harmonics (Lestrel 1982; Shen et al. 2009), as well as eigensurface analysis (Polly and McLeod 2008). These methods do not require the manual setting of anatomical landmarks and thus enable largely automated analyses. The price of this, however, is that point homology may not be represented well. While often still sufficient for simple classification analyses, averaging shapes with homologous tips or edges usually leads to a blurring or partial disappearance of these tips or edges.

Geometric morphometric analysis can also be combined with pixel- or voxel-based image analysis to quantify shape variation jointly with variation in two- or three-dimensional color patterns (*geometric morphometric image analysis*; Mayer et al. 2014). This approach starts with a detailed representation of point locations, curves, and surfaces by anatomical landmarks and semilandmarks. Based on these descriptors, the underlying two- or three-dimensional images are deformed to exactly the same shape for these structures (image unwarping based on TPS interpolation, a kind of non-affine image registration). In these shape-standardized images, the corresponding pixels or voxels are likely to represent comparable biological structures and can be statistically analyzed. Together with the shape coordinates, these standardized images can be averaged or regressed on external variables; they can also be subjected to principal component analysis or other ordination techniques. This combined approach allows for the morphometric analysis of well-defined body parts together with more diffuse patterns of cell density or morphogen gradients (see Fig. 4 for an example).

Further Applications in Evo-Devo

Many morphometric studies in evo-devo go beyond comparisons of mean shape across age groups or species; for instance, they combine morphometric analyses with computational modelling, genetic and epigenetic manipulation, or breeding experiments (e.g., Salazar-Ciudad and Jernvall 2010, Arif et al. 2013, Ramler et al. 2014, Powder et al. 2015, and Green et al. 2017). They also include more complex analyses of the spatiotemporal patterns of divergence of developmental trajectories across individuals, populations, or species. For instance, heterochrony – evolutionary changes in developmental timing – can be studied by searching for overlapping but differently timed ontogenetic trajectories in shape space (Mitteroecker et al. 2004).

Other classic morphometric applications, originating in the early twentieth-century approaches by Sewall Wright and Paul Terentjev as well as in Olson and Miller's 1958 book, are the study of morphological integration and modularity, which gained renewed interest in the 1980s and 1990s after their implementation into a quantitative genetic framework and then again in the 2000s through the application of geometric morphometrics. However, many geometric morphometric studies on modularity are difficult to interpret because of simplistic biological and statistical models (e.g., Mitteroecker et al. 2012, Armbruster et al. 2014, and Bookstein 2015).

Geometric morphometric approaches were also used to study the generation and canalization of phenotypic variation during development (e.g., Zelditch et al. 2004, Mitteroecker et al. 2012, Ramler et al. 2014, and Green et al. 2017) and to assess how developmental processes "funnel" the vast amount of genetic variation into comparatively few factors of morphological variation (Hallgrimsson and Lieberman 2008).

Current Challenges and Limitations

Landmark-based geometric morphometrics requires the same set of homologous landmarks to be present in all measured specimens, and the statistical methods commonly used proved most effective when variation in these landmarks is relatively small, such as typically occurring throughout fetal and postnatal development or between similarly aged individuals of related species (Bookstein 1991; Dryden and Mardia 1998). These limitations preclude the analysis of developmental and evolutionary emergence or loss of body structures, as this would entail different landmarks in different individuals. But geometric morphometrics has been successfully applied to similarly staged mouse embryos and embryonic structures such as limb buds (e.g., Hallgrimsson and Lieberman 2008, Martínez-Abadías et al. 2016, and Green et al. 2017) as well as to early stages of fish development (e.g., Mayer et al. 2014 and Powder et al. 2015). Landmark-free approaches were suggested to overcome this limitation; for instance, Rolfe et al. (2011) could successfully differentiate between different developmental stages and growth patterns of chicken embryos. However, the relaxation of biological homology in these approaches challenges the biological interpretation of mean and variance patterns as well as more complex geometries in shape space, such as (non)linearity of ontogenetic trajectories. In general, the wider the morphological diversity covered, the weaker the geometric structure in morphospace that can be meaningfully interpreted (Mitteroecker and Huttegger 2009).

Another methodological challenge is imposed by the spatial analysis of cell densities, morphogen gradients, and gene expression patterns, which are central to many contemporary evo-devo studies. Martínez-Abadías et al. (2016) used a combination of Procrustes-based geometric morphometrics and elliptical Fourier analysis to assess covariation between *Hox* gene expression pattern and limb bud development in mice. Mayer et al. (2014) combined geometric morphometrics with pixel-based image analysis to quantify spatial patterns of cell density in the developing tail fin of rainbow trouts (Fig. 4). While both approaches are promising, there is space for further methodological advancement.

Among the most pressing statistical challenges is the implementation of more realistic null models for landmark variation that incorporate the spatial distribution of measurements (Mitteroecker et al. 2012; Bookstein 2015). Such models will greatly facilitate the identification of morphological factors at various spatial scales as well as the computation of more meaningful shape distances and ordinations.

Cross-References

- ▶ Canalization: A Central but Controversial Concept in Evo-Devo
- ▶ Computational Modeling at the Cell and Tissue Level in Evo-Devo
- ▶ Developmental Homology
- ▶ Heterochrony
- ▶ Phenotyping in Evo-Devo

Acknowledgments PM is supported by the Austrian Science Fund (FWF P29397).

References

Alberch P, Gould SJ, Oster GF, Wake DB (1979) Size and shape in ontogeny and phylogeny. Paleobiology 5:296–317

Arif S, Hilbrant M, Hopfen C, Almudi I, Nunes MDS, Posnien N, Kuncheria L, Tanaka K, Mitteroecker P, Schlotterer C, McGregor AP (2013) Genetic and developmental analysis of differences in eye and face morphology between *Drosophila simulans* and *Drosophila mauritiana*. Evol Dev 15(4):257–267

Armbruster WS, Pélabon C, Bolstad GH, Hansen TF (2014) Integrated phenotypes: understanding trait covariation in plants and animals. Philos Trans R Soc Lond Ser B Biol Sci 369 (1649):20130245

Ashburner J, Friston KJ (2000) Voxel-based morphometry—the methods. NeuroImage 11:805–821

Bookstein F (1991) Morphometric tools for landmark data: geometry and biology. Cambridge University Press, Cambridge/New York

Bookstein FL (2015) Integration, disintegration, and self-similarity: characterizing the scales of shape variation in landmark data. Evol Biol 42(4):395–426

Coquerelle M, Bookstein FL, Braga J, Halazonetis DJ, Weber GW, Mitteroecker P (2011) Sexual dimorphism of the human mandible and its association with dental development. Am J Phys Anthropol 145(2):192–202

Dryden IL, Mardia K (1998) Statistical shape analysis. Wiley, New York

Green RM, Fish JL, Young NM, Smith FJ, Roberts B, Dolan K, Choi I, Leach CL, Gordon P, Cheverud JM, Roseman CC, Williams TJ, Marcucio RS, Hallgrímsson B (2017) Developmental nonlinearity drives phenotypic robustness. Nat Commun 8(1):1970

Gunz P, Mitteroecker P (2013) Semilandmarks: a method for quantifying curves and surfaces. Hystrix 24(1):103–109

Hallgrimsson B, Lieberman DE (2008) Mouse models and the evolutionary developmental biology of the skull. Integr Comp Biol 48:373–384

Klingenberg CP (2010) Evolution and development of shape: integrating quantitative approaches. Nat Rev Genet 11:623–635

Lele S, Richtsmeier JT (1991) Euclidean distance matrix analysis: a coordinate free approach for comparing biological shapes using landmark data. Am J Phys Anthropol 86:415–428

Lestrel P (1982) A fourier analytic procedure to describe complex morphological shape. In: Dixon A, Sarnat B (eds) Factors and mechanisms influencing bone growth. Alan R. Liss, Los Angeles, pp 393–409

MacLeod N (1999) Generalizing and extending the eigenshape method of shape visualization and analysis. Paleobiology 25(1):107–138

Martínez-Abadías N, Mateu R, Niksic M, Russo L, Sharpe J (2016) Geometric morphometrics on gene expression patterns within phenotypes: a case example on limb development. Syst Biol 65 (2):194–211

Mayer C, Metscher BD, Müller GB, Mitteroecker P (2014) Studying developmental variation with geometric morphometric image analysis (gmia). PLoS One 9(12):e115076

Mitteroecker P, Gunz P (2009) Advances in geometric morphometrics. Evol Biol 36:235–247

Mitteroecker P, Huttegger S (2009) The concept of morphospaces in evolutionary and developmental biology: mathematics and metaphors. Biol Theory 4(1):54–67

Mitteroecker P, Gunz P, Bernhard M, Schaefer K, Bookstein FL (2004) Comparison of cranial ontogenetic trajectories among great apes and humans. J Hum Evol 46:679–697

Mitteroecker P, Gunz P, Neubauer S, Muller G (2012) How to explore morphological integration in human evolution and development? Evol Biol 39(4):536–553

Polly P, McLeod N (2008) Locomotion in fossil carnivora: an application of eigensurface analysis for morphometric comparison of 3D surfaces. Palaeontol Electron 11(2):10A:13p

Pomidor BJ, Makedonska J, Slice DE (2016) A landmark-free method for three-dimensional shape analysis. PLoS One 11(3):e0150368

Powder KE, Milch K, Asselin G, Albertson RC (2015) Constraint and diversification of developmental trajectories in cichlid facial morphologies. EvoDevo 6:25

Ramler D, Mitteroecker P, Shama LNS, Wegner KM, Ahnelt H (2014) Nonlinear effects of temperature on body form and developmental canalization in the threespine stickleback. J Evol Biol 27(3):497–507

Rohlf FJ, Slice DE (1990) Extensions of the procrustes method for the optimal superim-position of landmarks. Syst Zool 39:40–59

Rolfe SM, Shapiro LG, Cox TC, Maga AM, Cox LL (2011) A landmark-free framework for the detection and description of shape differences in embryos. Conf Proc IEEE Eng Med Biol Soc 2011:5153–5156

Salazar-Ciudad I, Jernvall J (2010) A computational model of teeth and the developmental origins of morphological variation. Nature 464(7288):583–586

Shen L, Farid H, McPeek MA (2009) Modeling three-dimensional morphological structures using spherical harmonics. Evolution 63(4):1003–1016

Zelditch ML, Lundrigan BL, Garland TJ (2004) Developmental regulation of skull morphology. I. Ontogenetic dynamics of variance. Evol Dev 6(3):194–206

Phenotyping in Evo-Devo

Nico Posnien

Contents

Abstract

The description and comparison of morphological features has been an integral part of evolutionary developmental biology (evo-devo) from the early comparative embryology in the late nineteenth century to the revival of the discipline in the late twentieth century. The success of modern evo-devo research was fueled by an exciting accumulation of experimental data revealing central mechanisms underlying developmental processes in a few well-established model organisms. Recent advances in imaging and sequencing technologies allow for an in-depth genome-wide and highly quantitative comparison of developmental processes in a variety of organisms. The combination of this quantitative data with the establishment of theoretical and mathematical frameworks to integrate and analyze such data provides an excellent starting point to reveal the evolutionary, genetic, developmental, and ecological forces underlying the morphological diversification in Nature. In this chapter, I summarize key features of qualitative and quantitative phenotyping methods, highlighting advantages and potential limitations. Additionally, I argue that gene expression represents an intermediate

N. Posnien (✉)
Abteilung für Entwicklungsbiologie, Johann-Friedrich-Blumenbach-Institut für Zoologie und Anthropologie, Universität Göttingen, Georg August University, Göttingen, Germany
e-mail: nposnie@gwdg.de

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_121

phenotype that has the potential to link genotypic and epigenetic changes with the evolution of developmental processes and thus adult morphology.

Keywords

Evo-devo · Phenotyping · Morphology · Gene expression · Geometric morphometrics · Mathematical modelling

Introduction

It is one of the greatest challenges of biological research to reveal general concepts underlying the evolution of the breathtaking morphological variation in animals and plants. In the last decades, it has been established that the functional morphology of organisms or organs that interact with the environment and thus provide the basis for evolutionary adaptions are the result of changes during embryonic and post-embryonic development. Evolutionary developmental biology (evo-devo), therefore, provides an excellent conceptional framework to study the evolution of the size and shape of living organisms and their individual organs.

In most animals, key morphological structures upon which selection can act are restricted to specific stages of the life cycle. For instance, external copulation organs are important for species-specific reproduction and are present in sexually mature adults. Similarly, sexually selected exaggerated morphologies, such as stag beetle horns, the colorful feathers of peacocks, or the antlers of male deer, are only fully formed at adult stages. At different stages of the life cycle, different sensory organs may be present to perceive environmental information. The visual system in mammals, for instance, is only necessary and functional at the adult stage. In contrast, holometabolous insects have different requirements for the perception of visual cues; the eyes are usually small during larval stages, while the adults of flying insects can develop highly sophisticated and large compound eyes. Similarly, functional properties of the olfactory or chemosensory system vary clearly with the life style of insects at different developmental stages. Some structures that show a large morphological variety, such as insect extraembryonic membranes play only transiently important roles during embryonic development. In contrast to animals, plants usually grow throughout their life and they are exposed to environmental changes throughout that period.

Depending on the stage and morphological trait to study, different methods and tools may be necessary to capture and digitize them. For very small structures, high magnifications are necessary, while larger structures are easier to image and compare using conventional microscopy. For internal structures, one may need noninvasive methods such as computed tomography (CT) imaging. And for morphological traits that are only transiently present, it would be advantageous to have access to noninvasive life imaging methods in combination with fluorescent live dyes or transgenic organisms. Depending on the desired resolution of the trait under investigation, it may be necessary to reach tissue or single-cell resolution based on

histological sections or transmission electron microscopy (TEM). Once the trait of interest is captured, the most important decision is whether a qualitative assessment is sufficient for a proper comparison or whether quantitative differences should be evaluated. For the latter, plenty of mathematical and statistical methods, such as geometric morphometrics, to compare shape differences across species or populations have been established and improved in the recent years (see chapter ▶ "Morphometrics in Evolutionary Developmental Biology").

Another important readout of the genotype of a given organism is the spatial and temporal expression of developmental genes. Those gene products act in gene regulatory networks to direct developmental processes such as cell growth and proliferation, tissue patterning, and morphogenesis, and their expression needs to be tightly controlled at any time point. Key conceptional achievements of evo-devo research are based on the comparison of gene expression and gene sequences. The availability of molecular and genetic methods established in a few model organisms such as the fruit fly *Drosophila melanogaster*, the nematode *Caenorhabditis elegans*, or the mouse *Mus musculus* provided a substantial basis for the revival of evo-devo research in the late twentieth century.

Note that the development of an organism also influences, for instance, its behavior, life history, and physiology – three important phenotypes that also vary because of natural variation in developmental processes. Although the integration of these disciplines will inevitably contribute to exciting advances in the field, I will not discuss these aspects in this chapter.

In this chapter, I will first summarize major achievements obtained using thorough qualitative phenotyping. Afterwards, I will discuss advantages and applications of quantitative phenotyping methods. Gene expression as intermediate readout of the genotype-phenotype map will be briefly discussed, and the advantages of mathematical modelling in evo-devo will be highlighted. Eventually, I will provide some practical considerations aiming to foster the integration of a thorough experimental design.

Qualitative Phenotyping Is Prevalent in Macro-Evo-Devo Studies

Classical research questions in evo-devo dwell around the understanding of how different or similar body plans evolved. To understand the mechanisms underlying body plan changes, it is very often sufficient to study the entire organism by qualitative means. For instance, the groundbreaking identification of the highly conserved HOX cluster was possible by qualitatively comparing the impact of alterations in HOX gene expression on the body plan. The observation that some arthropod groups lack posterior body regions has been associated with the loss of posterior HOX genes in mites and tardigrades (Smith et al. 2016 and references therein). The homeotic nature of this gene family could, for instance, be observed by the qualitative comparison of different types of appendages that grow in different arthropod segments (Hughes and Kaufman 2002). In vertebrates, the regionalization of the vertebral column is also defined by the action of HOX genes. Recently, the

region-specific vertebral morphology has been quantitatively described and related to the activity of region-specific HOX protein activity in extant species. The identification of these region-specific unique features in extinct species from the fossil record allowed for the reconstruction of the evolution of regional patterning in fossils (Böhmer et al. 2015). Gene products with homeotic functions have also been shown to be involved in basic plant development. The MADS box genes are expressed in a combinatorial manner to define flower morphology. Loss of MADS box gene function has been implicated in different naturally occurring flower morphologies, which are easily identified by qualitative assessments (see chapter ▶ "Evolution of Floral Organ Identity").

Other traits that are easily accessible by qualitative measurements are gain and loss of structures. Prominent examples are pelvic skeleton structures in fresh and salt water stickleback fish that have been shown to be key adaptations to environmental changes, for example, the presence or absence of predators. The reduction or complete loss of eyes and body pigmentation in cave animals such as fish, crustaceans (Protas and Jeffery 2012) represents another qualitative trait variation. The loss of eyes in different fish lineages has been extensively studied and the underlying developmental mechanisms seem to be variable in different fish groups (Menuet et al. 2007; Stemmer et al. 2015). Body pigmentation is an adaptive trait that varies significantly in different animal groups. For instance, the evolution of the colorful wings of butterflies fascinated researchers for a long time. Research on the developmental basis of wing spot development and evolution has contributed to the formulation of central concepts in evo-devo (see chapters ▶ "Evo-Devo of Butterfly Wing Patterns" and ▶ "Evo-Devo of Butterfly Wing Patterns"). Among them is the observation that redeployment of certain developmental genes and subnetworks is a reoccurring method to increase functional complexity, while the number of developmental gene products encoded for most organisms is rather limited (i.e., toolkit). This has been shown for the involvement of the homeobox genes *Distall-less* (*Dll*) and *optix/six3* in wing spot formation. A similar observation was obtained for wing pigmentation spots in different *Drosophila* species that involve the redeployment of the *Dll* gene during pupal wing development to initiate the pigmentation cascade.

Besides adult traits, numerous morphological structures that are only transiently present have been studied applying qualitative methods. Prominent examples are extraembryonic membranes (i.e., the amnion and serosa) in insects. These membranes have been implicated in immune response (Jacobs et al. 2014), and they are relevant to support coordinated movement of embryonic tissue. Intriguingly, the size and morphology of these two membranes is highly variable among different insects ranging from clearly distinguishable amnion and serosa in beetles such as *Tribolium castaneum* to highly reduced amnioserosa in *Drosophila* and other higher Diptera (Panfilio 2008).

In summary, the qualitative analysis of morphological traits provides a powerful methodological framework to study the evolution of developmental programs in plants and animals and thus obtain valuable insights into phenotypic evolution. It is obvious, though, that qualitative measures are restricted to traits that are easily

accessible and differ clearly between studied species or taxa. Therefore, these approaches are mainly applied if macroevolutionary events are studied.

Quantitative Phenotyping Is Prevalent in Micro-Evo-Devo Studies

While morphological variation on a macroevolutionary scale is in many cases accessible by qualitative means, small-scale changes in morphology between closely related species (micro-evo-devo, sensu *lato*) or even across populations of the same species (micro-evo-devo, sensu stricto; Nunes et al. 2013) can only be identified unequivocally by applying quantitative methods.

The identification of morphological differences among genetically closely related (i.e., less diverged) organisms very often provides the opportunity to establish genotype-phenotype associations using quantitative genetics approaches. This combination already resulted in valuable insights into the developmental basis of variation in traits such as trichome number across various *Drosophila* species and populations (Stern and Frankel 2013). The accumulation of studies aiming at identifying causative genetic differences responsible for trichome number variation already allowed to draw general conclusions about the architecture of the developmental gene regulatory networks underlying trait formation. Most importantly, all identified loci converge in or are connected to the transcription factor Shaven baby (Svb) that seems to be a central hub in the underlying gene regulatory network. This finding was used to infer some level of predictability for genotype-phenotype associations (Stern and Orgogozo 2009). Recently, this view has been challenged by new data showing that trichome formation and evolution strongly depends on the developmental context and the architecture of the underlying gene regulatory network (Kittelmann et al. 2017), further highlighting the importance of a combination of developmental and quantitative genetics studies.

Similar general observations were obtained for pigmentation variation expressed as wing spots or body coloration. Intriguingly, quantitative mapping approaches repeatedly identified similar loci responsible for the observed variation in wing or body pigmentation in different *Drosophila* (Massey and Wittkopp 2016) and vertebrate (Hoekstra 2006) lineages. A more detailed analysis of the enzymatic pathway leading to the production of black pigment in *Drosophila* showed that the cascade is linear and rather simple and thus highly constrained. In the light of this knowledge, it is not surprising that only a limited number of evolutionary targets have been identified over the years and some level of predictability may be indeed possible. However, it remains to be established whether similar trends will be observed for different life stages or in different insect lineages.

Although the genetic architecture of traits like trichome number or pigmentation intensities may be highly complex with various loci interacting to shape the phenotypic outcome during development, the quantification of the morphological feature remains rather simple. But what about complex morphological traits such as leaf and flower shape in plants (Vuolo et al. 2016), eye size and shape (Posnien

et al. 2012), or exaggerated weapons in insects (Lavine et al. 2015), or skull shape in mice (Pallares et al. 2015)? To quantify morphological differences, much more sophisticated mathematical and statistical approaches are necessary. Especially the analysis of size and shape of complex organs has attracted much attention in the last 20 years since the advent of geometrics morphometrics (see chapter ▶ "Morphometrics in Evolutionary Developmental Biology"). More and more mathematical approaches are being established to quantitatively compare complex morphological features and most importantly to provide a solid statistical framework for these comparisons. Advanced noninvasive imaging technologies such as computed tomography (CT), even for small individuals, starts to allow for semi-high throughput imaging of complex morphological traits in three-dimensional space. Such imaging methods also provide new opportunities to capture high-resolution 3D information for fossils (Böhmer et al. 2015). Since the mathematical framework has been adopted to quantify and compare 3D image information, geometric morphometrics in combination with new imaging technology provides a fantastic opportunity to study complex trait evolution on a hitherto impossible detailed level.

Additionally, geometric morphometrics does not only provide the background to quantify differences in morphology, but it also allows visualizing variations in shape in even complex structures among groups. Another major advantage of geometric morphometric methodology is the fact that size and shape of a given organism or organ can be studied independently. This may allow to disentangle the developmental basis of shape differences related to overall growth regulation and those related to differential patterning processes or compartment-specific growth (see chapter ▶ "Morphometrics in Evolutionary Developmental Biology").

The combination of shape quantification with genetic information has a great potential to reveal the genetic architecture of divergence in complex traits. For instance, a thorough combination of genetic information and shape analyses allowed to establish a link between genetic variation, developmental plasticity, and sexual shape dimorphisms in wings (Testa and Dworkin 2016) in *Drosophila*. Similarly, the integration of quantitative mapping with three-dimensional shape analyses revealed candidate loci responsible for craniofacial shape variation in outbred mice (Pallares et al. 2015).

Once candidate loci are identified, a thorough functional characterization of the respective gene products allows to decipher the developmental basis underlying the evolution of shape differences. This latter aspect has been restricted to a few well-established model organisms such as mouse, *Drosophila,* or *Caenorhabditis elegans* until reverse genetics tools such as RNA interference (RNAi) and very recently the broadly functional genome editing method based on CRISPR/Cas9 started to change this picture. These days, functional manipulations to test and validate candidate genes are open to a much wider array of systems. The availability of these tools even allows, in the long run, to test the functional relevance of a given allelic variant in natural or seminatural conditions to include environmental and ecological information to assess the fitness-related aspects.

Gene Expression as Intermediate Phenotype

While purely morphological comparisons dominated the highly fruitful early years of comparative developmental biology, the resurrection of the discipline as evo-devo was triggered by advances in molecular biology. Active genomic loci are tissue- and time-specifically transcribed and translated into the functional proteins that control developmental processes. Therefore, the detection of gene expression throughout embryonic and postembryonic development provides an excellent readout of the genotype. For instance, transcription factors are fundamental DNA-binding proteins necessary to control the activity of many other genes, while structural proteins are integral parts of the cells of a given tissue. Hence, identifying the presence of a given gene product at a given time point in a given cell provides a powerful way to link the genotype and the resulting phenotype.

In the early days, molecular and genetics tools were primarily limited to a few prime model systems, such as *Drosophila melanogaster*, *Caenorhabditis elegans* and mouse. However, the establishment of cross-species whole mount in situ hybridization protocols in 1989 (Tautz and Pfeifle 1989) fueled the transfer of this method to plenty of emerging model systems. Since that time, gene expression has been extensively studied qualitatively by comparing expression patterns based on whole mount in situ hybridizations and in more rare cases on the protein level using immunohistological methods. Famous examples of the latter method were the use of cross-reacting antibodies against Engrailed (En) (Patel et al. 1989) and Distal-less (Dll) (Panganiban et al. 1997) in a variety of animals that resulted in the unexpected finding that the spatial distribution of the proteins was highly conserved in all studied species. This and many other studies led to the identification of a central finding in evo-devo research, namely the observation that the development of all living organisms is regulated by a limited set of genes, the so called developmental toolkit (Carroll 2008). Research in evo-devo dwells around this central dogma and tries to either use this toolkit to draw conclusions about organ, tissue, or even cell homologies or to understand, how the astonish morphological diversity present on earth evolved based on a limited set of toolkit genes. Hence, the qualitative assessment of spatial and temporal gene expression has been and still is a central instrument of evo-devo to develop new hypotheses. For instance, a comprehensive expression study in different stages of the life cycle in the sea anemone *Nematostella vectensis* allowed developing a new hypothesis about the evolution of the three germ layers present in Bilateria. Until recently, it was accepted textbook knowledge that the Mesendoderm and the Ectoderm of Cnidaria gave rise to the three bilaterian germ layers, ectoderm, mesoderm, and endoderm. The authors, however, found astonishing evidence for the presence of all three germ layers already in Cnidaria (Steinmetz et al. 2017).

The advent of next generation sequencing technologies and dropping sequencing costs in the last decade helped lifting gene expression studies to the genome wide level. These days, genomic resources can easily be generated, massively increasing the accessibility of more and more non-model systems for comparative molecular

studies. For instance, various large-scale consortia aim at providing genomic data for a broad taxon spectrum to support comparative genomics analyses and the reconstruction of phylogenetic relationships. A solid phylogenetic framework is crucial for evo-devo research to allow a proper reconstruction of developmental processes on a macroevolutionary scale (Telford et al. 2015). Additionally, the availability of genomic information will become highly valuable for various evo-devo questions; for instance, aiming at understanding the epigenetic underpinnings of developmental plasticity. Genome-wide expression comparisons are already broadly used to either answer specific questions or to facilitate the establishment of new hypotheses. For instance, the classical hourglass model based on comparative embryology observations has recently been revived and supported by gene expression studies in animals and plants (see chapter ▶ "The Developmental Hourglass in the Evolution of Embryogenesis"). Additionally, RNA sequencing helps reconstructing developmental gene regulatory networks on a more system wide level (Thompson et al. 2015). The generation of genome-wide expression profiles on the single-cell level will inevitably foster more in-depth analyses of the molecular fingerprint of various cell types (see chapter ▶ "Devo-Evo of Cell Types") and a highly detailed understanding of developmental processes (Karaiskos et al. 2017).

Besides advances in nucleotide sequencing, better protocols for fluorescent in situ hybridizations, even to the single-molecule level, and protein detection methods in combination with major innovations in microscopy technologies significantly improved the imaging resolution to quantitatively study temporal and spatial gene expression. This expression data can be used to feed mathematical models (Sharpe 2017) (see chapter ▶ "Modeling Evolution of Developmental Gene Regulatory Networks") to infer predictions that can be tested in the lab. This way, longstanding questions in evo-devo can be addressed with new methodology. It is, for instance, long known that segments are added sequentially in short germ insects and in other arthropods such as spiders and myriapods. This process is based on a gene expression oscillator mechanism reminiscent of that known in vertebrate somatogenesis (see chapter ▶ "The Evolution and Development of Segmented Body Plans"). Only a combination of thorough gene expression quantification with reverse modelling recently revealed that oscillatory segmentation gene expression does also exist in the long germ model system *Drosophila*, lending further support for the conservation of segmentation mechanisms in animals (Verd et al. 2018) (see chapter ▶ "Modeling Evolution of Developmental Gene Regulatory Networks").

While we can learn a lot from purely studying gene expression and function, it should be the ultimate goal to understand in detail how gene expression influences developmental processes. Mathematical modelling approaches integrating observed or predicted gene expression dynamics and cell and tissue behaviors have a great potential to sufficiently link the genomic architecture with developmental processes. Such models can subsequently also be used to implement an evolutionary scale to reconstruct the history of developmental changes on a mechanistic level. First results are already highly encouraging, for instance, the

understanding of the evolutionary forces that shape tooth morphology in seals (Salazar-Ciudad and Jernvall 2010).

Practical Considerations

While technological innovations seem to proceed with high speed, and new methods to assess organismal phenotypes pop up nearly every week, one should not forget about some central limitations in their application.

Since phenotypes are studied in living organisms, it is of major importance to obtain basic knowledge about the ecology and life cycle of the animal or plant in question. For instance, for some applications, it may be relevant to rear the organisms in laboratory conditions to have access to different life stages throughout the year, or organisms that have extremely long development times may be less well suited for in-depth phenotyping during development. While new genome editing technologies lower the obstacles of functional perturbations, one should not underestimate the time needed to transfer existing protocols from other species to a new one. Ironically, although the time needed to establish a new phenotyping method for a new model system is often a limiting factor, this information is rarely accounted for in the final publications summarizing the results.

Another focus of attention should be the amount of phenotypic data generated. Recent imaging and sequencing technologies result in massive amounts of data that needs to be stored, transferred, and handled. For most of these new applications, no easy-to-use analysis software is available since the analysis of such data requires special algorithms and analytical frameworks that are still under active development. One should thus be prepared to have access to high-performance computer clusters, and programming knowledge will become more and more relevant for biologists handling and especially integrating phenotyping data. Eventually, to be able to follow the rules of good scientific practice, it gets more and more relevant to share the large datasets in public databases and repositories to ensure on one side the reproducibility of the performed analyses and on the other side to allow the analysis of the data in another context or the integration in future meta-analyses.

As part of the experimental design and project planning, one should take special care about the actual phenotype to be studied. This applies specifically to the analysis of complex morphological traits. For instance, if one wants to understand the molecular and developmental basis underlying compound eye size variation in insects, it may be informative to measure and compare the area of the entire eye. However, since compound eyes are composed of functional subunits, called ommatidia, one should rather ask, whether different insects show differences in the number or the size of the individual ommatidia. This data may be much more informative for the establishment of a hypothesis about the underlying developmental differences. And what about the shape of the eye? Are different insect compound eyes always oval, or do different eyes show different proportions along different body axes? Answers to these questions may be helpful in revealing differences in patterning

processes during development. Hence, depending on the exact research question, the right phenotyping method should be applied.

In summary, we are living in exciting times with various new methodologies on the horizon to study the evolution of developmental processes in great detail. However, especially with the increase in the amount of data that can be generated, it is getting increasingly important to invest time in proper experimental design and extensive sharing of the data.

Cross-References

- ▶ Developmental Evolutionary Biology (Devo-Evo)
- ▶ Devo-Evo of Cell Types
- ▶ Evo-Devo of Butterfly Wing Patterns
- ▶ Evolution of Floral Organ Identity
- ▶ Macroevolution
- ▶ Modeling Evolution of Developmental Gene Regulatory Networks
- ▶ Morphometrics in Evolutionary Developmental Biology
- ▶ The Developmental Hourglass in the Evolution of Embryogenesis
- ▶ The Evolution and Development of Segmented Body Plans

Acknowledgments NP is supported by the Emmy Noether Programme of the German Research Foundation (G rant number: PO 1648/3-1). Many thanks to Elisa Buchberger and Micael Reis for critical comments on the manuscript. I would also like to thank Felix Quade for discussions on microscopy methods. And many thanks to all the great colleagues who contributed significantly to our currently knowledge in evo-devo but whose work I could not cite in this chapter due to space limitations.

References

Böhmer C, Rauhut OWM, Wörheide G (2015) Correlation between Hox code and vertebral morphology in archosaurs. Proc R Soc Lond B Biol Sci 282(1810):20150077. https://doi.org/10.1098/rspb.2015.0077

Carroll SB (2008) Evo-devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. Cell 134(1):25–36. https://doi.org/10.1016/j.cell.2008.06.030

Hoekstra HE (2006) Genetics, development and evolution of adaptive pigmentation in vertebrates. Heredity 97(3):222–234. https://doi.org/10.1038/sj.hdy.6800861

Hughes CL, Kaufman TC (2002) Hox genes and the evolution of the arthropod body plan1. Evol Dev 4(6):459–499. https://doi.org/10.1046/j.1525-142X.2002.02034.x

Jacobs CGC, Spaink HP, van der Zee M (2014) The extraembryonic serosa is a frontier epithelium providing the insect egg with a full-range innate immune response. eLife 3. https://doi.org/10.7554/eLife.04111

Karaiskos N, Wahle P, Alles J, Boltengagen A, Ayoub S, Kipar C, Kocks C, Rajewsky N, Zinzen RP (2017) The Drosophila embryo at single-cell transcriptome resolution. Science 3235 (August):eaan3235–eaan3235. https://doi.org/10.1126/science.aan3235

Kittelmann S, Buffry AD, Almudi I, Yoth M, Sabaris G, Franke FA, Couso JP, Nunes MDS, Frankel N, Gomez-Skarmeta JL, Pueyo-Marques J, Arif S, McGregor AP (2017) Gene

regulatory network architecture in different developmental contexts influences the genetic basis of morphological evolution. bioRxiv. https://doi.org/10.1101/219337

Lavine L, Gotoh H, Brent CS, Dworkin I, Emlen DJ (2015) Exaggerated trait growth in insects. Annu Rev Entomol 60(1):453–472. https://doi.org/10.1146/annurev-ento-010814-021045

Massey JH, Wittkopp PJ (2016) The genetic basis of pigmentation differences within and between Drosophila species. Curr Top Dev Biol 119:27–61. https://doi.org/10.1016/bs.ctdb.2016.03.004

Menuet A, Alunni A, Joly J-S, Jeffery WR, Rétaux S (2007) Expanded expression of Sonic Hedgehog in Astyanax cavefish: multiple consequences on forebrain development and evolution. Development 134(5):845–855. https://doi.org/10.1242/dev.02780

Nunes MDS, Arif S, Schlötterer C, McGregor AP (2013) A perspective on micro-evo-devo: progress and potential. Genetics 195(3):625–634. https://doi.org/10.1534/genetics.113.156463

Pallares LF, Carbonetto P, Gopalakrishnan S, Parker CC, Ackert-Bicknell CL, Palmer AA, Tautz D (2015) Mapping of craniofacial traits in outbred mice identifies major developmental genes involved in shape determination. PLoS Genet 11(11):e1005607. https://doi.org/10.1371/journal.pgen.1005607

Panfilio KA (2008) Extraembryonic development in insects and the acrobatics of blastokinesis. Dev Biol 313(2):471–491. https://doi.org/10.1016/j.ydbio.2007.11.004

Panganiban G, Irvine SM, Lowe C, Roehl H, Corley LS, Sherbon B, Grenier JK, Fallon JF, Kimble J, Walker M, Wray GA, Swalla BJ, Martindale MQ, Carroll SB (1997) The origin and evolution of animal appendages. Proc Natl Acad Sci U S A 94(10):5162–5166. https://doi.org/10.1073/pnas.94.10.5162

Patel NH, Martin-Blanco E, Coleman KG, Poole SJ, Ellis MC, Kornberg TB, Goodman CS (1989) Expression of engrailed proteins in arthropods, annelids, and chordates. Cell 58(5):955–968. https://doi.org/10.1016/0092-8674(89)90947-1

Posnien N, Hopfen C, Hilbrant M, Ramos-Womack M, Murat S, Schönauer A, Herbert SL, Nunes MDS, Arif S, Breuker CJ, Schlötterer C, Mitteroecker P, McGregor AP (2012) Evolution of eye morphology and rhodopsin expression in the *Drosophila melanogaster* species subgroup. PLoS ONE 7(5):e37346. https://doi.org/10.1371/journal.pone.0037346

Protas M, Jeffery WR (2012) Evolution and development in cave animals: from fish to crustaceans. Wiley Interdiscip Rev Dev Biol 1(6):823–845. https://doi.org/10.1002/wdev.61

Salazar-Ciudad I, Jernvall J (2010) A computational model of teeth and the developmental origins of morphological variation. Nature 464(7288):583–586. https://doi.org/10.1038/nature08838

Sharpe J (2017) Computer modeling in developmental biology: growing today, essential tomorrow. Development 144(23):4214–4225. https://doi.org/10.1242/dev.151274

Smith FW, Boothby TC, Giovannini I, Rebecchi L, Jockusch EL, Goldstein B (2016) The compact body plan of tardigrades evolved by the loss of a large body region. Curr Biol 26(2):224–229. https://doi.org/10.1016/j.cub.2015.11.059

Steinmetz PRH, Aman A, Kraus JEM, Technau U (2017) Gut-like ectodermal tissue in a sea anemone challenges germ layer homology. Nat Ecol Evol 1(10):1535–1542. https://doi.org/10.1038/s41559-017-0285-5

Stemmer M, Schuhmacher L-N, Foulkes NS, Bertolucci C, Wittbrodt J (2015) Cavefish eye loss in response to an early block in retinal differentiation progression. Development 142(4):743–752. https://doi.org/10.1242/dev.114629

Stern DL, Frankel N (2013) The structure and evolution of cis-regulatory regions: the shavenbaby story. Philos Trans R Soc Lond Ser B Biol Sci 368(1632):20130028. https://doi.org/10.1098/rstb.2013.0028

Stern DL, Orgogozo V (2009) Is genetic evolution predictable? Science (New York, NY) 323(5915):746–751. https://doi.org/10.1126/science.1158997

Tautz D, Pfeifle C (1989) A non-radioactive in situ hybridization method for the localization of specific RNAs in Drosophila embryos reveals translational control of the segmentation gene hunchback. Chromosoma 98(2):81–85. https://doi.org/10.1007/BF00291041

Telford MJ, Budd GE, Philippe H (2015) Phylogenomic insights into animal evolution. Curr Biol 25(19):R876–R887. https://doi.org/10.1016/j.cub.2015.07.060

Testa ND, Dworkin I (2016) The sex-limited effects of mutations in the EGFR and TGF-β signaling pathways on shape and size sexual dimorphism and allometry in the Drosophila wing. Dev Genes Evol 226(3):159–171. https://doi.org/10.1007/s00427-016-0534-7

Thompson D, Regev A, Roy S (2015) Comparative analysis of gene regulatory networks: from network reconstruction to evolution. Annu Rev Cell Dev Biol 31:399–428. https://doi.org/10.1146/annurev-cellbio-100913-012908

Verd B, Clark E, Wotton KR, Janssens H, Jiménez-Guri E, Crombach A, Jaeger J (2018) A damped oscillator imposes temporal order on posterior gap gene expression in Drosophila. PLoS Biol 16 (2):e2003174. https://doi.org/10.1371/journal.pbio.2003174

Vuolo F, Mentink RA, Hajheidari M, Bailey CD, Filatov DA, Tsiantis M (2016) Coupled enhancer and coding sequence evolution of a homeobox gene shaped leaf diversity. Genes Dev 30(21):2370–2375. https://doi.org/10.1101/gad.290684.116

Morphological Disparity

Melanie J. Hopkins and Sylvain Gerber

Contents

Abstract

Morphological disparity, the measure of morphological variation among species and higher taxa, has been at the core of an important research program in paleobiology over the last 25 years. Its quantification is based on the construction and exploration of morphospaces, multidimensional spaces spanned by a set of

Melanie J. Hopkins and Sylvain Gerber contributed equally to this work.

M. J. Hopkins (✉)
Division of Paleontology, American Museum of Natural History, New York, NY, USA
e-mail: mhopkins@amnh.org

S. Gerber
Institut de Systématique, Évolution, Biodiversité, UMR 7205 MNHN-CNRS-EPHE-UPMC-Sorbonne Universités, Muséum National d'Histoire Naturelle, Paris, France
e-mail: sylvain.gerber@mnhn.fr

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_132

morphological descriptors, and benefits from a well-established analytical protocol. Two main classes of indices are routinely used to describe the distribution of taxa in morphospace in terms of their spread and spacing. This unique focus on the morphological component of clade dynamics has promoted disparity as a distinct measure of biodiversity complementing traditional taxonomic proxies. Disparity studies have led to improved understanding of the evolutionary history of major clades and fostered new research on adaptive radiations, rates of evolution, and morphological innovation. Currently, active areas of methodological development focus on characterizing the geometric properties of morphospaces, devising indices that describe the structure of disparity, and incorporating phylogenetic information. There have also been increasing efforts to identify the determinants of disparity, from developmental to functional and ecological considerations, leading to conceptual extensions such as allometric disparity. The importance of trends, extinction, and chance as factors in the evolution of disparity remains relatively underexplored and needs more attention.

Keywords

Evo-devo · Morphospace · Morphometrics · Phenotypic evolution · Diversity · Macroevolution

Introduction

Many macroevolutionary patterns in deep time can and have been discussed in purely taxonomic terms. Significant episodes of radiation and extinction, for instance, can be detected from the documentation of changes in the number of species through time. However, even though our ability to distinguish species usually implies the existence of morphological differences between them, change in the number of species alone does not convey the magnitude of these morphological differences.

This is one of the reasons for the rejuvenation of morphological disparity analyses as an important research agenda in paleobiology and macroevolution since the 1990s. Morphological disparity is a macroevolutionary measure of morphological variation. Although it has been used historically in reference to the level of morphological variation observed among body plans, its current and most widespread use is as a description of the degree of morphological distinctness within a set of taxa at and above the species level (morphological diversity within species being intraspecific variation).

Estimates of morphological disparity have been employed primarily to document the evolutionary history of particular clades. Fossil data has been of primary importance for such studies. The fossil record contains examples of extinct morphologies that may not be inferable from modern morphological diversity and provides a direct record of temporal occurrence of different morphologies. Notable patterns include the tendency for disparity to peak early in the evolutionary history of

a clade (Hughes et al. 2013), and the frequent discordances between changes in taxonomic diversity and morphological disparity over the evolutionary history of a clade (Foote 1993a). Important reviews that also describe some of the impact that disparity studies have had on evolutionary biology, particularly in the area of adaptive radiations and rates of evolution, include Foote (1997), Wills (2001), Erwin (2007), and Wagner (2010). In this chapter, we will briefly review common methods for measuring disparity and then focus on the methodological and conceptual developments that occurred over the past decade.

Measuring Disparity

In empirical studies, the quantitative assessment of morphological disparity starts with the definition of an adequate set of morphological descriptors from which can be obtained a measure of dissimilarity between morphologies. In so doing, one establishes a morphospace, the multidimensional state space spanned by these morphological descriptors. The positioning of taxa relative to one another in the morphospace reflects their degree of morphological similarity: the closer, the more morphologically similar.

Morphological Descriptors

Different families of morphological descriptors exist:

1. Traditional morphometric descriptors. These are continuous data measured on a ratio scale. They may include length or perimeter measurements, angles between two linear features, estimates of area, or ratios between such measurements. These descriptors often require some sort of transformation to make them comparable in terms of scale or units.
2. Geometric morphometric descriptors. These are sets of two- or three-dimensional coordinate points whose configuration captures the geometry of the morphological feature of interest. Each point is associated with an intersection, junction or extreme (fixed landmarks), or with a curve (semi-landmarks and outlines).
3. Discrete character descriptors. These are categorical observations, such as the absence or presence of a trait, or qualitative descriptions of different states of expression of a trait that is present. They can have two or more states which may be ordered or not. In order to estimate disparity from discrete character data, the character-taxon matrix is converted to a pairwise distance matrix, or dissimilarity matrix (see Lloyd 2016 for review). Most recent disparity studies based on discrete character descriptors have co-opted character matrices that were originally constructed for phylogenetic purposes, and thus typically do not include autapomorphies. Whether this is a problem remains an open issue: there is currently no consensus on whether autapomorphies should be included in

character matrices intended for disparity analyses (e.g., Gould 1991; Ruta and Wills 2016).
4. Model-based descriptors. These are parameters that describe different shapes or morphological features under a specified model, such as one describing growth. A classic example is Raup's 1966 model for shell coiling, which was based on the geometry of the logarithmic spiral (Gerber 2016).

The use of traditional and geometric morphometric descriptors requires that all taxa express the traits being measured. Because discrete character data sets can also include information about the presence/absence of traits, a broader range of morphologies can be accommodated in the analysis compared to morphometric data. However, this is typically at the expense of a "cruder" description of morphologies. In general, different types of descriptors capture and emphasize different aspects of morphology, different levels of trait correlation and redundancy, and different scales of change. Disparity patterns may or may not be consistent across different types of descriptors even for the same set of sampled taxa; empirical studies to date are summarized in Hopkins (2017).

All of these descriptors are conducive to morphospaces as defined above. The term "morphospace" is thus a very broad designation that includes a great variety of mathematical spaces. Those can have quite distinct properties and geometries (e.g., Gerber 2016), and they may differ in their renditions of given evolutionary patterns. The investigator should therefore have an understanding of the properties of the morphospace employed in order to tease apart the biological signal from potential artifacts associated with a particular methodological approach.

Disparity Indices

Because morphological descriptors define a space, morphological disparity can also be defined as the quantitative characterization of the spread and spacing of taxa in this space, that is, the pattern of morphospace *occupation.* There are two components to morphospace occupation, the amount and structure of disparity, but most studies so far have exclusively focused on the former. The most commonly used disparity indices are based on the standard measures of statistical dispersion and describe the amount of disparity regardless of its structure.

1. Sum of (univariate) ranges, or total range. This metric represents the spread of the distribution in morphospace. The sum of ranges is sensitive to sample size and is thus frequently subjected to rarefaction analysis when comparisons are being made between groups or samples of different numbers of taxa. The sum of ranges is also dependent on orientation. This may have a nontrivial impact on comparison of subgroups ordinated in the same morphospace, since the major axis of variation of the entire group may differ from those of subgroups.
2. Sum of (univariate) variances, or total variance. Computed as the trace of the covariance matrix, or equivalently, the sum of its eigenvalues, it describes the

spacing of taxa in morphospace and is relatively insensitive to sample size. It is not redundant with the previous index. For a given total range, different values of total variance indicate a more or less densely occupied region of morphospace within stable boundaries. Subgroups may contribute differentially to the total variance; the contribution of the subgroup, or the partial disparity, is computed from the sum of the squared distances of each member of the subgroup to the overall centroid (Foote 1993b).

Wills (2001) described these and other indices in extensive detail, and Ciampaglio et al. (2001) ran a series of simulations to study their behavior for various types of morphospace patterns typically encountered in empirical case studies. However, many morphospaces may exhibit an affine rather than a Euclidean geometry. For such spaces, ratios of generalized variances (determinant of the covariance matrix, or equivalently the product of the eigenvalues) have been recommended as affine-invariant measures of disparity (Huttegger and Mitteroecker 2011). In the case of discrete character space, an alternative to total variance is the average pairwise dissimilarity. One benefit of this index is that it is estimated directly from the dissimilarity matrix and so does not require the use of ordination methods (see below).

Visualizing Morphospaces

Unless one is concerned with very simple description of morphologies, the high dimensionality of most morphospaces prevents the visualization of all their dimensions at once. Getting a visual assessment of the extent and structuring of variation thus requires the use of multivariate ordination methods such as principal component analysis, principal coordinates analysis, or nonmetric multidimensional scaling, which can extract the most relevant and salient features of the variation documented with fewer dimensions.

Importantly, such a representation is a *projection* of the morphospace and not the morphospace itself. This is often an informative and useful depiction but high-dimensional spaces cannot be displayed as bivariate or trivariate plots without loss of information, and thus these projections can be misleading. Fortunately, estimating disparity does not rely on such projections and can be assessed from the entire dataset (the true morphospace). The disparity indices mentioned above extend to any number of dimensions, and there is therefore no need to resort to dimension reduction techniques to measure disparity (e.g., keeping only the set of ordination components that describe 95% of the original variance), even when low-variance components contribute very little to the overall disparity.

It has been customary in the morphospace literature to distinguish empirical from theoretical morphospaces. The latter, constructed from model-based descriptors, are generally singled out as independent from the empirical sample of specimens studied and capable of producing nonexistent morphologies, thus revealing areas of morphospace that have not been occupied through evolution. These features,

however, do not pertain to theoretical morphospaces only but typify many of the so-called empirical morphospaces as well. The use of the empirical/theoretical distinction generally reflects a confusion between the morphospace and its ordination (e.g., projection on principal components). The addition of new taxa may alter the ordination but will not alter the relative distances between the previously measured taxa.

Current Issues in Methods

Recent methodological developments have focused primarily on improving the description of morphospace patterns and incorporating phylogenetic information in their exploration.

Fairly distinct patterns of morphospace occupation have been documented and common disparity indices can sometimes overlook their differences. For example, equally disparate clades can show drastically different structuring of the amount of morphological variation they display. Additional indices of morphospace occupation have been suggested to characterize these distinct patterns, particularly with respect to the dimensionality and the discontinuity of the distribution of taxa in morphospace (e.g., clustering, Wills et al. 2012). Efforts to describe morphospace structure have been complicated by two issues. First, tests for clustering lose statistical power as dimensionality increases. Second, clustering can occur for artifactual reasons, as well as biological reasons (see section "Explaining the Evolution of Disparity" below). For example, regions of morphospace that cannot be occupied may exist due to the use of character data which includes logically impossible character combinations.

Recent disparity analyses have tended to focus on clades for which phylogenetic hypotheses at the level of the OTUs described can be obtained. It is then possible to map the hypothesized tree onto the morphospace, producing what has come to be referred to as a phylomorphospace. Such a representation can help determine if phylogeny is a strong contributor of the structuring of morphological variation in a clade. Phylogenetic hypotheses have also been suggested as a means to account for the incomplete fossil record of clades. Models of character evolution such as parsimony can be used to assign character states to the internal nodes of the tree ("hypothetical ancestors"), fill in missing data, and correct for ghost-ranges. These approaches still need careful assessments of their statistical properties and heuristic values. They do alter the raw disparity signal in ways (e.g., asymmetric adjustment of stratigraphic range, superimposed model of character evolution) that might obscure the true nature of the processes underlying the clade dynamics.

Finally, there has also been considerable recent interest in inferring past clade disparity from the distribution of morphology among extant members of the clade, usually with the aid of a phylogenetic hypothesis of how clade members are related to one another evolutionarily. However, the robustness of such inferences has rarely been tested against the fossil record. One recent attempt found that the morphological disparity index was the most reliable for inferring an early burst of disparity in

birds, but still failed to recapitulate disparity patterns in the bird fossil record (Mitchell 2015). The inability to estimate extinct morphologies from extant taxa alone is the major obstacle facing this sort of approach, especially when the structure of morphospace occupation has changed, or when large or peripheral regions of morphospace are no longer occupied.

Explaining the Evolution of Disparity

Over the last 25 years, disparity curves have been constructed for many groups – although unevenly among vertebrate, invertebrate, and plant clades – following the methodological framework outlined above. Together with diversity curves, these disparity patterns have proved extremely useful in offering an expanded description of the evolutionary history of biodiversity by combining its taxonomic and morphological components. Comparative surveys and reviews of these studies have focused on the important task of documenting evolutionary histories patterns and assessing their relative frequencies. These include for instance the various modes of morphological diversification (with or without concordance with taxonomic diversification) and the selectivity versus randomness of extinction with respect to morphology.

The recognition of different patterns of disparity across different clades has also led to interest in determining the processes that underlie the evolution of disparity and the often heterogeneous patterning of morphospace. Historically, hypotheses about the determinants of morphospace occupation have fallen into three categories: extrinsic factors, biotic and abiotic; intrinsic factors related to development or growth; and chance. On the theoretical side, a few stochastic and analytical models have been implemented to explore the expected behavior of disparity in face of changing mode of morphological transitions, taxonomic turnover rates, and size of morphospace (e.g., Pie and Weitz 2005; Gerber et al. 2011 and references herein). These studies have highlighted the difficulties in distinguishing the drivers of disparity and isolating their signature from sampling error and stochastic variation. More work is needed in this area to define null models and characterize the expected signature of the various contributors of disparity.

In parallel, empirical efforts to understand the "mechanistic" bases of the dynamics of disparity have concentrated on functional and developmental aspects of morphological variation. These approaches have in common the (sometimes implicit) recognition of morphological phenotypes as being made up of quasi-independent units of evolutionary transformation, a phenomenon referred to as evolutionary modularity. Internally, these units are developmentally and functionally integrated and can be under distinct selective regimes and evolutionary constraints. Hence, while the disparity signal built from the entire set of morphological descriptors (characterizing the overall body) is relevant for documenting the evolutionary history of a particular clade (global pattern), this more comprehensive description of morphologies conflates the (quasi-independent) histories of its constitutive parts. Relevant subsets of the global library of descriptors can be used to build disparity signals attached to specific body parts corresponding to plausible modules, and may

in fact be more informative in identifying the underlying processes driving the evolution of disparity at the global level.

Accordingly, some authors have used character partitions to derive disparity curves reflecting differential functional and developmental contributions, such as external functional biology vs. internal anatomy, or ecological vs. nonecological characters (e.g., Ciampaglio 2002). More recent works have developed approaches directly targeting traits of specific functional and developmental relevance to phenotypic variation. We review them briefly below.

Developmental Morphospaces and Allometric Disparity

Morphological disparity analyses have traditionally focused on adult variation, but changes in adult phenotypes are mediated by development, and development therefore influences morphospace occupation and disparity dynamics (Gerber 2014). Fortunately, the morphological data retrievable from the fossil record are by no means restricted to the adult stage and disparity can be measured at any developmental stage. If the same set of descriptors is used to describe these different stages, then juvenile and adult morphologies can be displayed within the same morphospace. Earlier studies incorporating multiple developmental stages focused on changes in disparity through ontogeny (e.g., Zelditch et al. 2003; Eble 2003). More recent studies have focused on characterizing and analyzing the developmental trajectories themselves.

For example, Gerber et al. (2008) used coefficients describing allometric growth patterns in fossil ammonoids as descriptors for defining a multidimensional space in which each point represents an ontogenetic trajectory (allometric space). The distribution of points in this space, that is, the allometric disparity, can be quantified with the same indices as in standard disparity analysis. Allometric disparity can be compared to morphological disparity and their relative behaviors make it possible to distinguish different types of change in allometric trajectories, thus linking changes in adult disparity to specific modes of developmental evolution.

Functional Morphospace and Functional Disparity

Likewise, it is possible to focus on morphological descriptors that are tied to particular functions in the organisms under study. Commonly used characters relate to feeding ecology or biomechanics; there have also been developments in the use of models to derive functionally relevant descriptors (Anderson et al. 2011). Although functional properties of organisms are related to their morphology, functions that depend on multiple traits can potentially be met by many different morphologies (Wainwright 2007). As a result, the functional disparity and morphological disparity of a particular structure may only be weakly related in some systems. In general, the variation described by anatomical and functional datasets for the same taxa are likely to be correlated to some degree but not coincident (e.g., Anderson and Friedman 2012).

Spatial, Environmental, and Temporal Structure of Morphospace

Morphologies may be linked with environmental or ecological parameters in other ways when specific morphology-to-function associations are not known. For example, mapping group affiliations – where the group is defined by some ecological relatedness or habitat affinity – onto taxa in morphospace may reveal clustering associated with geographic occurrence or niche occupation, as well as the temporal changes in those associations (e.g., Hopkins 2014).

As should be obvious from any disparity curve (e.g., Foote 1993a), both disparity and morphospace structure are strongly associated with time as clades evolve into new areas of morphospace. Although morphological diversification is often conceptualized as a diffusive process of volume-filling, increases in disparity may be highly structured and uneven due to underlying trends (which are often due to developmental or functional constraints). Indeed, some trends may not increase disparity at all, if areas of morphospace are abandoned as the clade mean shifts (Hopkins 2016). Most of the literature on trends has been concerned with their documentation and categorization for univariate traits, such as body size. Categorical schemes are intended to be indicative of some underlying processes, such as species selection and constraints in the form of upper or lower bounds, but in general, the relationship between trends and the evolution of disparity is largely unexplored.

Extinction and Extinction Space

Areas of morphospace can only be abandoned through extinction. As such, extinction, particularly selective extinction, necessarily alters morphospace occupation and structure and therefore impacts disparity. For example, extinction selectivity has been associated with morphological specialization in some (but not all) clades. Korn et al. (2013) used indices which describe changes in morphospace occupation to define a multidimensional space for distinguishing between different selectivity modes during mass extinction events. One advantage of their approach was that it is not necessarily context-dependent. Because the indices summarize change in morphospace without recourse to the descriptors defining that morphospace, results based on disparate morphospaces can be compared to one another.

Chance and Historical Contingency

The importance of historical contingency in shaping patterns of disparity can be grasped most readily in the context of extinction, where the random culling of taxa can substantially alter the dynamics of disparity and put the evolutionary history of a clade on new tracks. Contingency pervades at all scales however, and extends beyond the case of random patterns of survivorship. The effect of particular contingent historical events on disparity is nevertheless difficult to apprehend, because they can be context-dependent and affect only one or a few lineages within a clade,

or be restricted to specific areas of the clade's geographic distribution. Their effects can still be significant at the global scale, however, and induce temporal shits in disparity. Contingent explanations can only emerge from detailed studies and "dissections" of disparity signals (e.g., geographic partitions and subclade components of global signals) and should not be confounded with general properties and common "laws" that might underlie all clades' histories. The difficult task of disentangling these classes of explanation invites a better characterization of the expected behavior of disparity, abstracted from the volatility of taxonomic rates, and the proposal of adequate models of diffusion in morphospace accounting for the properties of the mechanisms underlying evolutionary change in morphology.

Concluding Remarks

Since Gould's advocacy for the study of morphological disparity as an important macroevolutionary quantity (Gould 1991), paleontologists have successfully unearthed the history of many groups in terms of changes in diversity and changes in disparity. In so doing, they also have highlighted the frequent decoupling of these two facets of biodiversity. Over the years, methodological approaches to the study of disparity have been standardized in some ways, primarily through the maturation of morphometric techniques and the increasingly popular use of just a few informative indices of morphospace occupation.

These established analytical routines are powerful and will undoubtedly continue to be used to document disparity patterns for many clades. It is also clear, however, that many questions and issues, including some raised in the early years of the disparity research program, are still unresolved and/or lack appropriate conceptual and methodological frameworks for their analyses.

For example, recent research into the properties of different kinds of morphospaces has revealed situations in which classic measures of morphological distance (and thus disparity) might not be mathematically or evolutionary meaningful. Some features of morphospace occupation can also occur for both biological and artifactual reasons. All of these can affect measures of disparity and mislead our descriptions of evolutionary patterns.

Indices that measure the structure of disparity have been neglected compared to those that measure the amount of disparity, and we therefore know much less about the evolution of morphospace structuring (e.g., discreteness and dimensionality) as clades wax and wane, and from a technical viewpoint, the impact of sample size, taxonomic error, and morphospace dimensionality on such indices.

In terms of data, while cladistic matrices are increasingly used as discrete character spaces in disparity analyses and offer the possibility to combine morphospace and phylogenetic approaches, little is known with regard to the validity of their use as morphospaces (in particular with respect to their usually large amount of missing data, but see Lloyd 2016 for a start).

In parallel to continued empirical research and methodological development, there is a growing need for mathematical models of disparity and of diffusion in

morphospace. Explicit incorporation of stochastic effects is a major component in the study of trait evolution within lineages (e.g., "fossil time series") or across trees; in fact, in this area, there has been a recent shift away from the use of a stochastic model as a null hypothesis towards model selection approaches that use some criterion to select from among a set of models, of which one may represent stochasticity. Similarly, disparity studies may benefit from a shift towards statistical inference, with models that include potential determinants such as growth patterns, modular anatomical organization, functional constraints, selection, extinction, contingency, and chance, and away from post hoc explanations of descriptive patterns.

Cross-References

- ▶ Evolution of Complexity
- ▶ Macroevolution
- ▶ Methods and Practices in Paleo-Evo-Devo
- ▶ Morphometrics in Evolutionary Developmental Biology

References

Anderson PSL, Friedman M (2012) Using cladistic characters to predict functional variety: experiments using early gnathostomes. J Vertebr Paleontol 32:1254–1270

Anderson PSL, Bright JA, Gill PG, Palmer C, Rayfield EJ (2011) Models in palaeontological functional analysis. Biol Lett 8:119–122

Ciampaglio CN (2002) Determining the role that ecological and developmental constraints play in controlling disparity: examples from the crinoid and blastozoan fossil record. Evol Dev 4:170–188

Ciampaglio CN, Kemp M, McShea DW (2001) Detecting changes in morphospace occupation patterns in the fossil record: characterization and analysis of measures of disparity. Paleobiology 27(4):695–715

Eble GJ (2003) Developmental morphospaces and evolution. In: Crutchfield JP, Schuster P (eds) Evolutionary dynamics: exploring the interplay of selection, accident, neutrality, and function. Oxford University Press, Oxford, pp 33–63

Erwin DH (2007) Disparity: morphological pattern and developmental context. Palaeontology 50 (1):57–73

Foote M (1993a) Discordance and concordance between morphological and taxonomic diversity. Paleobiology 19(2):185–204

Foote M (1993b) Contributions of individual taxa to overall morphological disparity. Paleobiology 19(2):403–419

Foote M (1997) The evolution of morphological diversity. Annu Rev Ecol Evol Syst 28:129–152

Gerber S (2014) Not all roads can be taken: development induces anisotropic accessibility in morphospace. Evol Dev 16(6):373–381

Gerber S (2016) The geometry of morphospaces: lessons from the classic Raup shell coiling model. Biol Rev. https://doi.org/10.1111/brv.12276

Gerber S, Eble GJ, Neige P (2008) Allometric space and allometric disparity: a developmental perspective in the macroevolutionary analysis of morphological disparity. Evolution 62 (6):1450–1457

Gerber S, Eble GJ, Neige P (2011) Developmental aspects of morphological disparity dynamics: a simple analytical exploration. Paleobiology 37:237–251

Gould SJ (1991) The disparity of the Burgess Shale arthropod fauna and the limits of cladistic analysis: why we must strive to quantify morphospace. Paleobiology 17(4):411–423

Hopkins MJ (2014) The environmental structure of trilobite morphological disparity. Paleobiology 40(3):352–373

Hopkins MJ (2016) Magnitude versus direction of change and the contribution of macroevolutionary trends to morphological disparity. Biol J Linn Soc 118(1):116–130

Hopkins MJ (2017) How well does a part represent the whole? A comparison of cranidial shape evolution with exoskeletal character evolution in the trilobite family Pterocephaliidae. Palaeontology 60:309–318

Hughes M, Gerber S, Wills MA (2013) Clades reach highest morphological disparity early in their evolution. Proc Natl Acad Sci U S A 110(34):13875–13879

Huttegger SM, Mitteroecker P (2011) Invariance and meaningfulness in phenotypic spaces. Evol Biol 38:335–351

Korn D, Hopkins MJ, Walton SA (2013) Extinction space – a method for the quantification and classification of changes in morphospace across extinction boundaries. Evolution 67 (10):2795–2810

Lloyd GT (2016) Estimating morphological diversity and tempo with discrete character-taxon matrices: implementation, challenges, progress, and future directions. Biol J Linn Soc 118 (1):131–151

Mitchell JS (2015) Extant-only comparative methods fail to recover the disparity preserved in the bird fossil record. Evolution 69(9):2414–2424

Pie MR, Weitz JS (2005) A null model of morphospace occupation. Am Nat 166:E1–D13

Ruta M, Wills MA (2016) Comparable disparity in the appendicular skeleton across the fish-tetrapod transition, and the morphological gap between fish and the tetrapod postcrania. Palaeontology 59:249–267

Wagner PJ (2010) Paleontological perspectives on morphological evolution. In: Bell MA, Futuyma DJ, Eanes WF, Levinton JS (eds) Evolution since Darwin: the first 150 years. Sinauer Associates, Sunderland, pp 451–478

Wainwright PC (2007) Functional versus morphological diversity in macroevolution. Annu Rev Ecol Evol Syst 38:381–401

Wills MA (2001) Morphological disparity: a primer. In: Adrain JM, Edgecombe GD, Lieberman BS (eds) Fossils, phylogeny, and form: an analytical approach. Topics in geobiology. Kluwer Academic/Plenum Publishers, New York, pp 55–145

Wills MA, Gerber S, Ruta S, Hughes M (2012) The disparity of priapulid, archaeopriapulid and palaeoscolecid worms in the light of new data. J Evol Biol 25:2056–2076

Zelditch ML, Sheets HD, Fink WL (2003) The ontogenetic dynamics of shape disparity. Paleobiology 29(1):139–156

Anatomical Network Analysis in Evo-Devo

Borja Esteve-Altava and Diego Rasskin-Gutman

Contents

B. Esteve-Altava (✉)
Institut de Biologia Evolutiva (UPF-CSIC), Universitat Pompeu Fabra, Barcelona, Spain
e-mail: boresal@gmail.com

D. Rasskin-Gutman
Theoretical Biology Research Group, Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Valencia, Spain
e-mail: diego.rasskin@uv.es

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_57

Abstract

This chapter introduces the reader to anatomical network analysis (AnNA): a conceptual framework for the tolopological analysis of organismal form. AnNA focuses on the structural relations among anatomical parts, which allows for an evaluation of morphological organization in comparative analyses for both development and evolution. The nodes of the network represent anatomical elements, and the links that connect them represent structural relations or interactions among these elements. Network theory provides the methods to analyze these anatomical network models. The first and second sections present the historical and conceptual background of this framework. The third section explains the construction of anatomical networks and some of the basic parameters we can use to characterize the topology of these models and infer their morphological organization. The fourth section summarizes the interpretation of network parameters in terms of morphological complexity, hierarchy, integration, and modularity in the context of morphological evo-devo. The fifth section introduces the classical construction rules to build null models for networks and an example of the use of network null models in morphology. Finally, in the sixth section, we have explored some of the limits of AnNA.

Keywords

Morphology · Network theory · Quantitative methods · Topology

Introduction

The quantification of form is essential for the study of evolution (variation and diversification) and development (growth and patterning). Form is the result of the many processes acting at different levels of organization during development, from genetic control of cellular products and processes to developmental constraints on anatomy. Form is also a firsthand resource for evolutionary inquiry, since it is usually the only information preserved from extinct animals in the fossil record. The concept of organismal form covers the size and shape of the body and the structural arrangement and relations of body parts.

Anatomical network analysis (AnNA) is a conceptual framework that provides a set of tools for the analysis of the structural arrangement and relations among body parts in a morphological system. It relies on an old anatomical adagio, the *principe des connexions,* which identifies physical connections among anatomical elements (i.e., bones, muscles, cartilages) as carriers of important biological information, often more so than their size and shape. Indeed, this assumption is at the foundation of comparative anatomy itself; it was championed by the great French anatomist Étienne Geoffroy Sait-Hilaire in the nineteenth-century and it has been the focus of attention for comparative anatomy ever since. Geoffroy recognized that the shape and size of the same anatomical elements in different organisms vary greatly; so much that, to correctly identify them, it was more useful to analyze how they were

connected to their anatomical surroundings. The study of connections, although intuitively sound, lacked until very recently a suitable methodological framework to codify, manipulate, and analyze the patterns underlying these physical relationships in a meaningful way. Theoretical biologists, such as Joseph Woodger, Nicolas Rashevsky, and Rupert Riedl, proposed different ways to quantify and use the connections among anatomical parts to study the form of organisms but without a proper suite of methods. More recently, Rasskin-Gutman and Buscalioni (2001) proposed the use of graph theory to analyze anatomical connections among the skeletal elements forming the archosaurs pelvis (ischium, ilium, and pubis), setting the foundations for AnNA. In the past decade, AnNA has been developed further through its use in studies on the evolution and development (including pathology) of forms. From a methodological perspective, AnNA is based on mathematical tools from graph theory and network analysis that are available through many software packages (for a review on AnNA, see Rasskin-Gutman 2003; Rasskin-Gutman and Esteve-Altava 2014). This chapter presents the conceptual foundation of this latter approach and the methods adopted to use network analysis to study the evolution and development of form.

Connections in the Context of Organismal Form

The first step in a study of form using AnNA is to understand the biological meaning of the connectivity patterns one is about to analyze. The definition of what is a connection will determine the meaning of the results and the usefulness of the conclusions to be drawn from the study. But, why focusing on connections? Besides its classical appeal mentioned above, it is worth noting that any anatomical system can be teased apart on different levels of morphological organization. For example, Diego Rasskin-Gutman and Angela Buscalioni (2001) proposed a division into four related but semi-independent levels: (1) proportions, (2) connections, (3) orientations, and (4) articulations (Rasskin-Gutman and Buscalioni 2001; Rasskin-Gutman 2003). (**A/N**: in their system, the level of articulations refers to the range of motion among body elements.) Other levels could be think of, for example, that of relative positioning of parts (Woodger 1945). Each level gives insights into level-specific constraints and mechanisms that generate morphological variation and affect its evolution; hence, each level needs its level-specific type of formalisms and methods.

Of these four levels of morphological organization, the most studied one is that related to size and shape (proportions, level 1); it can be analyzed by using traditional morphometric tools with size and shape measurements or landmark-based geometric morphometrics with Cartesian coordinates (see chapter ▶ "Morphometrics in Evolutionary Developmental Biology"). AnNA is useful to analyze the structure and topology of forms (connections, level 2), where the formalism is a codification of the physical connections among elements; this codification results on an adjacency matrix, in its simplest form, filled by 1s (representing connections) or 0s representing absence of connections; cells in an adjacency matrix can also take continuous values representing relative degrees or areas of connection among elements (see section "The Network Model and Its Analysis"). The other two levels of morphological

organization capture the relative positioning of parts (orientations, level 3), which can be formalized a set of angles, and the mobility of joints (articulations, level 4), which can be formalized as tables of kinetic data.

The level of connections describes the topological relations among anatomical parts, that is, their arrangement in a morphological system; in addition, topological relations often capture the presence of functional and developmental relationships (codependences) among parts (Esteve-Altava 2017). For example, connections among skull bones not only represent the topological boundaries among bones but also primary sites of bone growth and remodeling, as well as sites of stress diffusion. Function and development are also important factors acting on the origin and variation of all other levels of morphological organization, making them codependent to some extent. For example, some studies posed that the covariation of landmark positions (commonly used in geometric morphometrics to analyze shape changes) are not independent as is sometimes assumed (Chernoff and Magwene 1999); instead, landmark covariation is constrained by the connections of the parts on which they are located. At the same time, changes in shape also affect the topological arrangement of parts, which might vary during evolution. The extent to which each level of morphological organization affects each other remains an open question (Esteve-Altava 2017).

Anatomical Network Analysis

Network models are sets of interacting elements. The analysis of network models requires a specific set of tools: concepts, descriptions, and algorithms; network theory is the branch of mathematics that supplies them. In this section, we first explain the creation of anatomical network models. Then, we introduce the most popular element and network parameters that we can quantify in anatomical networks, as well as the most popular organizational features and null models used to describe the structure and properties of anatomical networks. The lists of parameters, features, and null models are thus not exhaustive but limited to those that have been used before in the context of anatomical network analysis. Specialized literature, such as reviews by Albert and Barabási (2002), Dorogovtsev and Mendes (2003), Newman et al. (2006), and Mark Newman's book "*Networks: An Introduction*" (2010), offer comprehensive mathematical descriptions of the parameters introduced here and of others used in the analysis of various complex networks. In section "Anatomical Network Analysis in Morphological Evo-Devo," we provide the morphological interpretation of the statistics herein introduced within the framework of evo-devo.

Building an Anatomical Network: Elements and Interactions

Identifying the elements and interactions of an anatomical system is the first and most important step of the modeling process. Both, elements and interactions, must have a precise definition to identify them across all specimens of the study. This task

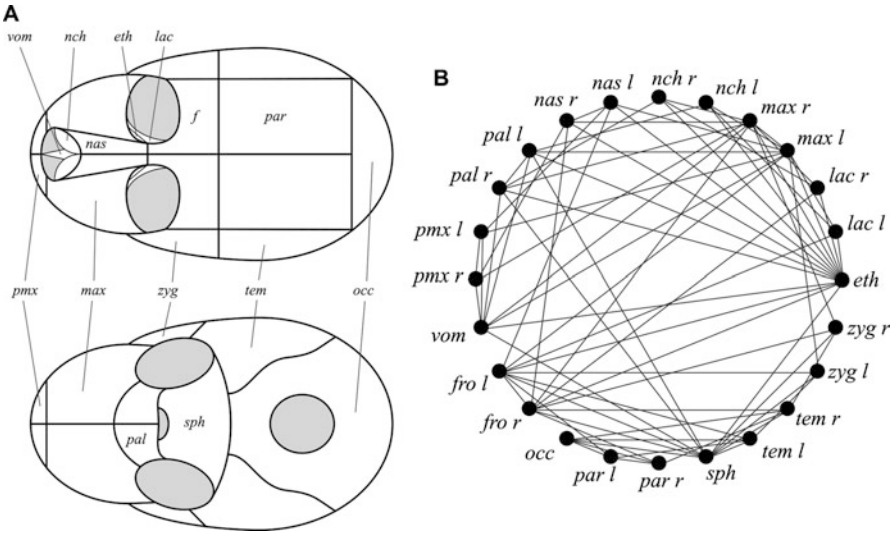


Fig. 1 Schema of a primate skull (**a**) and its corresponding network model (**b**). Labels *eth* ethmoid, *fro* frontal, *lac* lacrimal, *max* maxilla, *nas* nasal, *nch* nasal concha, *occ* occipital, *pal* palatine, *par* parietal, *pmx* premaxilla, *sph* sphenoid, *tem* temporal, *vom* vomer, *zyg* zygomatic, *l* left, *r* right. (Modified from Esteve-Altava et al. 2015a)

implies making some compromises between the generalization required in comparative studies and the accuracy required to uncover meaningful information.

Let's consider the skeletal parts of the vertebrate's head as an anatomical system to illustrate the process of defining elements and interactions to create an anatomical network (Fig. 1). At first glance, the natural elements of the skull are its bones: a rigid body of ossified tissue. Even such a straightforward definition like this makes it difficult to identify these elements in some cases. For example, identifying bones is tricky in an ontogenetic sequence, because some adult bones are formed by many ossification centers fused sequentially, which makes it difficult to trace back their origin. A different approach would be to use ossification centers instead of bones as elements of the network. However, this would prevent a broad comparison including both living and extinct forms, because ossification centers are barely observable in fossils.

Likewise, the physical junction between two bones is the most obvious interaction between them. However, various types of joints are possible between the bones of the head, namely: cartilaginous, dental, fibrous, and synovial joints. Because different types of joints have different functions and properties, the use of a specific type constrains the range of relations modeled and interpreted. For example, craniofacial sutures (fibrous joints) act as primary sites of bone growth, while the temporomandibular joint (a synovial joint) is responsible for jaw movements too. A network model that only uses sutures as interactions captures a developmental relation among bones (i.e., growth), while a model that also uses synovial joints

captures, in addition, biomechanical relations. Furthermore, other types of junctions, such as cartilaginous joints, introduce problems in the identification of their presence, for example, in fossils. It is worth noting that anatomical networks are not restricted to the modeling of the skeletal system; for example, muscles can be modeled as nodes of a muscular network and/or as links connecting skeletal parts (Esteve-Altava et al. 2015b; Dos Santos et al. 2017).

The example above shows how the same anatomical system can be modeled in different ways, according to different definitions of its elements and interactions, each option generating a different network model. Note that any additional information other than the modeled interactions, such as physical position in the space, orientation, or shape of the elements, is not included in the anatomical network model. Such information could be added, if needed, as additional descriptors that define types of nodes and links based on biological features. Finally, we can use AnNA as part of a comparison between topological features and other biological features of the anatomical system, for example, to test the relationship between topology and shape (Esteve-Altava 2017).

The Network Model and Its Analysis

A *network model* is the combination of two sets: a set of nodes and a set of links; each link has two endpoints, that is, it represents a connection between two nodes. In this mathematical abstraction, the nodes stand for anatomical elements and the links stand for interactions among elements (in the example above, nodes are bones and links are physical joints). The most common representation of a network is a drawing of dots joined by lines: a line connecting two nodes indicates the presence of a mutual relation. Directed links indicate nonreciprocal relations, while weighted links indicate the strength of the interactions. Notice that all network representations are equivalent if the same links between nodes are kept. For simplicity, here we only provide mathematical descriptions of the algorithms used to analyze undirected (reciprocal) and unweighted networks; derived algorithms exist for the specific analysis of directed and/or weighted networks (for details on such derived algorithms, see the recommended literature at the beginning of section "Anatomical Network Analysis").

The *adjacency matrix* ($A_{i,j}$) codifies the connections among the nodes of the network, that is, the number and the particular distribution of links between nodes. For undirected, unweighted networks, this is a symmetric binary matrix of size $N \times N$, where 1 indicates presence and 0 indicates absence of connection. Thus, the adjacency matrix defines the neighborhood, the connectivity context of each node as all the nodes to which it connects. An adjacency matrix is the main source of data in many programs used to analyze networks, but it is not the only one. For example, a list of edges (i.e., links) is also a very common source: a list in which each row indicates the origin and the destination of a link.

Network Parameters

Some important descriptors and parameters for individual nodes and the whole network are listed below. While node descriptors are very useful to study the properties of individual elements in relation to others, network descriptors are useful to compare one network to another.

Node degree: Sum of links a specific node has to other nodes in the network,

$$k_i = \sum_{i=1}^{j=n} A_{i,j}$$

Clustering coefficient: Ratio between the actual number of links among the neighbors of node i and the maximum number of links possible among them (i.e., $k_i(k_i-1)/2$),

$$C_i = \frac{2e_i}{k_i(k_i-1)}$$

where e_i is the number of links that exists connecting the neighbors of node i.

Shortest path length between two nodes: Their shortest distance measured as number of links to go from one node to the other,

$$l_{i,j} = d(n_i, n_j)$$

where $d(n_i,n_j)$ is the minimum distance in number of links to connect nodes i and j. Note that more than one path might have the shortest length.

Density: Total number of existing links, K, divided by the maximum number of possible links for a given number of nodes, N,

$$D = \frac{2K}{N(N-1)}$$

Average clustering coefficient: Arithmetic mean of the clustering coefficient of all nodes in the network,

$$C = \frac{1}{N}\sum C_i$$

Average shortest path length: Arithmetic mean of the shortest path length between all pairs of nodes,

$$L = \frac{1}{N-1}\sum l_{i,j}$$

Degree distribution: Frequency of occurrence of nodes with a given number of links,

$$P(k) = \frac{N_k}{N}$$

Clustering coefficient distribution: Clustering coefficient mean of all nodes with k links,

$$C(k) = \frac{\sum C_{i,k}}{N}$$

Types of Network Organization

In addition, the organization of the network can be informative about its properties for a given function. For example, networks are often seen as scale-free, hierarchical, and/or small-world, depending on the values of some of the parameters we just listed above compared to a null model (often a random network, see below). The presence of a community structure or modules inside the network is also very important in AnNA.

Hierarchical networks take their name from a very specific idea about hierarchy: nodes are organized as clusters within clusters – thus, C is high in these types of networks – , which promotes the formation of modules as clusters of highly interconnected nodes. Hierarchical networks are also scale free, which means that its structure is preserved at any scale of observation; in addition, these networks always host highly connected nodes or hubs. A network with a hierarchical organization shows a stratification of connections in various nested layers. Both the $P(k)$ and the $C(k)$ help assessing the presence of a hierarchical organization in a network. The functional form of these distributions (e.g., uniform, Poisson, or power law) characterizes the organization of connections among the nodes. Hierarchical networks have characteristic $P(k)$ and $C(k)$ functions that differ from those of random and scale-free networks. In random networks, $P(k)$ fits a Poisson function; in scale-free networks, it fits a power law function; in both, $C(k)$ fits a discrete uniform function. In contrast, hierarchical networks have a $P(k)$ and a $C(k)$ that fit power law functions. In general, a power law distribution in both parameters indicates that the neighborhoods of low-degree nodes are highly clustered, forming blocks, while those of high-degree nodes are sparsely connected, which suggest that high-degree nodes are acting as connectors between blocks. A hierarchical organization is commonly observed in anatomical networks with a community structure.

Small world networks have a special kind of organization between regularity and randomness; their low shortest path length (L) gives them special dynamic relationships among nodes, and their high clustering coefficient (C) provides them with distinctive structural features. Having a low L means that the communication of any kind of properties among nodes (e.g., stress forces among bones) is more efficient, thanks to shortcut links; having a high C means that there are many clusters or associations between nodes, which can be putative modules. A network with a small-world organization has a higher C and a lower or similar L to that of a random network, because of the presence of short-cut nodes. These nodes connect other nodes that would otherwise be far apart (i.e., high shortest path length). The presence

of a small-world is assessed by computing the values of C and L and then comparing them to those of random equivalent networks (i.e., networks with the same number of nodes and links but randomly rewired). A small-world organization is common in anatomical networks and is related to the identification of a community structure.

Anatomical Network Analysis in Morphological Evo-Devo

This section summarizes the current interpretation of the network parameters previously described in section "Anatomical Network Analysis" as features of the morphological organization of the body. At a connectivity level, morphological organization is the result of the overall interactions among anatomical parts. Finding the morphogenetic processes, from genetic regulatory networks to developmental constraints, that produce morphological organization is at the core of evo-devo research. To this end, we need first to describe quantitatively the morphology of organisms. Network parameters serve as proxies for features often related to the morphological organization of the body, such as complexity, hierarchy, integration, and modularity. The morphological interpretations of network parameters offered herein represent a work in progress (Rasskin-Gutman and Esteve-Altava 2014), open to discussion.

Morphological Complexity

Complexity can be defined in many ways: a state of order, unpredictability within a structured disorder, functional multitasking, structural stability, or amount of information for a minimal description to name a few (see chapter ▶ "Evolution of Complexity"). Morphological complexity has been usually defined as the number of different anatomical elements (McShea 1991). However, complex systems not only have many parts, they also achieve more structural and functional interactions. In this context, morphological complexity can be quantified explicitly by analyzing the connectivity patterns among anatomical elements using network analysis. This definition of morphological complexity, as the elements of an anatomical system and their interactions, resembles that of Herbert Simon (1962): *a large number of parts that interact in a non-simple way.*

The complexity, or simplicity, of the pattern of interactions of an anatomical network can be quantified by complementary network parameters, being the most straightforward the density of connections. The network density is the number of existing connections in the network, expressed as a fraction of the total possible connections between elements. In a network with density equal to 1, all elements are connected, that is, they are all interacting with each other. In nonanatomical systems, it is expected that more relationships would allow the system to perform more functions and show more complex behaviors. Complete connectivity is not present in anatomical structures; however, for a range of intermediate values of density, we would also expect greater values of density to correlate with more complex

behaviors. For example, a limb with more muscular connections could achieve a greater range of motion and complex behaviors (e.g., walking, climbing, manufacturing). Here, a greater number of connections would confer a greater potentiality of action; however, this does not entail that all interactions among anatomical parts are used at the same time. Analogously, the number of links of a given anatomical element can be a measure of its individual complexity, as related to the number of interactions it has, which is discussed in the literature using Rupert Riedl concept of burden (Riedl 1978; see chapter ▶ "Concept of Burden in Evo-Devo").

Hierarchical Organization

Morphological systems are hierarchical in two ways (Mayr 1982). The first way is by aggregation, that is, each anatomical part is composed of tissues, cells, and so on downwards in the hierarchy while parts make up organs, bodies, and so on upwards in the hierarchy (see chapter ▶ "Levels of Organization in Evo-Devo"). The second way is by constitution, that is, anatomical parts interact with each other as blocks within blocks, promoting hierarchical patterns of integration. A hierarchical integration of the body promotes body parts to change in form, growth, and function in a coordinate manner.

Network analysis can readily recognize constitutional hierarchy by analyzing patterns of connectivity among anatomical elements. We have already seen that, in the context of network sciences, the degree and clustering coefficient distributions help us identifying hierarchical networks as nested clusters (i.e., blocks) of anatomical elements (see section "Anatomical Network Analysis" on *hierarchical networks*).

Morphological Integration

Morphological integration means association between morphological traits, which is generally defined as the covariation among morphological traits due to common developmental and/or functional causes (Olson and Miller 1958). Depending on the definition of trait and unit of variation, the interpretation of integration varies in the context of genetics, development, and evolution, but also in morphology, depending on whether the focus is on proportions (shape and size) or connections (structure). On the one hand, when focusing on proportions, morphological integration is related to the study of correlations between body parts, that is, how much two traits (e.g., distances, landmark coordinates) change together. On the other hand, when focusing on connections, morphological integration refers to the interconnection of anatomical elements, that is, how many interactions tight them together; the statement is about the parts within the individual. For example, we would expect that two connected elements are more integrated than two disconnected elements, because a connection sets a developmental and functional dependency between them.

In a network structure, integration depends not only on direct contacts but also on indirect interactions. Two network parameters directly related to indirect interactions are the clustering coefficient and the shortest path length. Both parameters are related to information flow and correlation in networks: the clustering coefficient captures short-range information correlation due to redundancy among neighbor elements, while the shortest path length determines the speed of information transmission to distant elements depending on their effective proximity.

Morphological Modularity

Morphological systems are modular when they have differences in the degree of integration between parts, that is, the system has a heterogeneous integration of parts across different regions. Modularity, like integration, is a multilayered property that arises at different levels of organization: developmental, genetic, functional, and evolutionary, which converge in observable morphological modules. Traditionally, morphological modules are inferred from covariation among morphological traits, usually sets of distances or landmarks that tend to change together; thus, they are also called variational modules.

In network theory, a module is defined as a group of elements with more connections between them than to other elements outside the group (Fortunato 2010). This definition of module is also valid for any other biological system because of its generality. For instance, an anatomical module is a region of a body part with anatomical elements interacting more within the boundaries of the region than with other anatomical elements outside the region. Connectivity modules differ from the most common use today of variational modules (as related to shape covariation) in that they are inferred from the topological arrangement of anatomical units (Esteve-Altava 2017). Thus, the morphological information for variational and connectivity modules comes then from completely different sources. If, for example, the interactions modeled as links are developmental and/or functional, then connectivity modules have also a developmental and functional foundation.

Null Models in Anatomical Networks

A null model in network sciences is an idealized process-based model that generates a specific connectivity pattern of a network. A null model specifies the way nodes are added to the network and are connected by links. When this pattern is compared with that of an empirical network, it provides a comparative baseline to infer plausible mechanisms of network formation (i.e., construction rules). In an evo-devo context, null models are used, for example, to provide hypotheses about developmental mechanisms and to build generative morphospaces (as in Esteve-Altava and Rasskin-Gutman 2014b). The following are the null models that have been used

before to study the evolution and development of anatomical networks; this list is by no means exhaustive.

The Regular Model

A regular network is built so that all nodes have the same number of links. The properties of regular networks are determined by the number of nodes and the number of links per node. Biological structures conforming to a regular null model are, for example, the hexagonal prismatic wax cells of a honeycomb or the scutes of a turtle shell. These regular patterns are formed by different processes, in the case of the honeycomb, by selection of space-efficient packing while in the case of turtle shells, by stationary accretion of keratin in all directions.

The Random Model

In a random network, all pairs of nodes have the same chances to be connected. Paul Erdös and Alfréd Rényi (1959) originally proposed to build random networks by connecting nodes at random with a probability p. Some properties of random networks are size-dependent; for example, as the number of nodes increases, the degree distribution tends to a Poisson distribution, the average shortest path length increases as the logarithm of the number of nodes, and clusters tend to disappear. Likewise, the value of p affects the compactness of the network: the higher the value of p, the higher the network density and clustering coefficient, and the lower the shortest path length.

Pure random pattern of connection in biological structures are rare because of the many constraints that can prevent some links while facilitating others, thus, biasing the arrangement of connections away from randomness. For instance, the cranial cavity that hosts the human brain has a spherical shape that imposes a bias in the probability of connection between the occipital bone and the nasal bones, which is physically impossible given their range of shape (i.e., $p = 0$). However, this null model is still valid as a comparative model to establish the presence of some network features, for example, the small-world effect.

The Small-World Model

A small-world network is more clustered than a random network, but it has a similar (or slightly lower) average shortest path. Many complex systems have a small-world organization. Duncan Watts and Steven Strogatz (1998) proposed a mechanism to create small-world networks starting with a regular network that is sequentially rewired at random, with probability p (all nodes have an opportunity to change or keep their connection). By increasing the parameter p from 0 to 1, a network

switches from regular to random: the small-world organization is a transition state in this process.

A small-world organization has been found in most natural systems, including metabolic networks, brain networks, anatomical networks (e.g., skulls), and ecological networks. It is perhaps one of the default types of organization of living systems: between randomness and regularity. Mechanisms by which such systems are created are, however, very diverse, and, except perhaps for neurons, a rewiring process is not expected. An originally regular process that is later biased by emerging interactions could produce a small-world organization. In the case of skulls, for example, we could speculate about mechanisms biasing a homogeneous growth of bones from ossification centers and later interactions (fusion or boundary formation) among bones during development (see example in section "Using Null Network Models to Study Morphological Systems: An Example").

The Scale-Free Model

A scale-free network has a degree distribution that follows a power law, which means that most nodes have a few connections and a few nodes have many (these are called *hubs*). This organization is present in many natural systems. Albert-László Barabási and Réka Albert (1999) proposed a preferential attachment mechanism to build scale-free networks. New nodes are added sequentially to a network and connected to old nodes (already present in the network) with a probability that depends on the number of connections of the old nodes. Preferential attachment creates networks with a power law degree distribution and no clusters. However, the prevalence of the preferential attachment mechanisms to produce scale-free model has been questioned (Fox-Keller 2005). Note that only a preferential attachment to the most connected nodes will produce these types of networks; other forms of preferential attachments are possible in biological systems which may not derive from the previous number of interactions.

The Gabriel Model

A Gabriel network is a type of geometric network; these are networks that are spatially constrained: two nodes only connect if they satisfy a geometric requirement. The nodes of this type of networks occupy a position in the space (e.g., in a 3D Euclidean space). Kuno Gabriel and Robert Sokal (1969) proposed a mechanism to construct spatially constrained networks based on spatial interference between nodes. Two nodes are connected if, and only if, the sphere whose diameter is the line between both nodes does not have any other node within its volume. The properties of the network depend on the number and exact position of nodes.

The Gabriel model is useful to infer developmental or structural constraints imposed by geometry. Not only because of the physical distance between, and

exact position of, ossification centers but also by the presence of insurmountable obstacles between them during development: cavities, openings, and other structures. For this reason, Gabriel models have been used before in modeling structural constraints in skull evolution and development (see example in section "Using Null Network Models to Study Morphological Systems: An Example"; Esteve-Altava and Rasskin-Gutman 2014a). When modeling the development of anatomical structures, such as skulls, the Gabriel model simulates a homogeneous growth of anatomical parts in all directions.

Using Null Network Models to Study Morphological Systems: An Example

Null models can be used to understand the mechanisms that participate in the formation of complex anatomical systems like the human head (Esteve-Altava and Rasskin-Gutman 2014a). A common hypothesis to explain the development of the human head is the functional matrix hypothesis proposed by Melvin Moss (1968). This hypothesis argues that the presence of muscle inductions and functional cavities (e.g., brain, eyeballs) guide the growth of bones and, hence, determine the shape and articulations of bones. Here is an example of the use of a network null model to study the formation of suture contacts in the human skull.

In a developing skull, bones grow until they meet with another bone and form a suture joint. Let's assume, for simplicity, that each bone of the skull comes from only one ossification center that starts growing from its center. In the absence of any constraint on the speed and direction of growth, bones would expand as perfect spheres until they meet other bones and form a suture connection. The occupation of a region of the space by one bone will prevent the growth of other bones through this same space. Thus, an existing connection between two bones prevents other bones to connect. This is the case, for example, of the pterion region of the human skull, where the parietal, the frontal, the temporal, and the sphenoid bones meet. If the sphenoid and the parietal connect first, a fronto-temporal join is impossible; if the frontal and the temporal connect first, a spheno-parietal join is impossible. Moreover, the presence of cavities hosting soft tissues and sensory organs (i.e., functional matrices) also constrains which contacts among bones are feasible in a growing skull.

Imagine now that we model the human skull as a Gabriel network. Each bone is represented as a node, to which we give a position in a 3D space according to its center. The mechanism of linkage of the Gabriel model simulates precisely what would happen if bones had grown from its center at a uniform speed and direction (i.e., without constraints). Thus, the Gabriel model captures the impossibility of creating a suture contact between distant bones due to the presence of unavoidable obstacles between them during development, such as cavities, openings, and, in this case, other bones.

If we compare the existing connections in the human skull with those present in the Gabriel model of the human skull, we will have three types of outcomes (Fig. 2):

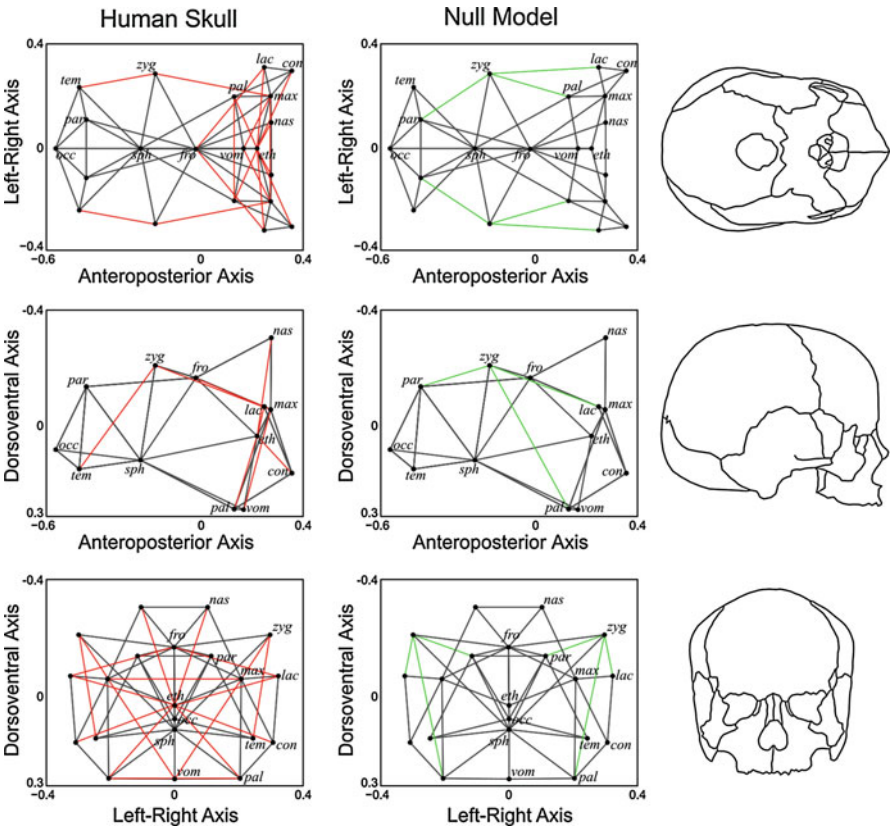


Fig. 2 Bone articulations in a human skull and in a Gabriel null model in various views. Solid gray lines show connections that are predicted by the null model as they actually occur in the human skull; dashed green lines show connections predicted by the null model but not realized; and dotted red lines show connections that are not predicted by the null model but occur in the actual human skull. Labels in Fig. 1. (Modified from Esteve-Altava and Rasskin-Gutman 2014a)

(1) a group of connections that exist in both the null model and the real skull: these are connections that can be explained by an unconstrained growth of bones. This does not mean that the formation of these connections lacks constraints. Rather, it means that there is no need to invoke constraints acting on bone growth to explain such pattern of connection. (2) Connections that exist in the null model but not in the real skull: these are connections that are prevented due to deviation from homogeneous bone growth. This can happen, for example, due to the presence of cranial cavities and openings. (3) Connections that do not exist in the null model but do exist in the real skull: these (as in case 2) are connections that require deviation from homogeneous growth of bones to exist. These connections would require a mechanism that actively pushes the bones to grow in a certain direction or at a faster rate. This difference might be important, for example, to test the validity of the functional

matrix hypothesis in the formation of sutures. In sum, by using null network models, and comparing them to real anatomical systems, we can test hypotheses about processes that participate in the formation of an anatomical system during development as well as in its evolution.

Limitations and Cautions in Anatomical Network Modeling

As it occurs in the construction of any model, the construction of anatomical networks is susceptible to some limitations that, if they are not identified (and fixed or assumed), can lead to some misunderstanding in the interpretation of results (Rasskin-Gutman and Esteve-Altava 2014). Most of these limitations arise from: (1) the definition and selection of elements and relations, (2) typological simplifications, and (3) a false sense of symmetry.

Identification of Elements and Relations

Building the appropriate network model for the system of interest and the questions asked is the first step in an anatomical network analysis. This requires a careful selection of the units of description and the relations modeled: model descriptors (nodes and links) must have precise definitions to enable their identification in all the objects of the study. However, this is not always a straightforward task neither in theory nor in practice (Butts 2009). Think, for example, of a skull network in which the units of description are bones and physical junctions. It is known that the bone unit may change in different ways during development (e.g., connections, proportions, and ossification). For instance, the frontal bone in the human skull is a single unpaired bone in the adult but two paired bones at birth. A different approach could use the ossification centers as elements of the skull network but that would exclude fossil skulls from a broader phylogenetic analysis. The same applies to the definition of physical junction as the structural relation between bones. To use suture joints is an easy way to identify most of the contacts occurring in the skull; however, it excludes from the network those bones that join the skull in a different way, such as the mandible. Moreover, a dichotomous definition of relation between bones (i.e., presence or absence) may obscure differences in the strength of junctions due to the size of their area of articulation. On the other hand, this binary definition allows one to consider all interactions even when the length of the contact is unknown, which is common in fossils.

Typological Simplifications

Even the most conserved anatomical structures show some degree of natural variation at the species or population level. For example, the pterion region of the human skull, where the temporal, parietal, sphenoid, and frontal bones converge shows

intraspecific variation in the way these bones are connected. Thus, suture contact between the sphenoid and parietal bones in this region (sphenoparietal pattern) prevents the frontal and temporal bones to contact (frontotemporal pattern); in some rare cases, the four bones meet in a single point (stellate pattern) or a wormian bone can form in their intersection (epipteric pattern). Another example will be the variable presence of some muscles or their attachments; for example, the forearm muscle, palmaris longus, is sometimes absent in humans.

Arguably, considering only the *type* form can lead to misrepresentation or, at least, oversimplification of the morphological system under study. Local variation in the connectivity pattern among anatomical parts could produce a slight variation in the network properties of a system. However, taking the *type* form (or the most common configuration) of a morphological system is a common idealization in anatomical network analysis.

Illusions of Symmetry

An example of the typological simplification in the construction of anatomical network models is the tendency to symmetrizing anatomical patterns when using *type* forms. Using the previous examples of the pterion region and the palmaris longus, variation is further increased by potential anatomical differences between the left and right side in one individual. Symmetry simplification is common in fossil descriptions, in which connectivity patterns may be obscured by conservation and taphonomic processes. The origin of this idealization lays in the well-established idea that the vertebrate body plan is bilaterally symmetric by default (although internal organs may show directional patterns of asymmetry). Small disruptions of bilateral symmetry can occur also due to errors or fluctuations during development because of alterations of the developmental program or environmental stress.

These current limitations may be overcome by new, innovative approaches that take into account – or empirically address – variability in connectivity patterns at a population or intraspecific level. Nonetheless, modeling is always an exercise of simplification, the limits of which depend on the problem at hand and the system of study.

Conclusion

Anatomical network analysis offers a conceptual framework along with the necessary tools to investigate the architecture of morphological systems at a structural (or topological) level. Thanks to its level of generality, the same tools can be applied to the study of organization and change of organismal form both at developmental and evolutionary scales. Within its limits and idealizations, an anatomical network analysis provides a systematic way of measuring structural complexity and integration, as well as exploring morphological modularity in evolution and development.

Cross-References

- ▶ Concept of Burden in Evo-Devo
- ▶ Developmental Homology
- ▶ Mechanisms of Pattern Formation, Morphogenesis, and Evolution
- ▶ Morphometrics in Evolutionary Developmental Biology

Acknowledgments BE-A is funded by a European Union's Horizon 2020 Marie Skłodowska-Curie Grant No. 654155. DRG is funded by MINECO-FEDER BFU2015-70927-R.

References

Albert R, Barabási A-L (2002) Statistical mechanics of complex networks. Rev Mod Phys 74:47

Barabási A-L, Albert R (1999) Emergence of scaling in random networks. Science 286:509–512

Butts CT (2009) Revisiting the foundations of network analysis. Science 325:414–416

Chernoff B, Magwene PM (1999) Afterword. In: Olson EC, Miller PL (eds) Morphological integration. University of Chicago Press, Chicago, pp 319–353

Dorogovtsev R, Mendes JFF (2003) Evolution of networks: from biological networks to the internet and WWW. Oxford University Press, Oxford

Dos Santos DA, Fratani J, Ponssa ML, Abdala V (2017) Network architecture associated with the highly specialized hindlimb of frogs. PLoS ONE 12(5):e0177819

Erdös P, Rényi A (1959) On random graphs. Publ Math Debr 6:290–297

Esteve-Altava B (2017) Challenges in identifying and interpreting organizational modules in morphology. J Morphol 278:960–974

Esteve-Altava B, Rasskin-Gutman D (2014a) Beyond the functional matrix hypothesis: a network null model of human skull growth for the formation of bone articulations. J Anat 225:306–316

Esteve-Altava B, Rasskin-Gutman D (2014b) Theoretical morphology of tetrapod skull networks. C R Palevol 13:41–50

Esteve-Altava B, Boughner JC, Diogo R, Villmoare BA, Rasskin-Gutman D (2015a) Anatomical network analysis shows decoupling of modular lability and complexity in the evolution of the primate skull. PLoS One 10(5):e0127653

Esteve-Altava B, Diogo R, Smith C, Boughner JC, Rasskin-Gutman D (2015b) Anatomical networks reveal the musculoskeletal modularity of the human head. Sci Rep 5:8298. https://doi.org/10.1038/srep08298

Fortunato S (2010) Community detection in graphs. Phys Rep 486:75–174

Fox-Keller E (2005) Revisiting scale-free networks. BioEssays 27:1060–1068

Gabriel KR, Sokal RR (1969) A new statistical approach to geographic variation analysis. Syst Zool 18:259

Mayr E (1982) The growth of biological thought. Harvard University Press, Cambridge

McShea DW (1991) Complexity and evolution: what everybody knows. Biol Philos 6:303–324

Moss ML (1968) A theoretical analysis of the functional matrix. Acta Biotheor 18:195–202

Newman M (2010) Networks: an introduction. Oxford University Press, Oxford

Newman M, Barabási A-L, Watts DJ (2006) The structure and dynamics of networks. Princeton University Press, Princeton

Olson EC, Miller RL (1958) Morphological integration. The University of Chicago Press, Chicago

Rasskin-Gutman D (2003) Boundary constraints for the emergence of form. In: Müller G, Newman S (eds) Origination of organismal form. MIT Press, Cambridge, pp 305–322

Rasskin-Gutman D, Buscalioni AD (2001) Theoretical morphology of the Archosaur (Reptilia: Diapsida) pelvic girdle. Paleobiology 27:59–78

Rasskin-Gutman D, Esteve-Altava B (2014) Connecting the dots: anatomical network analysis in morphological Evo-Devo. Biol Theory 9:178–193
Riedl R (1978) Order in living organisms: a systems analysis of evolution. Wiley, New York
Simon HA (1962) The architecture of complexity. Proc Am Philos Soc 106:467–482
Watts DJ, Strogatz SH (1998) Collective dynamics of 'small-world' networks. Nature 393:440–442
Woodger JH (1945) On biological transformations. In: Gross WEL, Medawar PB (eds) Essays on growth and form presented to D'A. W. Thompson. Oxford University Press, Oxford, pp 95–120

Computational Modeling at the Cell and Tissue Level in Evo-Devo

Miquel Marin-Riera and Isaac Salazar-Ciudad

Contents

Abstract

Computational models of development integrate empirical knowledge about the dynamics of development, including the interactions at the level of genes, cells, and tissues. These models are capable of predicting the relationship between

M. Marin-Riera (✉)
Center of Excellence in Experimental and Computational Biology, Institute of Biotechnology, University of Helsinki, Helsinki, Finland

Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Barcelona, Spain
e-mail: mmriera@helsinki.fi; miquel.marin@embl.es

I. Salazar-Ciudad
Genomics, Bioinformatics and Evolution, Departament de Genètica i Microbiologia, Universitat Autónoma de Barcelona, Barcelona, Spain

Evo-Devo Helsinki Community, Center of Excellence in Experimental and Computational Biology, Institute of Biotechnology, University of Helsinki, Helsinki, Finland
e-mail: isaac.salazar@helsinki.fi; Isaac.Salazar@uab.cat

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_56

genotype and phenotype for a specific organ or embryo part, that is, the association of specific genetic differences with specific phenotypic differences. Thus, they can provide insights into the evolution of specific lineages by predicting what phenotypic variation is present at each generation for selection to act on. In this chapter we explain how computational models of development are designed and describe several approaches using them in order to address specific and general questions in evolution. Models of development can be used to infer the range of possible phenotypes for a given organ or structure and predict the genetic and developmental bases of specific evolutionary transitions. By including realistic developmental dynamics in population-based models of evolution, one can assess the effect of a complex relationship between genotype and phenotype on the dynamics of adaptation in populations. Furthermore, when the structure of development is allowed to change by mutation in these models, general patterns in the evolution of the mechanisms of development can be inferred.

Keywords

Modeling and simulation · Genotype-phenotype map · Evolution · Evo-devo

Introduction

One of the main challenges of evolutionary biology is to understand how organisms change over generations. Classically, natural selection acting on phenotypic variation has been proposed to be the main driver of phenotypic change. Thus, in order to predict phenotypic evolutionary change, one needs to understand both natural selection and which heritable phenotypic variation arises in each generation in populations. The largest part of this heritable phenotypic variation ultimately arises from genetic variation, but we do not completely understand how specific genetic variants give rise to specific phenotypic variants. This is because an organism's phenotype, especially its morphology, arises through, and because of, a complex process of embryonic development based on complex networks of interaction between genes, cells, and tissues (see chapter ▶ "Mechanisms of Pattern Formation, Morphogenesis, and Evolution").

Development is a process in which a single cell transforms, over time, into an organism composed of multiple cells and cell types arranged in specific and complex spatial patterns. This process can be understood as a series of transformations of specific spatial distributions of cell types, or patterns, into other, usually more complex, distributions of cell types in space. Any morphological difference between two individual organisms arises first as a difference in these transformations at some stage during development. Each pattern transformation in development involves complex networks of genetic, cellular, and tissue interactions over time. Consequently, any morphological difference between two individuals arises as a difference in some of these interactions. Thus, in order to understand, for example, why some change in a specific gene leads to a specific set of morphological changes, we need to understand this genetic variant in the context of the dynamic networks of interactions among genes, cells, and tissues in a specific pattern transformation in development.

In this sense, a "developmental mechanism" can be described as any gene network that regulates at least one cell behavior and is involved in a pattern transformation (Salazar-Ciudad et al. 2003).

Generally, in order to understand how morphology in a population changes over evolutionary time, one needs to address two questions: (1) which heritable phenotypic variation appears at each generation, and (2) which of this generated variation is filtered out by ecological factors? To address question 1, it is necessary to understand development. A good understanding of the development of a species or lineage at a certain point in time significantly enhances the capacity to understand its evolutionary trajectory (especially if something is known about question 2). Additionally, one can ask a third question that derives from 1 and 2, which is (3) how does development itself evolve? While questions 1 and 2 need to be answered for each separate generation and species, by addressing question 3, one might be able to understand some aspects of the evolution of morphology over much larger evolutionary and phylogenetic scales. To address question 1, the two aspects of development that should be studied are:

1. *Variational properties.* They are defined as the ensemble of morphologies that can be produced by a developmental system in the face of changes in the environment or small genetic mutations (i.e., mutations that do not change the topology of the genetic network of a developmental mechanism) (Salazar-Ciudad et al. 2003; Salazar-Ciudad 2006).
2. *The genotype-phenotype map* (GPM). The GPM is a theoretical function that describes which specific genetic differences are associated with specific morphological differences within the variational properties. The GPM maps each possible genotype to a single specific phenotype, and, since two different genotypes can be connected by a single or a series of mutations, it can also show how the phenotype of a specific mutant form differs from the wild-type phenotype.

Both concepts can be used in the context of the development of a whole organism, a part of an organism, or a specific developmental mechanism in a specific pattern transformation (Fig. 1). Thus, the variational properties describe what development, or a developmental mechanism, can make at the phenotypic level, and the GPM describes the association between each of those phenotypic variants and specific genetic variations (Fig. 1) (note that these two concepts are not completely independent from one another, since the GPM links the variational properties to the genetic landscape, and different GPMs will contain different variational properties).

While it is possible to estimate the GPM by statistical methods without understanding the underlying developmental dynamics, that is, by either collecting large datasets of associations between specific genetic mutations and their corresponding phenotypes (Orgogozo et al. 2015) or by measuring the variance-covariance matrix of crossed genetic effects on trait values within a population (i.e., G-matrix, Lande and Arnold 1983), this approach is not very informative about why the GPM is the way it is. Alternatively, it is possible to achieve a deeper and more mechanistic understanding of the GPM by studying development, since it is mostly responsible for bridging genotype and phenotype at the morphological level.

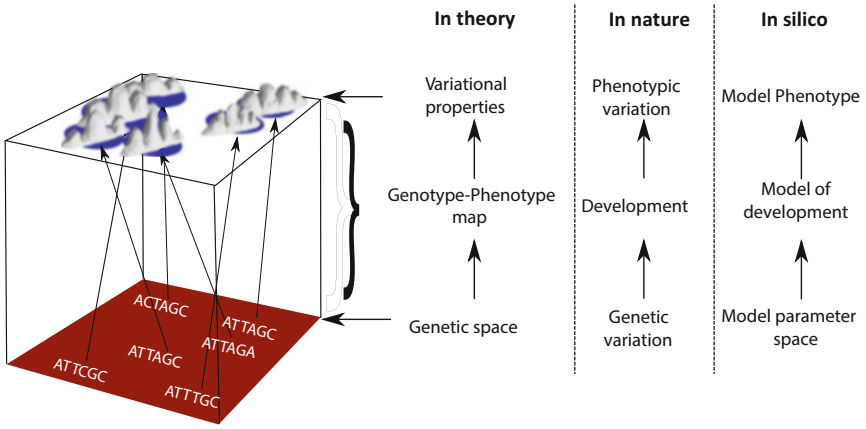


Fig. 1 The genotype-phenotype map (GPM) and the variational properties. Given a certain developmental process in an individual, a population, or a species, there is a limited number of different phenotypes that can arise through changes in the genotype. The ensemble of phenotypes that can arise through a developmental process is called variational properties, and the association between each genetic change and a phenotypic change is the GPM. The GPM can be reproduced *in silico* by integrating our knowledge of development into a mathematical model. Variation in the model parameters will result in different model phenotypes. By systematically exploring the model parameter space, one can infer the variational properties of that specific developmental system

Development involves the interactions among genes, cells, and tissues, and there is usually abundant but incomplete experimental information about these interactions. Most morphologies are complex, that is, composed of many measurable traits whose variation is highly interdependent. Thus, in order to be able to predict the variational properties and GPM of development, it is usually necessary to create mathematical models incorporating information and hypotheses about how genes, cells, and tissues interact during development. These models can then be used to understand how subtle and complex morphological variations arise. Mathematical models are just a way to explore the phenotypes and phenotypic variation that should be expected from a specific hypothesis of development. In this sense, models make quantitative predictions on the variational properties and the GPM based on developmental mechanisms. This approach is cheaper and less cumbersome than to experimentally check the phenotypic effect of each genetic variation in the GPM and, if successful, can provide a general understanding of the developmental logic that generates certain morphological variants and not others.

In this chapter we will discuss how mathematical models of development are built and how they can be used to predict phenotypic change both in the lab and in an evolutionary context. We will also explain how models can be used to study the phenotypic variation arising from development (question 1), to infer which types of selective pressures facilitate or prevent adaptive change in complex organisms with realistic complex GPMs (question 2), and to find regularities in the emergence and replacement of different types of development along the course of the evolution of a lineage (question 3).

Mathematical Models of Development as Tools to Integrate Experimental Evidence and Predict the Outcome of New Experiments

There is a long list of realistic computational models in development involving both cell communication and cell movement by mechanical interactions, such as models of tooth development (Salazar-Ciudad and Jernvall 2010), limb development (Raspopovic et al. 2014; Zhu et al. 2010), turtle shell formation (Moustakas-Verho et al. 2014), and blood vessel development (Merks et al. 2008), to cite just a few. Mathematical models of development implement hypotheses that are based on previous experimental observations (e.g., tissue composition and architecture) and perturbations (e.g., gene knockout experiments). For some organs there is enough experimental data to raise one or several hypotheses about the mechanisms driving the pattern transformations. Experimental data need to include at least information about the main morphogenetic (movement of cells and tissues) and inductive (spatial changes in gene expression) events taking place during development.

The model is then built by formulating the mechanistic hypothesis in mathematical terms. In order to validate the model, and thus to accept or reject a mechanistic hypothesis, the model should be used to reproduce the wild-type phenotype of the system and each of the developmental stages under study. For a more stringent and informative test, however, the model should also reproduce the phenotypic variation observed in the system, either in mutants, experimental manipulations of development, or as natural phenotypic variation between individuals within a species or between species. If the outcome of the model (the phenotype) matches the observed variation, then the mechanistic hypothesis cannot be rejected. If it does not, then the hypothesis has to be rejected and another one needs to be devised. Failure, however, is informative about which aspects of the real dynamics are more poorly understood and, thus, orients future experiments. Furthermore, a validated model of development may be able to predict the outcome of experiments that have not been yet carried out. Thus, good models of development can become powerful tools to assist and guide experimental research programs.

Mathematical models of development usually include:

1. The initial conditions. Usually studies on development focus on a specific time range limited by two developmental stages. The developmental pattern at the initial stage should be used in the model as the initial conditions.
2. The basic mechanics of cells and tissues. The mechanical interactions between different cells and between cells and the extracellular matrix taking place in the developmental system under study (e.g., cell-cell adhesion, cell volume conservation, and cell migration) should be implemented in the model. Cell mechanics can be ignored in systems where cells do not move.
3. Intracellular gene networks and extracellular signals diffusing between cells. Extracellular signals can also be ignored in models where there is no cell-cell communication.

4. Cell behaviors (e.g., cell division, cell death, cell adhesion, cell contraction) and their regulation by the gene network.

In order to illustrate the structure and implementation of a development model including all the previous elements, we will describe here the tooth model (Salazar-Ciudad and Jernvall 2010) (Fig. 2).

The Tooth Development Model

Tooth development consists of the growth and folding of the dental epithelium over a mesenchymal condensation. During this process, epithelial signaling centers, called

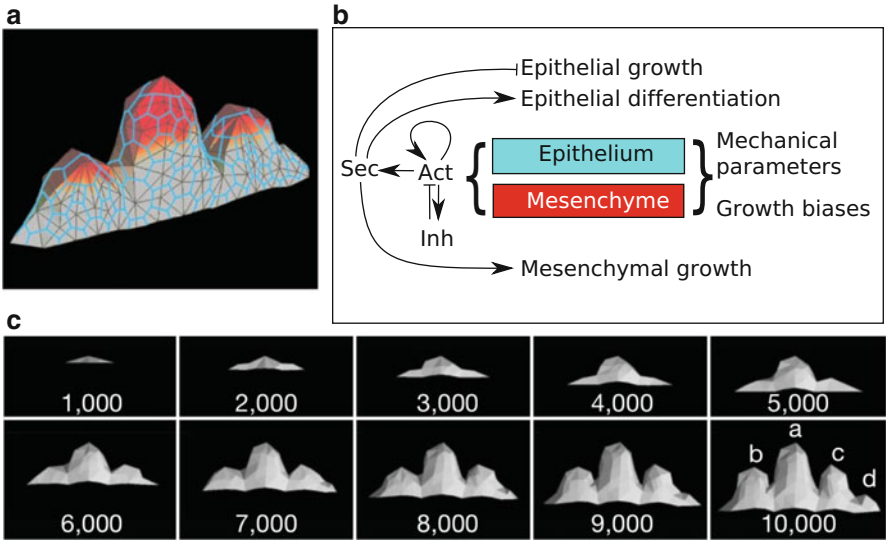


Fig. 2 The tooth development model. (**a**), depiction of cells and their mechanical interactions in the developing tooth epithelium. Epithelial surface depicts the concentration of activator signal (in red), blue lines indicate cell borders, and black lines depict mechanical interactions between neighboring cells. (**b**), schematization of the gene network interactions, cell behaviors, and mechanical parameters included in the model. Cell growth and differentiation are regulated by a signaling network. Activator signal Act promotes its own expression, the expression of Inh and Sec. Inhibitor signal Inh inhibits the expression of Act. Secondary signal Sec promotes cell differentiation in the epithelium and inhibits its growth (in the absence of Sec, epithelial cells grow at a default rate) and also promotes growth in the mesenchyme. (**c**), time sequence of a model simulation from a lateral view. A small, flat epithelium starts growing over the underlying mesenchyme. When some cells receive enough concentration of Act, they differentiate into enamel knots, stop growing, and start forming the tips of the cusps. New enamel knots arise at a certain distance from existing knots because of Inh being secreted by the differentiated knots. Inh prevents the differentiation of cells close to the knots. The undifferentiated epithelium between existing knots continues growing downward and forms the valleys between the cusps

enamel knots, are induced in specific positions and regulate cell proliferation and differentiation through cell signaling to neighboring cells. Epithelial proliferation is downregulated at the enamel knots, but not in the space between them. As a result, the epithelium grows and folds down between the knots, thus creating valleys between them, and the knots end up at the tips of what become the tooth cusps. The first knot is induced by the underlying mesenchymal condensate, but, once formed, all knots secrete a second inhibitory signal that precludes the formation of new knots at a certain distance from the existing knots. The tooth model summarizes those processes by defining a spatial context for cells, their mechanics, and a gene network that regulates the cell behaviors of proliferation and differentiation (Salazar-Ciudad and Jernvall 2010).

In the tooth model, molecules are produced by cells and diffuse in the extracellular space between them. The model considers only three of these extracellular signals. (1) An activator signal comes in through the borders of the system and promotes its own synthesis and secretion on epithelial cells. It also induces the differentiation of epithelial cells into enamel knots and thus inhibits cell proliferation (Fig. 2b). (2) An inhibitor signal is only produced at the enamel knot cells and inhibits the secretion of the activator in the cells that receive it (Fig. 2b). (3) A third extracellular signal is secreted from the enamel knots and induces cell differentiation (Fig. 2b).

The model allows one to simulate the real-time dynamics of tooth development (Fig. 2c). The simulation starts with a small, flat epithelium over a block of mesenchyme (closely resembling the earliest stages in tooth development). As a result of the model dynamics, the epithelium starts growing downward and soon the first enamel knot appears. As the tooth epithelium keeps growing, new knots appear separated from the existing ones, and the growing epithelium between them becomes the valley between the cusps (Fig. 2c). Thus, signaling and induction are taking place while the shape of the tissues (where diffusion is taking place) is constantly changing. Only the initial conditions and the model parameters are specified before the simulation. It is important to note that the final tooth phenotype and its change over developmental time arise as a result of the intrinsic dynamics between cells and gene products during the course of the developmental simulations. The outcome or phenotype of the model is, at each time in development, the morphology of the developing tooth, which is the distribution of cells in three-dimensional space, and the concentration of each molecule in each cell.

The model defines a number of parameters that quantify certain aspects of the cellular and signaling interactions (Fig. 2c), such as the growth rate of cells, mechanical properties of cells, diffusivity constants of molecular signals, and strength of regulatory interactions between genes. Different values of these parameters will change the dynamics of development during the simulation and thus alter its final outcome. For example, decreasing the parameter value that specifies the secretion rate of the inhibitor in the enamel knots will reduce the distance between cusps in the final phenotype, and that happens because during the model simulation, the amount of inhibitor secreted around knots is smaller, which allows for new knots to differentiate closer to existing ones.

The model does not include all the known genes and gene products involved in tooth development (for simplicity, the only gene products considered are the extracellular signals themselves). In many cases, we do not know where they are located in the gene network of tooth development, and thus they cannot be included in the model. In other cases, variation in these genes produces variation in the tooth that is not interesting from the point of view of evolution and the GPM. This is the case when, for example, the mutation produces either no effect on tooth morphology or prevents teeth from forming at all. In fact, any gene relevant for the production of morphology and its variations necessarily does so because it affects one or more of the cell behaviors involved in tooth development (i.e., cell signaling, cell division, cell adhesion, and cell differentiation).

The effect of these non-included genes is actually encapsulated within the model parameters. In general, the parameters are dependent on a large number of gene products and thus are, in a way, genetically encoded. Instead of including all possible genes, a model can include, for simplicity and as a first step, a subset of genes that is judged to be the most important. Also, for simplicity, model parameters take continuous values within a range.

From an evolutionary perspective, teeth are often the only traces left by extinct mammalian species, and so tooth morphology is very important for studying the mammalian fossil record. In that sense, the tooth model can shed light on the developmental bases of specific morphological transitions during mammalian evolution (Salazar-Ciudad and Jernvall 2002) as well as assist with empirical research trying to address the same questions in vitro (Harjunmaa et al. 2014). Perturbation experiments or mutants that affect tooth development can be reproduced in the model by changing parameter values from the wild-type phenotype (which could be seen as in silico mutations). For example, the inhibition of the Shh signaling pathway in vivo and in vitro causes a shortening in the separation distance between cusps, which results in an increase in their number due to a tighter packing of the cusps (Harjunmaa et al. 2012). This phenotype is predicted by the model by decreasing the parameters affecting the rate of inhibitor secretion at the enamel knots, which suggests that Shh is most likely acting as, or contributing to, the pathway that inhibits the differentiation of the enamel knots. It is important to note that in this case the tooth model does not need to integrate any information from the Shh inhibition experiment in order to predict its outcome. Given a solid mechanistic hypothesis of the developmental process under study, theoretical models of development can provide predictions of the outcome of an experiment before it is carried out.

Predicting Phenotypic Variation from Development and the Developmental Basis of Evolutionary Transitions

Knowing the ensemble of phenotypic variation that a developmental process can produce is relevant in order to make predictions of the evolution of a lineage. The variational properties will tell us the distribution of phenotypic variants that can arise in each generation. By determining in which direction phenotypic variation is possible by means of genetic variation, development can have a strong effect on

the direction of evolutionary change for natural selection to act on (note that natural selection can also have a strong effect on such direction, but only by eliminating the phenotypic variation produced by development); developmental processes tell us which regions of the morphospace are available for populations to occupy throughout evolution (note that a developmental mechanism may not be able to produce just any possible morphology, even when considering all possible genetic variants).

In order to know the variational properties of development in an organism, one should study all the genetic variants or mutants that are known to affect the phenotype through that developmental process (either for a whole species or for a population in a given generation). For most species and organs, this is likely to be difficult and quite time-consuming. Computational models of development can offer a first theoretical glance at how these variational properties might look and may provide some theoretical predictions of evolutionary relevance. The computational exploration of the variational properties of development is often performed through a parameter screening of the model. In a parameter screening, a large number of parameter combinations are generated by changing all the parameter values and are fed to the model in order to get the set of possible morphologies (Salazar-Ciudad and Jernvall 2004; Prusinkiewicz et al. 2007) (Fig. 1).

Models of development can also be used to understand the specific developmental origin of the standing phenotypic variation within a population. Gathering empirical data on how variable development is in a natural population is methodologically challenging. Computational models of development can be used to generate different sets of phenotypic variation by giving different values to one or a few model parameters, creating different *in silico* populations. Then, by assessing which virtual population most resembles the natural population, we can infer which parameters of development may contribute to the natural phenotypic variation (Salazar-Ciudad and Jernvall 2010). For instance, when comparing virtual populations generated by the tooth model with teeth from a natural population of seals, it was found that variation coming from the model parameters involved in the diffusivity of the inhibitor and the activation of the activator by itself were able to explain the largest part of the natural variation observed (Salazar-Ciudad and Jernvall 2010). Thus, computational models can provide predictions about which specific aspects of a developmental mechanism (encoded as parameters in the model) account for phenotypic variation within a population or across taxa. The genetic basis of each of these aspects is usually quite complex and lies beyond the scope of this type of models. Thus, ultimately, in order to identify the specific loci regulating these developmental aspects, further genetic studies would be required, although the theoretical predictions provided by computational modeling may help narrow down the search.

Predicting the Effectiveness of Natural Selection on Evolving Populations In Silico Using Models of Development

Natural selection favors the perpetuation of phenotypes that are better adapted to the environment. This can be envisioned using the metaphor of the adaptive landscape (Wright 1932). Adaptive landscapes are relief maps in which each point on the

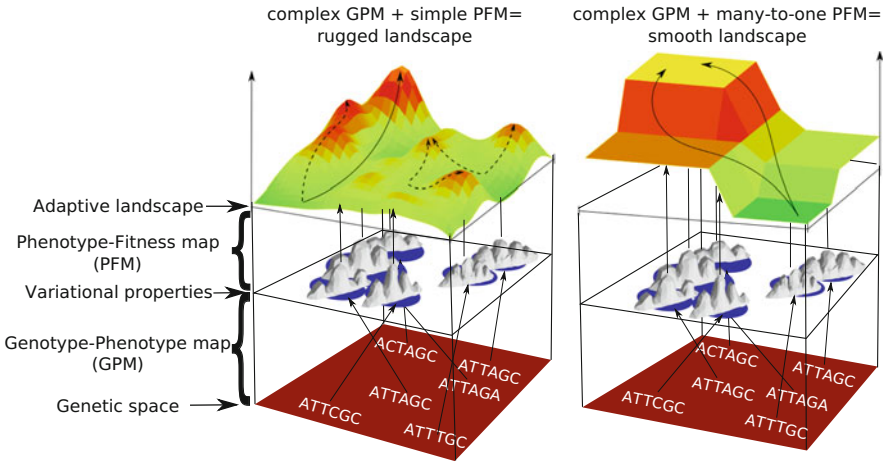


Fig. 3 Adaptive landscapes can be split into genotype-phenotype maps (GPMs) and phenotype-fitness maps (PFM). Two adaptive landscapes with their respective GPM and PFM are depicted. An adaptive landscape is an abstract space, depicted here as a relief surface, that determines the possible evolutionary trajectories of a population. The height of the surface depends on the fitness of the phenotype; thus populations will tend to climb uphill and seldom downhill. Once a peak is reached, adaptive change is precluded since it is not possible to further increase fitness. Rugged adaptive landscapes (left) consist of many peaks of different fitness values, and so populations are more likely to get trapped in peaks of low fitness. Smooth landscapes usually have fewer peaks; thus populations will often reach the peak of maximum fitness (right). A combination of a complex GPM and a simple PFM will result in a rugged adaptive landscape (left), whereas if a complex GPM is combined with a many-to-one PFM, the result will be a smooth adaptive landscape (right)

surface is a genotype, and the height of the surface at that point is equal to its fitness. One individual at a certain point of the landscape can only move to other, nearby points in the landscape through mutation. In the landscape metaphor, populations can be seen as clouds of points. Since natural selection favors higher fitness values, populations tend to climb uphill, although downhill movements can also happen because of genetic drift or high mutation rates. It has been theoretically argued that the shape of an adaptive landscape has important implications for the ability of populations to adaptively evolve. Populations evolving on smooth landscapes with a single fitness peak will easily reach this fitness maximum, whereas in rugged landscapes (i.e., a rough surface and many fitness peaks), populations will often get trapped on suboptimal local peaks (Fig. 3) (Wright 1932; Kaufmann 1993).

The fitness of an individual is determined by both its phenotype and the environment (i.e., the ecological factors determining whether the phenotype is suitable for survival and/or reproduction). Then the adaptive landscape can in fact be decomposed into two different landscapes (Fig. 3): a phenotype-fitness landscape mapping all possible phenotypes and their corresponding fitness (also called a phenotype-fitness map or PFM) and a genotype-phenotype landscape mapping all possible genotypes and their corresponding phenotypes (the GPM). The structures of both landscapes have important implications for evolutionary dynamics. Complex

GPMs have been suggested to be the most prevalent, since they are present from the level of RNA secondary structure (Stadler 2006) to the level of developing organisms (Salazar-Ciudad and Jernvall 2005). Moreover, they may prevent adaptive change by contributing to the overall ruggedness of the adaptive landscape.

Even if most morphological structures have a complex GPM, they often exhibit millions of years of adaptive evolution. Thus, there have to be, in theory, other factors that counteract the effect of the complex GPMs on the ruggedness of the adaptive landscapes for the adaptability of populations. This question has been addressed by simulating the evolution of populations in which the individual's phenotype arises through a realistic model of development. In a recent study (Salazar-Ciudad and Marín-Riera 2013), this has been done by using the abovementioned tooth model.

During the evolutionary simulations, individual fitness is determined by a function assessing how much an individual phenotype resembles an arbitrary target phenotype. In this evolutionary model, populations walk on the adaptive landscape (technically they walk at the same time on the phenotype-fitness landscape and the GPM); thus the evolutionary process and the dynamics of adaptation can be monitored. The degree of ruggedness of the adaptive landscape can be inferred by measuring how often populations reach the optimum (i.e., the target phenotype). When the function used to calculate fitness was fine-grained (i.e., comparing the degree of match between an individual's morphology and a target morphology at a cellular resolution), the target morphology was rarely reached, and the amount of adaptive change accumulated during evolution was small. Consequently, the phenotype-fitness landscape was rugged. When the function used to calculate fitness was coarse-grained (i.e., by comparing the level of tooth surface complexity to a target value of surface complexity), the optimum was often reached.

These results suggested that the fine-grained phenotype-fitness landscape combined with the tooth GPM created a rugged adaptive landscape that prevented substantial adaptation, whereas the coarse-grained phenotype-fitness landscape in combination with the GPM created a smooth adaptive landscape (Fig. 3). The coarse-grained phenotype-fitness landscape has a many-to-one mapping between phenotype and fitness that effectively makes the adaptive landscape smoother, since by that criterion phenotypes with quite different morphologies may have the same level of surface complexity, hence the same fitness value (Fig. 3). From these data it becomes clear that there are some types of phenotype-fitness landscapes that are unable to drive adaptive change. Thus, natural selection on real populations will effectively drive adaptive evolution when fitness is determined by a many-to-one or other simpler phenotype-fitness landscapes that only take into account a small proportion of the phenotype's information. This approach looks very promising in simulating the link between development, evolution, and ecology. However, it is computationally very demanding, and thus the model of development needs to be relatively simple (e.g., the tooth organ consisted of a few hundred cells by the end of each developmental simulation). New modeling approaches will overcome this limitation by increasing simulation speed thanks to parallel computation.

Studying the Emergence and Replacement Dynamics of Different Types of Development over the Course of Evolution

When a new phenotypic variant is fixed by selection in a population during evolution, so are the changes in development that lead to these phenotypic changes. In this way development evolves along with the phenotype. Two questions related to the evolution of development can be addressed with the help of mathematical models of development: i) which types of development can evolve within a lineage under different types of selective pressures, and ii) how different types of development can replace others during evolution under a single constant selective pressure? In these models of evolution of development, however, mutations affect not only the developmental parameters but also the number of genes involved in development and how they are connected, that is, the gene regulatory network topology (see chapter ▶ "Modeling Evolution of Developmental Gene Regulatory Networks").

So far, computational studies on the evolution of development have mainly focused on the evolution of gene expression patterning mechanisms that included cell signaling as the only cell behavior (Salazar-Ciudad et al. 2001; ten Tusscher and Hogeweg 2011). In most cases, these models of development assume a simple organism made up of a string of nonmoving cells, each having a gene regulatory network that involves one or several extracellular signals. The resulting phenotype is the spatial pattern of gene expression arising from the inductive interactions between gene products within and between cells.

By simulating the evolution of populations under different selective pressures, it is possible to make predictions about how development (in this case the gene network topology) will evolve in each case (question i). In one of these studies (Salazar-Ciudad et al. 2001), populations were selected either for a phenotype with a low number of gene expression stripes or for a large number of stripes, and it was found that different types of gene networks appeared in each case. Hierarchic networks, in which the rate of secretion of an extracellular signal by a cell is not affected by the response of other cells to this signal, were found more likely to evolve when organisms were selected for a low number of stripes. In contrast, Turing-like reaction-diffusion networks, in which the rate of secretion of an extracellular signal by a cell is affected by the response of other cells to this signal, were found more likely to evolve under a selective pressure for a larger number of stripes. It was argued that, in order to generate patterns with a large number of stripes, hierarchic networks required a larger number of genes than reaction-diffusion networks. Thus, the latter were more likely to appear in evolution when there was selection for a large number of stripes, since large hierarchic gene networks would take more time to appear through a process of random mutation and selection.

Models of the evolution of development can also make predictions about how different types of development may be replaced by other types during evolution (question ii). In the same study (Salazar-Ciudad et al. 2001), simulations were carried out in which individuals were selected for an increase in pattern complexity (here, a higher complexity is roughly equivalent to a larger number of stripes). In those simulations, the first networks to emerge were of the hierarchic type, with

rather simple phenotypes. Those networks were soon replaced by emergent networks, which rapidly increased the complexity of the phenotype over evolutionary time. In hierarchic networks, phenotype complexity strongly correlates with network size (number of genes), which means that in order to increase phenotypic complexity, the network has to incorporate new genes, a process that is slow at an evolutionary scale. In contrast, a simple emergent network can generate phenotypes of different complexities just by mutating the parameters related to the regulation of gene-gene interactions, which means that once an emergent network appears during evolution, phenotype complexity will rapidly increase through small genetic changes that do not affect network topology.

This is potentially one of the most powerful approaches that computational modeling can provide to evo-devo, since it is capable of predicting large-scale trends for the evolution not only of the phenotype but also of the underlying developmental mechanisms and the GPM as well. Nonetheless, this approach is usually limited by the heavy computational requirements of simulating the complexity of development for all the individuals in a population and by the length of the simulations. That is why these types of studies have been restricted to modeling development as the process of gene expression patterning in a static field of cells (Salazar-Ciudad et al. 2001; ten Tusscher and Hogeweg 2011), with a few exceptions (e.g., Hogeweg 2000). However, with the advent of faster computational methods, it will be possible to include models of development that consider morphogenetic processes such as growth and cell- and tissue-level deformations in three dimensions in these evolutionary simulations.

Future Directions

Computational models can bring insights into general and specific questions in evolutionary developmental biology since they allow the variational properties and the GPM of developmental systems to be systematically explored, thus providing an understanding of which phenotypic variation natural selection can act on at each generation. Most models of development used in earlier studies either focus on one specific organ/structure or do not include all the cell behaviors known to be involved in animal development. That has not prevented those models from providing insights into some general questions in evolution. In the future, however, more complete models including a wider range of cell dynamics and cell behaviors could be used to study the evolution of developmental systems. This is because, in most of animal development, cells do something more than just signal to each other to change patterns (e.g., cell division, apoptosis, cell adhesion, and so on). The combination of these cell behaviors with cell signaling has been suggested to give rise to GPMs and variational properties quite different from the ones found in models of development including only cell signaling (Salazar-Ciudad and Jernvall 2004). Recently, some modeling frameworks have implemented part (Swat et al. 2012; Starruß et al. 2014) or all of those cell behaviors (Marin-Riera et al. 2016).

Organ-specific models of development have been shown to reproduce quite accurately the relationship between genotype and phenotype of their real counterpart (Salazar-Ciudad and Jernvall 2010; Zhu et al. 2010), and that has allowed for predictions about evolutionary transitions of the organ. Nonetheless, the design of those models constrains them to only reproduce the GPM of a single organ, so it is not possible to infer evolutionary transitions between different organs (e.g., the transition between reptilian scales and avian feathers). Thus, the next step in the modeling of developmental mechanisms should be to design models that are able to reproduce a variety of organs that are supposed to be evolutionarily related. By reproducing the development of different organs with the same model, it would be possible to quantitatively compare their differences at the level of development and infer which changes would be required to evolve from one organ to the other.

Cross-References

- ▶ Mechanisms of Pattern Formation, Morphogenesis, and Evolution
- ▶ Modeling and Simulation in Evo-Devo
- ▶ Modeling Evolution of Developmental Gene Regulatory Networks

References

Evans AR, Wilson GP, Fortelius M, Jernvall J (2007) High-level similarity of dentitions in carnivorans and rodents. Nature 445(7123):78–81. https://doi.org/10.1038/nature05433

Harjunmaa E et al (2012) On the difficulty of increasing dental complexity. Nature 483 (7389):324–327

Harjunmaa E et al (2014) Replaying evolutionary transitions from the dental fossil record. Nature 512(7512):44–48

Hogeweg P (2000) Evolving mechanisms of morphogenesis: on the interplay between differential adhesion and cell differentiation. J Theor Biol 203(4):317–333. https://doi.org/10.1006/jtbi.2000.1087

Kaufmann S (1993) The origins of order. Oxford University Press, Oxford, UK

Lande R, Arnold SJJ (1983) The measurement of selection on correlated characters. Evolution 37(6):1210–1226

Marin-Riera M et al (2016) Computational modeling of development by epithelia, mesenchyme and their interactions: a unified model. Bioinformatics (Oxford, England) 32(2):219–225

Merks RMH et al (2008) Contact-inhibited chemotaxis in de novo and sprouting blood-vessel growth. PLoS Comput Biol 4(9):e1000163

Moustakas-Verho JE et al (2014) The origin and loss of periodic patterning in the turtle shell. Development 141(15):3033–3039

Orgogozo V, Morizot B, Martin A (2015) The differential view of genotype–phenotype relationships. Front Genet 6:1–14

Prusinkiewicz P et al (2007) Evolution and development of inflorescence architectures. Science (New York, NY) 316(5830):1452–1456

Raspopovic J, Marcon L, Russo L, Sharpe J (2014) Modeling digits. Digit patterning is controlled by a Bmp-Sox9-Wnt Turing network modulated by morphogen gradients. Science 345:566–570

Salazar-Ciudad I (2006) Developmental constraints vs. variational properties: how pattern formation can help to understand evolution and development. J Exp Zool B Mol Dev Evol 306 (2):107–125

Salazar-Ciudad I, Jernvall J (2002) A gene network model accounting for development and evolution of mammalian teeth. PNAS 99(12):12

Salazar-Ciudad I, Jernvall J (2004) How different types of pattern formation mechanisms affect the evolution of form and development. Evol Dev 6(1):6–16

Salazar-Ciudad I, Jernvall J (2005) Graduality and innovation in the evolution of complex phenotypes: insights from development. J Exp Zool B Mol Dev Evol 304(6):619–631

Salazar-Ciudad I, Jernvall J (2010) A computational model of teeth and the developmental origins of morphological variation. Nature 464(7288):583–586

Salazar-Ciudad I, Marín-Riera M (2013) Adaptive dynamics under development-based genotype-phenotype maps. Nature 497(7449):361–364

Salazar-Ciudad I, Newman SA, Solé RV (2001) Phenotypic and dynamical transitions in model genetic networks I. Emergence of patterns and genotype-phenotype relationships. Evol Dev 3(2):84–94

Salazar-Ciudad I, Jernvall J, Newman SA (2003) Mechanisms of pattern formation in development and evolution. Development 130(10):2027–2037. https://doi.org/10.1242/dev.00425

Sharpe J (2017) Computer modeling in developmental biology: growing today, essential tomorrow. Development 144(23):4214–4225. https://doi.org/10.1242/dev.151274

Stadler PF (2006) Genotype–phenotype maps. Biol Ther 1(3):268–279

Starruß J et al (2014) Morpheus: a user-friendly modeling environment for multiscale and multicellular systems biology. Bioinformatics (Oxford, England) 30(9):1331–1332

Swat MH et al (2012) Multi-scale modeling of tissues using CompuCell3D. Methods Cell Biol 110:325–366

ten Tusscher KH, Hogeweg P (2011) Evolution of networks for body plan patterning; interplay of modularity, robustness and evolvability. PLoS Comput Biol 7(10):e1002208

Wright S (1932) The roles of mutation, inbreeding, crossbreeding and selection in evolution. In: Proceedings of the sixth international congress on genetics, vol 1, pp 356–366

Zhu J et al (2010) Bare bones pattern formation: a core regulatory network in varying geometries reproduces major features of vertebrate limb development and evolution. PLoS ONE 5(5): e10892

Modeling Evolution of Developmental Gene Regulatory Networks

Renske M. A. Vroomans and Kirsten H. W. J. ten Tusscher

Contents

Abstract

The field of evo-devo studies what, how, and why developmental patterning processes have evolved. While comparative approaches based in experimental data are essential for answering the first two types of questions, evo-devo simulations studies are critical to answer why questions. By simulating evo-devo processes, the evolutionary tape can be replayed both under the same and different conditions, enabling us to answer questions on contingency, convergence, and constraints and their roles in determining evolutionary outcomes.

In this chapter, we describe the basic ingredients of computational models simulating evo-devo processes: gene expression regulation; cell and tissue behavior; and mutation-selection driven evolution. We describe for each of these model ingredients the choices that need to be made, e.g., whether the model simulates a one, two, or three-dimensional tissue, and how these affect computational efficiency as well as modeling outcomes. We focus on the importance of

R. M. A. Vroomans (✉) · K. H. W. J. ten Tusscher
Theoretical Biology Group, Department of Biology, Utrecht University, Utrecht, The Netherlands
e-mail: renske.vroomans@gmail.com

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_118

incorporating a realistic, nonlinear, and evolvable genotype-phenotype map in evo-devo simulation models.

We end with an illustration of how evo-devo models have helped answer why questions in the field of animal body plan segmentation.

Keywords

Computational modeling · Evolutionary simulations · Gene regulatory networks · Genotype-phenotype mapping · Robustness and evolvability

Introduction

The field of evo-devo studies the reciprocal impact of evolution on development and development on evolution. The ultimate aim is to determine what, how, and why particular developmental patterning processes have evolved. Comparative approaches, such as anatomical comparisons, spatio-temporal gene expression mapping, or bioinformatic analysis of genome composition, may uncover which changes underlie the phenotypic differences we observe between extant species. They are instead less suitable for addressing *why* developmental programs have evolved and diverged along particular trajectories; for instance, it may be the case that a particular patterning process is discovered through mutations more easily than other patterning mechanisms, or instead, it may confer a selective advantage. The observation that evolution has followed a certain path in a certain species is insufficient to discern whether this path is just one of many possible evolutionary outcomes (all leading to different developmental programs), or rather that given a second or third chance, a similar trajectory would have been followed. In the latter case, even when unrelated species appear to have evolved similar developmental traits, it is hard to determine whether this convergence arose because this developmental mechanism confers the highest fitness advantage or because constraints arising from a limited toolkit of developmental genes or prior evolutionary processes reduced the evolutionary accessibility of alternative mechanisms Chipman (2010).

In-silico modeling provides us with a means to address these types of *why* questions. Depending on the particular approach, models can be used to investigate the evolutionary accessibility of different theoretically inferred developmental mechanisms that are capable of generating the same phenotype or to study their robustness to a variety of perturbations (Cotterell and Sharpe (2010); Jiménez et al. (2015); Salazar-Ciudad et al. (2001)). Furthermore, computer simulations allow us to "replay the evolutionary tape": letting the same developmental character evolve multiple times in silico to assess the likelihood of finding various alternative mechanisms to generate this character. With such simulations we can also compare the evolutionary consequences of a variety of different conditions, for instance, the presence or absence of gene expression noise or a morphogen gradient (Vroomans et al. 2016) – or what happens if two developmental

traits evolve at the same time (ten Tusscher and Hogeweg 2011). Finally, such simulations generate a perfect "*in-silico* fossil record" of both the genotype and phenotype of all ancestors leading up to an evolutionary outcome. This enables one to reconstruct the precise mutational trajectory leading up to this outcome and investigate whether convergent evolutionary outcomes arise from similar or different mutational routes (see chapter ▶ "Convergence").

In this chapter, we will discuss how *in-silico* models of evo-devo are built up and the different ways they have been used to tackle the *why* questions of developmental processes and their evolution.

Simulating Development

Development occurs on multiple levels: it involves processes that range from the subcellular polarization patterns within cells, via division, movement, cell fate and shape changes of individual cells to overall tissue-scale growth, patterning, and morphogenesis (see chapter ▶ "Levels of Organization in Evo-Devo"). Evo-devo models are necessarily simplified to keep them manageable in terms of required computational time and the ease with which results can be analyzed and understood. As a consequence, these models typically incorporate two organizational levels of development – cells and tissues – while subcellular patterning is usually ignored. Despite these simplifications, many choices still need to be made: how gene expression regulation and dynamics are modeled, what types of cell-cell communication are considered, whether cell division and growth are modeled explicitly, and more.

These modeling choices may have consequences for the types of developmental mechanisms that can be captured in the model, as well as the evolutionary questions that can be answered. In this section, we first discuss the building blocks necessary to simulate developmental processes. We discuss some of the different modeling choices that can be made, their advantages and disadvantages, and their consequences for the evolutionary process.

Within Cells

Gene Expression Levels There are two main ways in which gene expression levels can be modeled: Boolean or continuous (Fig. 1A). In models using boolean gene expression, only two levels of gene expression are distinguished: no expression (0) or full expression (1). Boolean models are computationally much more efficient and therefore attractive if one aims to investigate large networks containing many genes. Furthermore, Boolean models contain few parameters and therefore enable a qualitative analysis of network behavior when there is little information available on kinetic constants (Spirov and Holloway 2013). However, with the Boolean modeling formalism, the gradual activation or inhibition of a gene, or the graded expression of a gene across a tissue, cannot be simulated. To overcome this limitation and yet maintain the computational efficiency advantages, some modelers have extended the

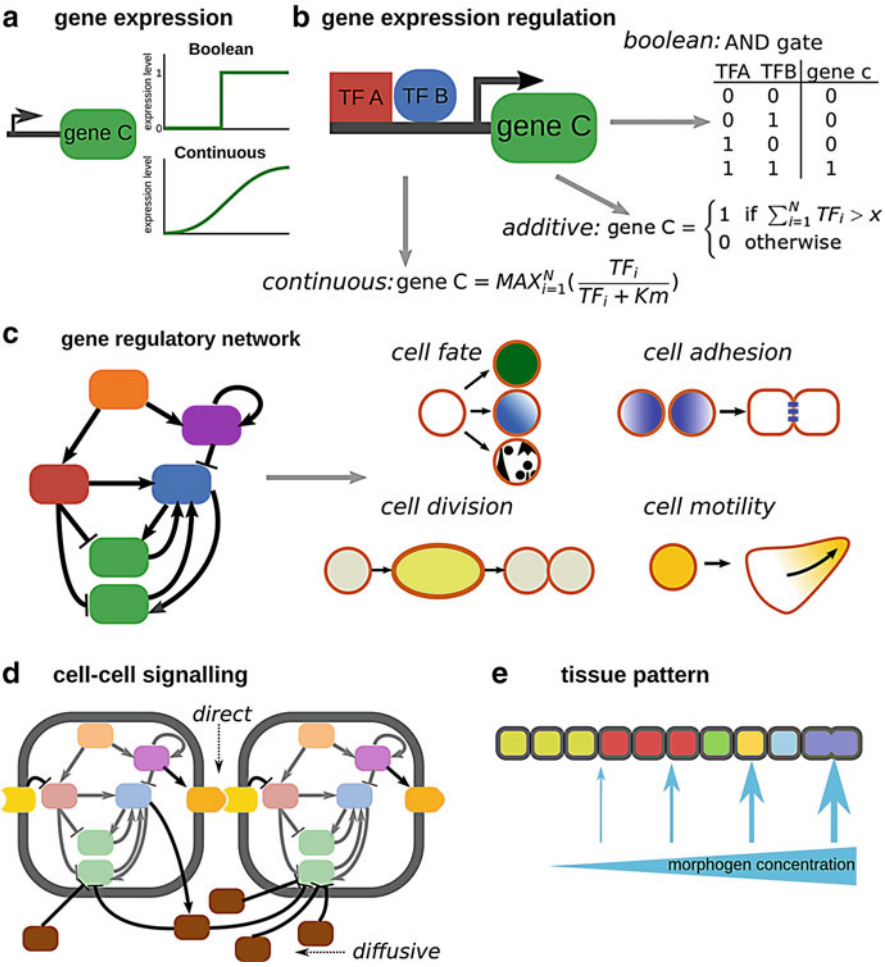


Fig. 1 Overview of the building blocks of evo-devo models

Boolean approach to include multiple discrete expression levels, for examples 0, 1, 2, and 3.

In models applying continuous gene expression, gene expression levels can take on any arbitrary expression value between zero and a superimposed or dynamically evolving maximum. While computationally less efficient, this approach is necessary if more gradual changes in expression are expected to be important for the developmental process under study, for example, a long-time-lag between the switching on and reaching full expression of a gene or the gradual amplification of initially small differences between cells to break symmetry.

Regulation of Gene Expression In multicellular eukaryotes, gene expression is regulated by a complex array of processes. Nuclear localization of the gene and its epigenetic state influence how easily the DNA can be accessed. Next, transcription factors control gene transcription in a complex, combinatorial manner via the promoter near the gene and via potentially multiple, modular enhancers that may even be present on a different chromosome. Finally, post-transcriptional processes like alternative splicing, protein modifications, and regulation of protein degradation are also involved. Current evo-devo models typically consider only the regulation of gene expression via transcription factors binding to the promoter, making use of one of three approaches: Boolean, additive, and continuous nonlinear integration of transcription factor input (Fig. 1B) (although one study did consider alternative control regions (Fujimoto et al. 2008)).

The Boolean approach uses so-called logical functions, or gates to integrate inputs, is typically combined with Boolean modelling of gene expression levels and usually assumes a constant number of transcription factors influencing each gene. For example, an AND gate requires that transcription factor A (TFA) and transcription factor B (TFB) are both expressed for the downstream gene to become expressed, while for an OR gate, the downstream gene becomes expressed if either TFA or TFB or both are expressed (Fig. 1B). To integrate larger numbers of inputs, more complex logical functions and combining of multiple logical functions are necessary. The disadvantage of this approach is that often, only a few regulatory inputs are relevant for the gene output, with others inconsequential due to the switch-like nature of Boolean gene expression. As a consequence, these models frequently overestimate the actual robustness of a developmental mechanisms (reviewed in Spirov and Holloway (2013)).

An alternative approach that is often used in combination with Boolean gene expression levels is additive integration. In this approach, TFs have an assigned weight – a positive value for activating and a negative value for repressive TFs – and gene expression is computed as a weighted sum of the expression levels of the TFs (ten Tusscher and Hogeweg 2009; Wagner 1996). This approach more easily allows for a variable number of TFs influencing each gene. The trade-off is that the additive approach is linear and therefore lacks some of the complex, nonlinear character typical of real gene expression regulation. When additive approaches are combined with continuous gene expression levels, the weighted sum of transcription factor inputs is often mapped to transcription rate via an additional, nonlinear function, thereby overcoming this linearity drawback (Salazar-Ciudad et al. 2001; Vroomans et al. 2016).

A final approach is continuous, nonlinear integration of gene inputs. Although existing in several varieties, they have in common that the input of an individual transcription factor on a downstream gene is modeled via a saturating Hill function (Fig. 1B). This mimics the saturation effect that occurs in vivo, where depending on TF concentration and binding site affinity, beyond a certain threshold all available regulatory sites will be occupied, so that an increase in TF concentration cannot further increase transcription of the downstream gene.

In Silico Gene Expression Dynamics During development, once a cell is formed it starts with an initial gene expression state that subsequently changes. At the start of development, this state is often maternally determined, while cells arising in the course of development typically inherit their state from the parent cell. To simulate this in evo-devo models, new *in-silico* cells are endowed with a particular initial gene expression state, where the extent to which different gene expression levels are discerned depends on the gene level model formalism chosen (Boolean or continuous). For cells already present from the start of *in-silico* development, a predefined, imposed gene expression state is used. Upon division, cells inherit their parental state. The subsequent unfolding of gene expression is dictated by the combination of activating and inhibiting signals from transcription factor genes upstream of each gene. Again, the chosen formalism of the model impacts exactly how transcription factor input is translated into gene expression levels at the next time instance.

Due to the small number of molecules of transcription factors and DNA polymerases, gene expression is an inherently noisy process. Thus, if one, for example, wishes to investigate whether noisy gene expression impacts the type of evolutionary outcomes by imposing selection for *developmental* robustness one needs to incorporate noise. In models with Boolean gene expression levels, noisy expression can be incorporated by using probabilistic update rules. For example, for an AND gate, if TF1 and TF2 are both expressed, the gene will become expressed with a probability of 90%, yet with a probability of 10% it remains not expressed. In case of continuous gene expression, a noise term can be added to the differential equations governing expression dynamics that modulates the average gene expression level (ten Tusscher and Hogeweg 2011).

Cell Behavior The differentiation of cells into distinct cell types is marked by the convergence of different cells on different subsets of stably expressed cell type defining genes. Thus, in evo-devo models, gene expression is required to converge to a stable pattern for successful differentiation. Apart from influencing the particular cell type, gene expression also influences cell behavior: adhesion to neighboring cells and extracellular matrix, growth, division, shape, and motility (Fig. 1C). These processes are crucial for understanding the interplay between tissue growth, morphogenesis, and patterning. Thus far, only a limited number of evo-devo models have incorporated genes affecting cell behavior beyond cell fate determination (Hogeweg 2000; Vroomans et al. 2016). However, recently new model formalisms have been developed to this end (Marin-Riera et al. 2016). Cell division, for example, may be implemented by incorporating a designated division gene whose levels need to exceed a threshold for division to occur (Vroomans et al. 2016). In the case of cell adhesion, the expression of a number of "adhesion" genes may generate a complex, cell-type-dependent adhesion profile (Hogeweg 2000).

Between Cells

Direct Cell-Cell Signalling In many developmental processes, extensive signaling takes place between directly neighboring cells to coordinate their gene expression dynamics. This can be used to minimize differences, as with Delta-Notch-mediated synchronization of the segmentation-clock in vertebrate somitogenesis. Cells can also use signaling to coordinate their polar orientation, as, for example, during *Drosophila* trichome patterning. Conversely, signaling may be used to amplify small initial differences, thereby enabling symmetry breaking. This process is often referred to as lateral inhibition, and, for example, patterns the hair cells of the chick inner ear.

Direct cell-cell signaling, emulating Delta-Notch-type signaling, has been incorporated into a few evo-devo models. To do so, a subset of the modeled genes is designated as signaling – rather than transcription factor genes. To simplify matters, expression of a signaling gene is assumed to directly regulate expression of downstream genes in the neighboring cells but not the cell in which it is expressed (Fig. 1D). Thus, one basically represents an entire signal transduction pathway as a single unit that evolution can use. Modeling separate ligands, receptors, kinases, nuclear receptors, etc., would make it highly unlikely for the *in-silico* evolutionary process to discover a functional cell-cell signaling system. Furthermore, all major signal transduction pathways were present in the evolutionarily most ancient, simple multicellular organisms, and multicellular complexity has mostly increased through the frequent reusage of these modules rather than inventing new pathways from scratch (Chipman 2010). Thus, implementing signaling genes in this simplified manner is deemed a reasonable approach.

A special type of direct cell-cell signaling is cell adhesion, which has been implemented in several developmental models but only a single evo-devo study (Hogeweg 2000). Differential cell adhesion, with cells either preferring to adhere to similar or to different cell types, has been shown to be a major driver of morphogenetic processes such as cell mixing, cell sorting, tissue engulfment (Graner and Glazier 1992), and convergent extension (Vroomans et al. 2015).

Long-Range Cell-Cell Signaling In addition to the short-range cell-cell signaling mediated by membrane bound receptor ligand pairs, long-range signaling mediated by diffusion of signaling molecules plays an important role in development. Well-known examples are the antagonistic FGF and RA gradients involved in vertebrate somitogenesis and the Bicoid gradient in early *Drosophila* development. Long-range signaling can be easily incorporated in evo-devo models by allowing diffusion of some gene products between cells (Cotterell and Sharpe 2010; Fujimoto et al. 2008) (Fig. 1D). Alternatively, morphogen gradients can be

superimposed (Fig. 1E). If one only wishes to investigate evolutionary processes arising after the prior evolution of the morphogen gradient, this latter approach is more computationally efficient (François et al. 2007; ten Tusscher and Hogeweg 2011). If in contrast, the question is how signaling centers and morphogen gradients may evolve, one needs to incorporate that certain genes may evolve the potential to be excreted and diffuse.

Tissue Level

Tissue Structure Developmental processes occur inside the complex, three-dimensional bodies of organisms. However, many developmental processes are restricted to a limited body region (e.g., eye development), occur on a largely flat surface (e.g., patterning wing veins in insects), or along a particular dimension (e.g., patterning along the anterior-posterior axis). This often allows one to focus modeling efforts to particular regions of the body or restrict simulations to two or even one dimension, reducing computational requirements and model complexity. Indeed, in many *in-silico* evo-devo studies of axial patterning, only a 1D tissue is considered, where cells form a single row (Fig. 1E) (Cotterell and Sharpe 2010; François et al. 2007; Fujimoto et al. 2008; Salazar-Ciudad et al. 2001; ten Tusscher and Hogeweg 2011; Vroomans et al. 2016). While such an approach is sufficient to study the basics of how gene regulatory networks underlying axial patterning may evolve, it also has clear limitations. For example, to investigate how patterning mechanisms evolved that ensure coherent boundaries, models should incorporate at least a two-dimensional tissue; Similarly, to take into account how cell movement contributes to patterning, considering higher dimensional tissues is essential.

Tissue Dynamics Depending on the developmental process under study, tissue patterning into different cell types may occur prior to or after processes such as cell division and motion that change overall tissue architecture (coined morphostatic patterning) or co-occur with tissue shape changes (morphodynamic) (Salazar-ciudad and Jernvall 2004). Axial patterning coincides with tissue growth and extension, and depending on the animal under study may also coincide with convergent extension (Vroomans et al. 2015). Still in many evo-devo studies this growth process is ignored and a fixed-size, one-dimensional tissue architecture is used (Cotterell and Sharpe 2010; François et al. 2007; Fujimoto et al. 2008; Salazar-Ciudad et al. 2001; ten Tusscher and Hogeweg 2011). In many cases, this is a reasonable approximation when no major reorganization of tissue occurs. However, cell division and tissue growth need to be explicitly incorporated in a model if one wishes, e.g., to investigate how the process of posterior elongation itself evolved. Depending on the exact research question, this incorporated growth process can be either imposed or regulated by the GRN (Hogeweg 2000; Vroomans et al. 2016) with levels of a designated gene deciding whether a cell is ready for division.

Tissue Pattern Development Given that all cells in a multicellular organism share the same genome, and hence the same regulatory networks governing gene expression dynamics, an initial symmetry breaking event is essential to enable different cells to obtain different fates. A famous example is the maternally deposited Bicoid mRNA in *Drosophila* that gives rise to a protein morphogen gradient via diffusion and enables different cells to start expressing different sets of genes. Somewhat similar to this, in sequentially segmenting animals such as vertebrates, but also in the beetle *Tribolium*, segmentation is controlled by gradients arising from the localized production of a stable mRNA or protein combined with localized growth. Alternatively, as is the case in, for example, *C. elegans,* development, fertilization may trigger a polarization process leading to the asymmetric division of the zygote into two cells with distinct fates.

In many evo-devo studies, the research question concerns developmental patterning downstream of the initial symmetry-breaking event. In this situation, simply superimposing the symmetry-breaking signal, such as a morphogen wavefront (ten Tusscher and Hogeweg 2011), differential gene expression (Salazar-Ciudad et al. 2001), or gradient (François et al. 2007; Fujimoto et al. 2008; Vroomans et al. 2016) is a valid approach. However, if the research question is concerned with the evolution of this symmetry-breaking event, either noisy gene expression or initial but non persistent differences between cells should be implemented, to investigate how these can be exploited by the *in-silico* evolutionary process as a trigger for symmetry breaking (Vroomans et al. 2016).

Evo-Devo Models

The field of evo-devo aims to answer how and why particular developmental mechanisms evolved. To illustrate how models have been used for this purpose, we will focus on a well studied developmental process: the subdivision of the animal anterior-posterior (A-P) axis into regular, repeating segments. The property of a segmented major body axis is shared among the distantly related vertebrate, arthropod and annelid clades. Furthermore, a number of animals in other clades seem to have a repeated A-P pattern (metamers) in some embryonic tissues. Most segmented animals generate their repeating units in a regular, sequential, anterior-to-posterior fashion from a posterior growth zone. Within the arthropods however, certain unrelated species develop their body segments simultaneously, the most famous example being the fruitfly *Drosophila*.

Together, these observations lead to many evolutionary questions. For example, it is still debated whether sequential segmentation evolved at least three times in parallel, or evolved once in the ancestor of bilateral animals and was subsequently fully or partially lost in many clades. For the first case, an obvious followup question is why this particular developmental mode would have evolved multiple times. Another major open question is why *Drosophila* uses such a complex, hierarchic regulatory cascade, where each segment is patterned by a unique combination of

genes. These questions make animal axial segmentation an excellent evo-devo study case, and computational modeling has been widely applied to it.

When answering *why* questions in evo-devo, we need to distinguish between why a particular developmental pattern – such as a segmented body axis – arose, and why a particular mechanism generating that pattern arose (see chapter ▶ "Proximate Versus Ultimate Causation and Evo-Devo"). The first question is hard to answer because it ultimately requires us to answer what purpose the developmental pattern may have originally served. In case of segments, perhaps there was selection for a larger body size, and segments were a simple, modular way to achieve that goal. Alternatively, there may have been selection for improved locomotive control of a large body, with segmental modules allowing independent control of different body regions.

Evo-devo models are particularly well suited for answering the second type of why questions. Central to answering these questions is an understanding of the nature of the genotype-phenotype map, and how it is molded by evolutionary processes. In biological organisms, the mapping of the genome into a phenotype via regulatory network architecture, gene expression dynamics, cell behaviour and developmental process is highly complex and non-linear. Since developmental models explicitly incorporate this genotype-phenotype mapping, they enable us to investigate which mutations are being buffered by the overall network dynamics and hence have no phenotypic effect, and which mutations cause a full collapse of the phenotype because they affect a regulatory hub impacting a large part of the network.

These models also allow us to determine -within the given boundary conditions- how many different types of developmental mechanism exist to generate a particular pattern and how often these different mechanisms occur. This may indicate that certain developmental mechanisms are more likely to occur than others. We can compare these different developmental mechanisms in terms of robustness to determine fitness advantages of one mechanism over the other. Alternatively, we can investigate their evolutionary nearness in terms of number of mutations and fitness of intermediate genotypes to assess the likelihood of evolutionary drift between equivalent mechanisms.

Finally, in models explicitly simulating the evolution of developmental processes we can investigate how mechanisms differ in evolvability, the ease in which evolution discovers and subsequently extends them. In these models we can trace how evolution shapes the genotype-phenotype mapping, tuning robustness and evolvability, and how this impacts the potential for incremental evolution of complex patterning.

Different Approaches

There are three main approaches to studying evo-devo questions with computational models. First, one can simulate the developmental process of interest, focusing on the robustness of the mechanism to noise in gene expression or mutations. With

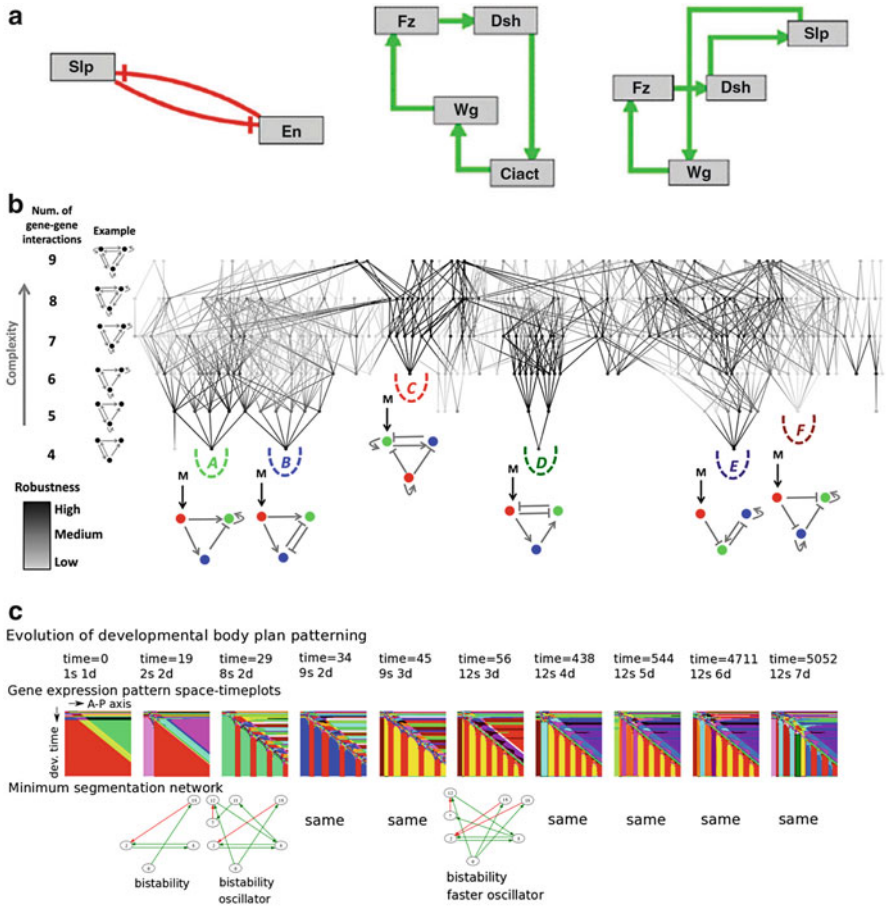


Fig. 2 *Three different approaches*. (**a**) The functional intracellular gene regulatory motifs identified for *Drosophila* segmentation gene network (Image from Sánchez et al. 2008). (**b**) The meta-network for segment-producing mechanisms. The letters indicate groups of networks that differ in developmental mechanism (Image from Cotterell and Sharpe 2010). (**c**) The *in-silico* fossil record of the evolution of a segmentation mechanism. Top row: the space-time plots of the development of several individuals in the evolutionary simulation (horizontal: space, vertical: developmental time). The colours indicate the different cell types. Bottom row: The corresponding minimal evolved gene regulatory networks that generate the cellular dynamics (Image adapted from ten Tusscher and Hogeweg 2011)

regards to body axis segmentation, such studies have been performed for both the pair rule and segment polarity networks (Sánchez and Thieffry 2003; Sánchez et al. 2008) (Fig. 2A). For both networks, the presence of mutual repression between genes was identified to play a major role in generating robust network dynamics. These results could be taken to suggest that these particular patterning mechanisms were selected for their high robustness. However, in absence of a comparison with

alternative patterning mechanisms resulting in similar downstream phenotypes, no strong claims of larger robustness than expected can be made.

A second approach is the so-called ensemble approach. In this approach, one investigates either all possible topologies of small size networks, or a large collection of randomly generated networks of a particular size (Cotterell and Sharpe 2010; Jiménez et al. 2015; Solé et al. 2002). Typically, the networks are sorted based on both the phenotype and the underlying developmental mechanism they encode. This method is efficient at finding many, if not all possible mechanisms for generating a certain phenotype, making it easier to compare them. For the small networks for which all possible topologies can be investigated, a meta-network can be created that connects similar gene regulatory networks (separated by a single difference) generating the same phenotype (Fig. 2B).

This meta-network has been used to study the mutational robustness of segmentation mechanisms, as this is determined by the number of interconnected networks generating the same mechanism (Cotterell and Sharpe 2010). Based on this approach, an alternative Turing-type mechanism for vertebrate segmentation was proposed which was found to be more robust than the classical clock-and-wavefront mechanism generally assumed to govern somitogenesis (Cotterell et al. 2015). The ensemble approach has also been used to study the evolvability from one segment-generating mechanism to the next, either for different mechanisms producing the same (Cotterell et al. 2015) or different phenotypes (Jiménez et al. 2015). A drawback of the ensemble approach is that it is thus far only feasible for small networks, that can perhaps best be interpreted as motifs of realistic, more complex developmental networks.

A final approach is to explicitly simulate the evolution of a developmental process (Fig. 2C). Darwinian evolution arises from the combination of reproduction with inheritance of parental properties, mutation to produce variety in offspring relative to parents, and selection which biases reproduction and survival to better adapted individuals. Simulating these processes requires the simulation of a population of individuals over many generations, imposing significant constraints on the complexity of the developmental process within a single individual that can be modeled.

In Silico Evolution

To build models that simulate Darwinian evolution, critical choices are the nature of the genome, the mutations operating on it, and the applied fitness criterion. In most evo-devo studies, the gene regulatory network is also considered the genome, and mutations operate directly on this network. Some studies, however, explicitly model a genome with genes and transcription factor binding sites, which encodes a gene regulatory network. Mutations then occur on the genome rather than on the regulatory network. Although this seems a minor difference, it may have important consequences for the evolutionary dynamics by impacting the mapping from genotype to phenotype.

In terms of mutations, most evo-devo models consider mutations that change which TFs influence a target gene, whether this influence is activating or repressive, the strength of this influence, deletion of a regulatory interaction, and insertion of a new regulatory interaction. In addition, some models incorporate mutations changing the maximum expression and degradation rate of a gene (Vroomans et al. 2016) and the diffusion constant of a gene product (Fujimoto et al. 2008). Finally, the models with an explicit genome incorporate duplication and deletion of genes, thereby allowing for variations in genome size. This substantially increases the degrees of freedom for the evolutionary process, and may hence impact the findability and evolvability of more complex developmental mechanisms. By implementing gene duplications such that the regulatory regions are duplicated together with the genes, evolution can tinker with one regulatory module, while another functional copy can be maintained.

These higher-level mutations have been suggested to increase evolvability (Spirov and Holloway 2013). However, large and complex genomes and networks may arise as a side effect of these extra degrees of freedom, with a high level of redundancy and many genes and interactions that have little effect. Unraveling how these genomes and networks translate into the observed developmental dynamics and final tissue pattern in these cases often requires pruning of the genomes and networks to identify the core mechanism.

In evo-devo simulations, a fitness criterion is typically used to ensure that the developmental pattern of interest evolves. The fitness score of an individual determines the reproduction rate of that individual, while leaving its death rate constant. Thus, in case of evo-devo studies which focus on body axis segmentation, fitness criteria evaluate the segmental pattern generated at the end of the development of an *in-silico* individual. However, different criteria may be applied, which vary in specificity. For example, one may simply let fitness increase with the number of generated segments, select for a particular number of segments, or even select for a particular spatial pattern of segments. The stricter the target, the more difficult it will be to evolve the desired phenotype because fewer evolutionary routes with intermediate fitness steps will be available (ten Tusscher 2013). Still, such a strict target may be important if one wishes to investigate how the regulatory mechanism changed due to evolutionary systems drift, while the developmental outcome remained constant (see chapter ► "Developmental System Drift").

A number of studies applied evolutionary simulations to evo-devo questions on segmentation. Collectively, these studies show that only a few distinct classes of mechanisms evolve for generating segments, and that which class emerges strongly depends on the applied morphogen dynamics and fitness criterion (reviewed in ten Tusscher (2013)). When the fitness criterion is very strict and/or the morphogen consists of a non-moving peak or gradient, segments are typically generated all at the same time. The mechanism used entails either a hierarchical cascade of gene expression involving many regulatory genes that mostly interact unidirectionally, or a self-organised emergent mechanism involving a limited number of mutually

interacting genes (François et al. 2007; Fujimoto et al. 2008; Kohsokabe and Kaneko 2016; Salazar-Ciudad et al. 2001).

The models show that while the emergent mechanisms more easily generate a larger number of segments the hierarchic mechanism is more robust to mutations as different segments depend on different genes. As a consequence, hierarchic mechanisms tend to replace emergent mechanisms over longer evolutionary time. The *in-silico* hierarchical mechanisms do to some extent resemble the *Drosophila* segmentation cascade. As such, the fact that they evolve under strict fitness criteria supports the idea that *Drosophila's* mechanism is secondarily evolved – hence, segment positions had to be strictly maintained relative to those generated by the ancestral mechanism (ten Tusscher 2013).

When, instead, simulations applied more general fitness criteria (supporting de-novo evolution of stripe patterning), and the morphogen was simulated to retract from anterior to posterior (emulating posterior growth), the most common evolutionary outcome is a sequential segmentation mechanism. This mechanism involved a continuous A-P transition from gene expression oscillations to a fixed segment pattern (François et al. 2007; ten Tusscher and Hogeweg 2011; Vroomans et al. 2016). By using the *in silico* fossil record generated in these simulations, it could be shown that this complex developmental mechanism evolves through the incremental evolution of network motifs, first generating bistability, then an oscillator and subsequently a sped up oscillator increasing the number of segments generated (François et al. 2007; ten Tusscher and Hogeweg 2011).

Thus, these studies provide powerful counterargument against the argument of irreducible complexity that is often made for complex novel phenotypes. Furthermore, by suggesting that evolution of sequential segmentation is relatively straightforward, they support the possibility of parallel evolution of this segmentation mode in the vertebrate, annelid and arthropod clades. These simulations also demonstrated that, compared to alternative mechanisms that occasionally evolved *in silico*, the sequential mode evolved more rapidly, was more evolvable and was more robust to noise in gene expression and division timing, and to mutations (François et al. 2007; Fujimoto et al. 2008; ten Tusscher and Hogeweg 2011; Vroomans et al. 2016).

Together, these studies thus suggest that, when growth occurs through posterior elongation, sequential segmentation is the expected evolutionary outcome. However, they leave open the question whether sequential segmentation is still the most likely evolutionary outcome if posterior elongation has not yet evolved (and therefore has to co-evolve). To address this, Vroomans and ten Tusscher (Vroomans et al. 2016) performed evo-devo simulations in which they selected both for axial growth and segmentation. In these simulations two mechanisms evolved, one in which growth and patterning occurred at the same time and across the entire tissue, and one in which both occurred sequentially from a posterior growth zone.

The simultaneous mechanism evolved tissue growth and segmentation concurrently, with new segments evolving as the tissue evolved to become larger. The sequential mechanism instead first evolved a large tissue and then evolved more and more segments. The simultaneous mechanism was the dominant outcome in simulations where only a transient morphogen signal was provided, while the sequential mechanism dominated when the morphogen was assumed to be maintained at a high

level in the posterior-most cell. Given the predominance of posterior elongation and sequential segmentation in extant organisms, these results suggest that these growth and segmentation modes arose after the earlier evolution of a posterior signalling center.

Discussion and Concluding Remarks

Most evo-devo research addresses *what* and *how* questions – what specific developmental mechanisms have evolved and how these have come about through mutations and selection. In addition to these types of questions, evo-devo modeling studies aim to also address *why* questions. We have shown here how computational models of evolution of development are constructed, and how they may provide deeper insights in why extant organisms use particular developmental mechanisms and not a theoretically possible alternative mechanism.

Answers that evo-devo models may provide could be that certain mechanisms are more easy to find for an evolutionary process given the nature of biological mutations or the prior evolutionary history and hence are statistically more likely to appear as an evolutionary outcome. Model outcomes may also demonstrate that certain mechanisms are more robust against mutations or developmental noise and therefore confer secondary fitness advantages, enabling them to evolutionary outcompete alternative patterning mechanisms. Finally, simulations may show that the need for coordination with simultaneously occurring other patterning mechanism may affect evolutionary outcome.

Constructing evo-devo models ultimately entails defining a genotype-phenotype mapping and how this mapping can be changed through evolution. Like all models, evo-devo models are by necessity simplifications. The choices made in terms of simulating gene expression, cell behaviour and tissue dynamics may affect the number and type of mechanisms that can generate a certain pattern in silico. As an example, in absence of diffusing gene products, no Turing-type patterning can arise. Similarly, choices for initial conditions, genome structure and mutational operators may affect evolvability and the potential for evolution to shape the genotype-phenotype mapping. Additionally, the choice for Boolean versus continuous gene expression modeling may substantially affect cellular differentiation dynamics and robustness of patterns to perturbations, again affecting what types of evolutionary outcomes are most likely to arise and persist. Thus, ideally, conclusions obtained in evo-devo modeling studies should be tested for their dependence on the modeling choices made.

As an example, if a study suggests the predominant evolution of a particular patterning mechanism, it is important to determine whether this depends on modeling assumptions or truly is a general outcome. If it is a general outcome, we can safely conclude that this particular mechanism is the expected evolutionary outcome. If instead the mechanism only dominates if particular assumptions are made, for example, the presence of a certain signaling center, this may reveal the critical dependence of the evolution of a trait on prior evolutionary events or certain aspects of the developmental genetic toolkit.

In this light, it is important to consider that while current evo-devo models are already quite complex, they do not yet incorporate major properties of metazoan genetic regulation. Incorporating cooperative activation and repression by nearby bound transcription factors, regulation by multiple modular enhancers and epigenetic regulation is likely to further increase the complexity of the genotype-phenotype mapping and the potential for evolution to fine-tune this mapping. It will be interesting to see how this may effect earlier modeling conclusions, and how it will increase our ability to explain how evolution converged to the developmental patterning mechanisms observed in extant organisms. Additionally, current evo-devo models focus on regulatory mutations affecting developmental patterning. Except for gene duplications, they ignore coding region mutations that could expand and modify the genetic toolkit available for development. Incorporating these types of mutations in evo-devo models is necessary to contribute to the debate on the relative importance of mutations in coding versus regulatory regions in evo-devo.

Cross-References

- ▶ A Macroevolutionary Perspective on Developmental Constraints in Animals
- ▶ Convergence
- ▶ Computational Modeling at the Cell and Tissue Level in Evo-Devo
- ▶ Evolvability
- ▶ Levels of Organization in Evo-Devo
- ▶ Modeling and Simulation in Evo-Devo
- ▶ Proximate Versus Ultimate Causation and Evo-Devo
- ▶ The Evolution and Development of Segmented Body Plans

References

Chipman AD (2010) Parallel evolution of segmentation by co-option of ancestral gene regulatory networks. BioEssays 32(1):60–70

Cotterell J, Sharpe J (2010) An atlas of gene regulatory networks reveals multiple three-gene mechanisms for interpreting morphogen gradients. Mol Syst Biol 6(1)

Cotterell J, Robert-Moreno A, Sharpe J (2015) A local, self-organizing reaction-diffusion model can explain somite patterning in embryos. Cell Syst 1(4):257–269

François P, Hakim V, Siggia ED (2007) Deriving structure from evolution: metazoan segmentation. Mol Syst Biol 3(1)

Fujimoto K, Ishihara S, Kaneko K (2008) Network evolution of body plans. PLoS One 3(7):e2772

Graner F m c, Glazier JA (1992) Simulation of biological cell sorting using a two-dimensional extended potts model. Phys Rev Lett 69:2013–2016

Hogeweg P (2000) Evolving mechanisms of morphogenesis: on the interplay between differential adhesion and cell differentiation. J Theor Biol 203(4):317–333

Jiménez A, Munteanu A, Sharpe J (2015) Dynamics of gene circuits shapes evolvability. PNAS 112(7):2103–2108

Kohsokabe T, Kaneko K (2016) Evolution-development congruence in pattern formation dynamics: bifurcations in gene expression and regulation of networks structures. J Exp Zool B Mol Dev Evol 326(1):61–84

Marin-Riera M, Brun-Usan M, Zimm R, Välikangas T, Salazar-Ciudad I (2016) Computational modeling of development by epithelia, mesenchyme and their interactions: a unified model. Bioinformatics 32(2):219–225

Salazar-ciudad I, Jernvall J (2004) How different types of pattern formation mechanisms affect the evolution of form and development. Evol Dev 6(1):6–16

Salazar-Ciudad I, Newman SA, Solé RV (2001) Phenotypic and dynamical transitions in model genetic networks I. Emergence of patterns and genotype-phenotype relationships. Evol Dev 3(2):84–94

Sánchez L, Thieffry D (2003) Segmenting the fly embryo: a logical analysis of the pair-rule cross-regulatory module. J Theor Biol 224(4):517–537

Sánchez L, Chaouiya C, Thieffry D (2008) Segmenting the fly embryo: logical analysis of the role of the segment polarity cross-regulatory module. Int J Dev Biol 52:1059–1075

Solé RV, Salazar-Ciudad I, Garcia-Fernández J (2002) Common pattern formation, modularity and phase transitions in a gene network model of morphogenesis. Physica A 305(34):640–654

Spirov A, Holloway D (2013) Using evolutionary computations to understand the design and evolution of gene and cell regulatory networks. Methods 62(1):39–55

ten Tusscher K (2013) Mechanisms and constraints shaping the evolution of body plan segmentation. Eur Phys J E 36(5):1–12

ten Tusscher KH, Hogeweg P (2009) The role of genome and gene regulatory network canalization in the evolution of multi-trait polymorphisms and sympatric speciation. BMC Evol Biol 9(1):159

ten Tusscher KH, Hogeweg P (2011) Evolution of networks for body plan patterning; interplay of modularity, robustness and evolvability. PLoS Comput Biol 7(10):e1002208

Vroomans RMA, Hogeweg P, ten Tusscher KHWJ (2015) Segment-specific adhesion as a driver of convergent extension. PLoS Comput Biol 11(2):1–24

Vroomans RMA, Hogeweg P, ten Tusscher KHWJ (2016) In silico evo-devo: reconstructing stages in the evolution of animal segmentation. EvoDevo 7(1):14

Wagner A (1996) Does evolutionary plasticity evolve? Evolution 50(3):1008–1023

Part X

Evo-Devo and Population Genetics

Developmental Evolutionary Biology (Devo-Evo)

Mihaela Pavličev

Contents

Abstract

The field of evolution and development consists of two main approaches, the first, the evolutionary developmental approach studying changes in development, and the second, the developmental evolutionary approach, studying how developmental properties affect the evolutionary process, thus using a systems-biological approach to integrate development into evolutionary theory. The evolution of development has been covered and conceptualized generously. In contrast, in spite of single highly influential concepts, such as variational modularity or robustness, there are only rare attempts to solidify the conceptual basis of developmental evolutionary biology, or devo-evo. Moreover, the opinions diverge strikingly as to whether there is a distinction between the two at all or whether devo-evo is not the actual core of the field anyhow. The naming and the

M. Pavličev (✉)
Department of Evolutionary Biology, Unit for Theoretical Biology, University of Vienna, Vienna, Austria
e-mail: mihaela.pavlicev@univie.ac.at

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_104

primacy issues aside, it is argued here for acknowledging a clear distinction between the two approaches, to what I refer to here as evolution *and* development, for the purpose of clarity. I show that the roots of devo-evo go far back, even to the early years of the Modern Synthesis, and discuss some of the main insights that were necessary to crystalize the approach as an autonomous one. Finally, I outline the agenda of developmental evolutionary biology and its past and future questions.

Keywords

Modularity · Robustness · Evolution of dominance · Variability · Evolvability

Introduction

The discipline of evolutionary and developmental biology as an organized research program that we know today has grown out of the general sentiment that the mainstream formulation of evolutionary theory – essentially the population genetic formulation of the Modern Synthesis (MS) – neglects the role that the organism plays in shaping the evolutionary process, ostensibly to the detriment of MS's explanatory power (see chapter ▶ "Evo-Devo's Contributions to the Extended Evolutionary Synthesis"). By "organism" is meant the organizing context generated by development and physiology, through which alleles realize their fitness contributions given a certain environment. It is emphasized that reducing evolution to changes in allele frequencies requires us to assume that the organismal context is either invariant or without consequence, i.e., that it allows for an arbitrary heritable variation to arise. Both of these assumptions are valid for some, but not all evolutionary change. Further criticisms include those of leaving important evolutionary phenomena, such as innovation (see chapter ▶ "Developmental Innovation and Phenotypic Novelty"), plasticity (see chapter ▶ "Developmental Plasticity and Evolution"), or gene-environment interaction (see chapter ▶ "Eco-Evo-Devo") out of sight, to name a few.

Today the community of researchers and thinkers in evolutionary and developmental biology is firmly established, with its own institutions: societies, university courses, focused events, scholarships, and professorships. In this community, the central idea to consider the organism when addressing evolutionary change is clearly shared. There is less uniformity however in *how* the integration is to be pursued (see chapter ▶ "Interdisciplinarity in Evo-Devo"). The rationales put forward in favor of the importance of development (and physiology) in the evolutionary process and therefore in evolutionary explanation are manifold, but can, for the present purpose, be roughly summarized in two leading notions:

1. Any phenotypic change must be mediated by a change in the way the phenotype is generated during development.
2. Development determines what kind of variants can be produced in the first place and therefore can be accessible to selection.

These two notions reflect what today are somewhat different foci and correspondingly different research programs addressing the role of development in evolution. The first one focuses on the evolutionary change in development itself. The second one addresses how the features of development, by determining the kind of variation that a particular developmental system is able to generate, influence their microevolutionary process – linking conceptually the developmental structure with microevolutionary processes.

The views about the status of the two approaches differ somewhat. For example, Hendrikse et al. (2007) very explicitly call for the ability of the developmental system to evolve (the nr. 2 above) to be *the* central question driving research in the *whole* discipline. Indeed, many researchers would claim that this is in fact what the field *is*. It would however be hard to overlook that the present community casts its research much broader and that an important, and large, portion of the community addresses how development itself evolves, using a comparative approach (nr. 1 above). Acknowledging that there exist two distinct motivations in the community, rather than claiming primacy for either one, can hopefully assist in developing the clarity of research scope. After all, the two approaches are certainly conceptually continuous, as the evolutionary change in development will eventually also change the way development affects the propensity to evolve.

Others have argued for the above distinction before (Hall 2000; Müller 2007; Wagner 2000), and the two approaches have originally been captured under different names: evolution of development (evo-devo) and developmental evolution (devo-evo). The community eventually adopted the name evo-devo for the whole discipline. To avoid misunderstandings when referring to either of the two approaches (evo-devo and devo-evo), I will in this paper refer to the discipline as a whole (i.e., encompassing both approaches), as "evolution and development."

Here I will focus on developmental evolution – an approach that has experienced less conceptual attention than that of the evolution of development. As will be shown, the approach has nevertheless been productive all along and is of central importance in achieving a true integration of development into existing evolutionary theory. I will begin by briefly outlining the two approaches. Next I will point to two older lines of research that show that the roots of developmental evolutionary biology reach far back, even to the early decades of the Modern Synthesis. This also demonstrates how much potential for integration has existed at least since the 1980s, even if it has not been acknowledged or fully developed at the time.

The Two Approaches in Evolution and Development

The first of the two focal questions above asks how a particular developmental feature *has originated* or *has changed*. This question is usually answered by elucidating developmental mechanisms and comparing them across species to find differences and commonalities, in much the same way as adult traits can be compared across species to understand their change. From this approach, we can determine the type of change in development (e.g., heterochrony, heterotopy, but also associated changes in DNA) that generated differences in the adult phenotype.

The evolution of development (evo-devo) approach does not aim at integrating development into the MS evolutionary theory. It explicitly addresses the evolutionary changes in domains where MS assumptions (such as constant developmental architecture) do not hold. Note that evolution of development does not exclude microevolution. Well-established research attempts to explain the evolutionary changes in development, at least among closely related species, by microevolutionary means, focused on the role of selection (see chapter ▶ "Micro-Evo-Devo").

In contrast, the developmental evolution approach (devo-evo) addresses how the features of developmental systems, by determining the kind of variation that can be generated, consequently influence the microevolutionary process. The well-known concepts belonging to this approach are robustness and modularity, describing the structure of the genotype-phenotype map that enhances or constrains the potential to vary and respond to selection in certain directions. Also questions on the role of context-dependency of variation, such as canalization, are part of this body of thinking. This second approach aims at integrating development into evolutionary theory, but in contrast to MS, explicitly addresses questions about the organism, i.e., the mediator between genes and fitness.

Early Research Lines in Developmental Evolution

Before discussing the core work of developmental evolution, it is worth pointing out that general attempts to integrate systems properties into evolutionary thinking appeared early. A very explicit proponent was, for example, Rupert Riedl (1975). Yet even within the MS, interest in developmental systems properties was present among its core architects – then mostly to do with the explanation of dominance of the wild type over the mutant. They again became particularly interesting in the 1980s – along with the initiation of the main evo-devo agenda. Two important examples will be presented here. Although not quite formulated the same way, they show the ideas of developmental system thinking very clearly.

Minding Physiological Constraints

In 1981, Kacser and Burns in their seminal paper on the mechanistic basis of dominance directly addressed the interface between the physiological processes and the population-level phenomenon (Kacser and Burns 1981). In this paper, the authors showed that the manifestation of dominance of the wild-type allele (the average heterozygote being phenotypically closer to the wild-type homozygote than to the mutant homozygote) is a direct consequence of the buffering kinetics of the long enzymatic chains and requires no additional external explanation, such as selective advantage. Kacser and Burns thus addressed a long-standing controversy about dominance evolution, going back to the discussions of Fisher, Wright, and Haldane, in which Wright proposed that the dominance of wild type is inherent to physiological structure (Wright 1934).

By their work, Kacser and Burns initiated the field of metabolic control theory, the systems study of enzyme kinetics. Several authors to follow have noted the opportunities that the connection of this field with population genetic principles may bring to evolutionary biology and have subsequently investigated metabolic chains with respect to various population-level phenomena such as the maintenance of variation in mutation-selection balance, selection response, the origin of variational pleiotropy in branching pathways, or epistasis (e.g., Clark 1991; Keightley and Kacser 1987; Szathmary 1993). The place of genotype-to-phenotype mapping here is taken by the physiology, which plays the same role as development does in other models – they both translate genetic variation into phenotypic variation in a highly structured way. Perturbations are introduced to the parameters in the model of metabolic pathway (e.g., the maximal velocity at which an enzyme can turn one substrate into another), representing the mutational effects. The observed variables are either flux, the maximal velocity of reaction transforming one substrate into another, or the pool, i.e., the concentration of single substrates. These "phenotypes" are recorded when the reaction reaches a dynamical equilibrium in the model, that is, when the amounts of products of single steps in the chain (i.e., pools) stop changing.

This field largely lost its intensity, but it, in principle, directly addressed the interface between the structure of the genotype-phenotype map and its variational properties, questions that can be traced back to the work of Wright on dominance in the 1930s. There are recent works that follow up on this way of thinking, in particular in gene regulation as will be shown below.

Minding Mutational Anisotropy

Another example shows how far the integration of development has come already in the 1980s – even if this may have become obvious only in retrospect. This example pertains to the independent works of Pere Alberch and Russ Lande, two contemporary figures frequently seen as representatives of rather different approaches to evolutionary biology.

Alberch was a passionate and prolific advocate for the role of development in evolution. His and Emily Gale's work on early perturbations of growth in limbs of salamander and frog is the first experimental demonstration of the role that development plays in translating early developmental perturbation (proxy for mutational effects) to phenotypic variation (see chapter ▶ "Pere Alberch (1954–1998)"). They showed that the same perturbation leads to different effects in frog and salamander, the difference corresponding to their diverged, species-specific developmental sequence – meaning that the pattern of variation that we observe among species is not the sole product of selection acting on ubiquitously present (i.e., isotropically distributed across traits) mutational variation, but rather that variation is already patterned in the developmental system in which it arises, before it is selected. Consequently, selection cannot be invoked as the sole evolutionary explanation of the phenotypic patterns of evolution but is rather only a part of the story.

At about the same time, Russ Lande conveyed a closely related idea in a quantitative genetic model: he expressed the distribution of mutational variation across traits as it arises *before* selection (**M** matrix) as a separate conceptual entity from the distribution of variation *after* selection (**G** matrix) (Lande 1979). The explicit separation of multi-trait **M** and **G** matrices enabled formulating assumptions about the distribution of the effects of mutation on the phenotypic traits, separate from the effect of selection. This corresponds precisely to the effects that Alberch demonstrated in comparative experimental embryology. Consequently, at least since 1979, the mutational isotropy – a notion often criticized from the standpoint of evolution and development – is not in fact a constitutive assumption of population or quantitative genetics at all. That is, the quantitative genetic models do not *require* the assumption of isotropy, in order to work. The structuring of mutation by development can readily be introduced into such models in terms of **M** and its consequences for the evolutionary dynamics studied. Whereas experimental work addressing mutational matrices is challenging, it is not absent (e.g., Zhu et al. 2014).

The Core Concepts of Developmental Evolution

Developmental structure influences the potential to evolve by modulating the expression of heritable variation in any phenotypic direction. Whether it thereby causes an enhancement or constraint depends on whether it funnels the heritable variation in the direction of selection or away from it, and whether it buffers variation from being exposed or enhances its expression. The focus of developmental evolution on how development affects evolutionary change thus aims to understand the potential of organisms to vary in the context of their complex developmental structure (see chapter ▶ "Evolvability"). Developmental evolution conceptualizes adaptive change as an interaction between the existing developmental system and selection.

Two important conceptual insights were key to establishing developmental evolution as a field in evolutionary biology: the consideration of the genotype-phenotype map independently of fitness and a distinction between variation and variability (Wagner and Altenberg 1996). Emancipating the GP component of the gene-to-fitness (G-P-W) mapping made it possible to study phenotypic diversity as such and the interaction between GP map structure and selection in the first place. Distinguishing between segregating variation and variability enabled grasping the long-term effect of the developmental system on the course of evolution. Variation thereby refers to the phenotypic effects of presently segregating mutations in a population, whereas variability refers to the propensity of the genome to generate variation through the developmental system. Population genetics generally focuses on segregating variation in a population, and its effects on variation in fitness, whereas it is blind to the processes that do not vary, and also to any consistency or bias of future mutations. In contrast, developmental evolution points out that the effect of developmental structure is not limited to presently segregating variants but

has persistent effects in future mutations. Development is thus actively involved in structuring evolutionary change.

What are these effects of development and how do they affect the potential to evolve? The field of developmental evolution worked out several recurring systemic properties of development and physiology that modify the ability to respond to selection, such as variational modularity and robustness. More cell-physiologically oriented authors Gerhart and Kirschner (2007) add to these several physiological principles such as developmental autonomy or exploratory behavior leading to the same kind of effect.

Together these properties are proposed to increase the ability to respond to selection beyond what would be expected under random mutation and selection, and these ideas have led to an immensely progressive and productive way to study the role of genotype-phenotype mapping in the evolutionary process. Much recent research in this field is dedicated to detecting, confirming, or rejecting the presence of modularity of robustness in the population, determining the degree of its distinctiveness, or developing methods to do so.

Other work is focused on developing this field further. Modularity and robustness inherit a coarse-grained view of the G-P map of the conventional quantitative genetics – in part due to limited information about the exact system-wide mechanisms of development and physiology. In such models of the GP map, organizational levels, genetic interdependencies, and physical interactions are not ignored (Cheverud 1984), but they are necessarily subsumed into average effects of mutational change onto the end phenotype. For example, a mutation causing covariation in two traits is called pleiotropic, regardless of the underlying mechanisms; no distinction is made at this level between impacting both traits directly and impacting the second trait indirectly by the change in the first one. At the molecular level, there can be many more and finer-grained distinctions. In other words, the developmental structure is reflected in the variational pattern, but whole classes of different developmental structures can generate the same variational pattern (Cowley and Atchley 1992).

Some of the discussions on the role of development have been played out on the distinction between additive and nonadditive portions of variation. Given the ubiquity of physical interactions (between proteins, metabolites, enzymes) in physiological and developmental systems, it is sometimes hard to believe that this complexity can be meaningfully summarized by the variational patterns of additive effects. Rather one is tempted to conclude that since nonlinearity of developmental processes is prevalent, this *must* lead to largely nonadditive genetic variation. However, the relationship between physical interaction and nonadditivity of variation is less straightforward. Already in simple model systems, the perturbations of physically interacting factors can translate into large proportions of additive genetic variation (Gjuvsland et al. 2013; Hansen and Wagner 2001; Pavlicev et al. 2016). Yet another role of development often discussed is that of multifunctional genes and the variational constraint they introduce. Given the predominance of gene reuse in different developmental and physiological contexts, one is tempted to think that covariance and constraint between traits *must* be ubiquitous. Yet even highly multifunctional

factors can generate much independent genetic variation of characters. For example, it is well-known that modularization of total variance does not require a lack of shared genes but can arise in a very delicately structured GP map, with much pleiotropy, or by epistatic compensation (Cheverud 1984).

These considerations lead to the question: do the developmental details matter for the potential of organisms to evolve, and if so, to what extent and how? More technically, different GP maps may generate the same variance, but do they also generate the same variability and the same potential for long-term evolution? Authors have used different approaches to address this question, and the results of these inquiries tend to show that the details indeed do matter.

The question is often cast in terms of predictability of selection response over short- versus long-term evolution (see chapter ▶ "Variational Approaches to Evolvability: Short- and Long-Term Perspectives"). Barton and Turelli (1989) pointed out that the statistical population-level summarizations of Mendelian Genetics involve too few variables (i.e., summarize over too many) to remain accurate over the long term. They list various factors that will affect the accuracy of long-term prediction, including those that are a consequence of the GP map structure (e.g., pleiotropy, epistasis).

In addition to this general assessment of the need to account for (developmental) detail in long-term predictions, several authors have focused on specific aspects. Gromko (1995) simulated GP maps and found that even when the GP maps resulted in the same trait correlations, their responses to selection varied depending on the exact GP structure, implying that the population-level correlation between traits is not a sufficient predictor of the response to selection. Trait correlation is due mostly to pleiotropic effects, but as mentioned, the same genetic correlations of traits can be generated by different arrangements of pleiotropy and also different underlying mechanisms. Baatz and Wagner (1997) showed one possibility of how an effect of pleiotropy can exceed its effect via genetic correlation between traits. This requires considering a selection regime in which most traits are under stabilizing selection, while a focal trait is evolving (the so-called corridor model). Pleiotropy among these traits can then cause a constraint not only because selection on the mean of one trait also changes the mean of the other traits – the well-known correlated response which is potentially disadvantageous. Baatz and Wagner have shown that in their model, pleiotropy can also be disadvantageous by increasing the variance of the traits under stabilizing selection but not the mean, which is the case when pleiotropy doesn't generate correlation. On the other hand, if pleiotropy reduces the variance of the trait under stabilizing selection, the response to selection may be enhanced. Hansen et al. (2019) have used simulations and the corridor model on a range of different GP maps and have further shown that the divergence between short-term and long-term predictions is dependent on the detailed structure of the GP map. In addition, the models show large variance in predictions.

Using an analytical approach, Pavlicev and Hansen (2011) systematically compared the potential of traits to evolve when their modular GP maps are based on very different pleiotropic structures. They show that the GP map determines the evolvability of traits by affecting the distribution of variation at the mutation-

selection balance. Importantly, the computational model assumptions used will influence the predictions about the evolvability in different GP maps. The models that assume that summary measures are based on many loci with very small effects estimate the response well, regardless of the developmental detail, in particular when short-term predictions are involved (Barton and Turelli 1989). But as the period of time increases and as the evolutionary response depends on new mutations with large effects, the developmental detail plays a considerable role (Pavlicev and Hansen 2011). In other words, the greater the dependence in our predictive models on variability rather than variation, the more apparent the influence of developmental structure becomes.

Hansen (2003) showed a yet different effect of developmental detail on the potential of GP maps to generate variation: he pointed out that modular genetic variation, if achieved by the restriction of pleiotropic effects to subsets of traits, also restricts the mutational domain for each trait and thus the maximal amount of genetic variation that can be generated for this trait. This can be interpreted as an argument against variational modularity. Alternatively, it may underscore the evolutionary advantage of GP map which can generate variational modularity without restricting pleiotropic effects.

Having shown that developmental detail clearly does matter opens the door widely to trying to understand the systemic effects of the more fine-grained "developmental rules." Given the developmental and physiological detail that is available in modern-day biology, questions can be asked on specific contexts – at the level of gene regulation (see chapter ▶ "Modeling Evolution of Developmental Gene Regulatory Networks"), developmental interactions during morphogenesis or growth (see chapters ▶ "Inherency" and ▶ "Mechanisms of Pattern Formation, Morphogenesis, and Evolution"), or physiology. Modularity in gene regulation and its evolutionary consequences are well-established (e.g., Carroll 2005). Furthermore, we know that certain regulatory network motifs in transcriptional regulation have a very high occurrence. One may ask about what kind of population variation in traits, which they underlie, they are able to generate. Similar, character identity networks have been proposed to individuate traits or cell types (Wagner 2014) – how do these systems translate variationally? Note that this kind of inquiry is in principle related to Kacser and Burns' work on dominance and its later population genetic integration. The difference is that while their focus was on physiology, the focus of developmental evolutionary biology is the effect of physiology and development on evolvability.

Gjuvsland et al. (2007) queried the patterns of variation in gene expression that can be generated by the most common transcriptional network motifs. Similarly, Pavlicev and Widder (2015) focused on two specific motifs and asked about the possibility of transition between them, motivated by questions of evolutionary decoupling of variation. Many more avenues of work are open in this field in the future. For example, what are the variational properties of the common feedback or cross-regulation structures in development and physiology? How do they behave under different selective regimes in long-term selection? What are the possibilities of transition between the GP substructures? To what extent can sub-circuits (motifs) be

used to answer these questions, and what is the role of their network context, the way they are embedded?

Finally, this kind of work need not be limited to the query of population-level variation. Akin to Alberch and Gale's demonstration of different mutational spectrum in salamanders and frogs, the consequences of developmental structure for variation can be compared between taxa (Jernvall 2000).

Similar questions about the effect of structural-developmental detail as discussed here on the structure of pleiotropy (i.e., the direction of effects in phenotypic space) can be asked about robustness (i.e., the size of effects). Robustness can contribute to evolvability by generating a pool of selectively neutral genetic variants. Under mutation, such populations can access an even larger pool of genotypes and thus potentially a large phenotypic space. Even without mutation, the presently neutral genetic variation can itself become heterogeneous and selectively relevant under environmental change (Wagner 2008). Mayer and Hansen (2017) have shown that this logic is not independent of assumptions about the structure of the genotype-phenotype map: namely, the assumption that the more robustness, the greater number of neighboring phenotypes are accessible. The GP map structure may limit this accessibility, and consequently even large pools of genetic variation may not automatically increase the accessibility of alternative phenotypes, while other small pools may be highly capable to generate potentially adaptive change.

Changing Developmental Rules

Wagner (2000) has described the domain pertaining to evolutionary explanations of population genetics to be the one in which the rules of development do not change and the domain of development and evolution as the one where they do. How does developmental evolution relate to the question of the evolution of developmental rules, that is, the evolution of the GP map structure itself?

The above section focused on the evolutionary change within the confines of constant developmental rules and has shown that even there the developmental details play a role. So far the attempts to understand the effect of developmental detail were discussed with additive changes in multiple traits in mind, without reference to context-dependency of genetic effects. When effects of mutations on one or several traits are additionally dependent on the genetic or developmental context (see chapter ▶ "Epistasis"), this may introduce many additional interesting structural effects (Carter et al. 2005) and also enable the GP map structure to itself evolve in a manner that could increase or decrease evolvability by modifying the effects of pleiotropy and canalization (see chapters ▶ "Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics" and ▶ "Canalization: A Central but Controversial Concept in Evo-Devo").

Therefore, understanding and distinguishing different underlying mechanisms involved is even more important and illuminating when the question is asked about their evolution. Several mechanisms for the evolution of novelties have been proposed, and to understand them from the perspective of developmental evolution

means to translate them into the following question: what properties of the system are involved, do they enhance or constrain the origin of novelty, and how do systems properties change in this process? Mechanism can significantly enrich the understanding of this evolutionary process. Two somewhat related models will be addressed briefly as examples that involve systemic mechanisms.

The first example is the variation and evolution of the pleiotropic structure (see chapter ▶ "Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics"). The model developed by Pavlicev and Wagner (2012) points out that the constraint due to the pleiotropic effects of genes can differ depending on the genetic background this gene finds itself on. There is thus an *interaction* between the loci generating pleiotropic effects and other genomic loci (the so-called relationship loci, rQTL). The model proposes the evolutionary mechanism by which divergence in degree of pleiotropy may occur between populations, where the background loci are selected to compensate for the potential deleterious side effects of otherwise advantageous pleiotropic mutations. Note that in this model variational pleiotropy evolves not by removing the functional effects of genes on certain traits. Rather the correlation between traits is modified by generating a specific systemic structure which modifies the variation. In other words, this process generates variational modularity and potentially individuation of a trait without generating structural modularity. This model has been tested theoretically in population genetic context and shown that correlations between traits can indeed evolve in a way that increases evolvability (see chapter ▶ "Evolvability").

The final example comes from cellular molecular research rather than from the study of variational patterns of GP maps. Cells are the basic building blocks of organisms and cell type origination is thus a basic example of evolutionary novelty (see chapter ▶ "Devo-Evo of Cell Types"). Researchers working on various instances of cell type evolution independently noted the involvement of stress reaction pathways in the identity of novel cell types (e.g., Wagner et al. 2019). They suggested that cell type innovation may arise from internalizing and stabilizing portions of the stress response pathway. This idea appears related to the ideas of genetic assimilation of plastic, induced responses such as described in the context of "plasticity first" or genetic assimilation models (see chapters ▶ "Developmental Plasticity and Evolution" and ▶ "Eco-Evo-Devo") – yet another proposed mechanism of evolution of novel traits. However, it also entails important differences: the initial "plastic" response in this case is not an alternative better-suited phenotype but rather a costly attempt of the organism to reestablish homeostasis, followed by the attempts to compensate for the consequences of a stressful situation. Furthermore, the evolutionary consequence is not to stabilize one of the alternative advantageous phenotypes but to mold the costly reaction into a new stable outcome that lacks the damaging consequences of stress response. Both stress reaction/internalization and genetic assimilation are mechanisms that will individualize the new entity and thus also in a variational sense generate a novel character. This empirical work thus sheds light on a possible mechanistic developmental structure conferring the individuality of a novel character and informs a plausible sequence of evolutionary events, but it does not immediately reveal the steps involved in changing the variational properties

in terms of transitions or the intermediate steps. Whether and how this can be done is a challenge for the future work in developmental evolution.

Conclusions

Brian Hall (2000) noted that while evolution of development asks for expanding the evolutionary synthesis by the evolution of development, developmental evolution asks for a revolution of the evolutionary theory by replacement. This for many practitioners of devo-evo is too strong a requirement. Evolutionary theory, centered on population genetics, need not be replaced, but some of the common assumptions should clearly be revised to accommodate the effect of organismal structure. That organismal structural detail, which is currently not sufficiently accounted for, affects evolutionary change has been demonstrated in a rich body of literature partially covered here, including authors working within the confines of MS, and in fact not limited to the recent decades. What is less well understood is what mechanisms and structures – beyond the coarse-grained variational modularity and robustness – cause what kind of variational properties, how they foster the ability of characters to evolve, and how these particular structures arise and themselves change.

Cross-References

- ▶ Canalization: A Central but Controversial Concept in Evo-Devo
- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Developmental Plasticity and Evolution
- ▶ Devo-Evo of Cell Types
- ▶ Eco-Evo-Devo
- ▶ Epistasis
- ▶ Evo-Devo's Contributions to the Extended Evolutionary Synthesis
- ▶ Evolvability
- ▶ Inherency
- ▶ Interdisciplinarity in Evo-Devo
- ▶ Mechanisms of Pattern Formation, Morphogenesis, and Evolution
- ▶ Micro-Evo-Devo
- ▶ Modeling Evolution of Developmental Gene Regulatory Networks
- ▶ Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics
- ▶ Variational Approaches to Evolvability: Short- and Long-Term Perspectives

References

Baatz M, Wagner GP (1997) Adaptive inertia caused by hidden pleiotropic effects. Theor Popul Biol 51:49–66

Barton N, Turelli M (1989) Evolutionary quantitative genetics: how little do we know? Annu Rev Genet 23:337–370

Carroll SB (2005) Evolution at two levels: on genes and form. PLoS Biol 3(7):1159–1166

Carter AJ, Hermisson J, Hansen TF (2005) The role of epistatic gene interactions in the response to selection and the evolution of evolvability. Theor Popul Biol 68:179–196

Cheverud JM (1984) Quantitative genetics and developmental constraints on evolution by selection. J Theor Biol 110:155–171

Clark AG (1991) Mutation-selection balance and metabolic control theory. Genetics 129:909–923

Cowley DE, Atchley WR (1992) Quantitative genetic models for development, epigenetic selection, and phenotypic evolution. Evolution 46:495–518

Gerhart J, Kirschner M (2007) The theory of facilitated variation. Proc Natl Acad Sci USA 104(Suppl 1):8582–8589

Gjuvsland AB, Hayes BJ, Omholt SW, Carlborg O (2007) Statistical epistasis is a generic feature of gene regulatory networks. Genetics 175:411–420

Gjuvsland AB, Wang Y, Plahte E, Omholt SW (2013) Monotonicity is a key feature of genotype-phenotype maps. Front Genet 4:216

Gromko MH (1995) Unpredictability of correlated response to selection: pleiotropy and sampling interact. Evolution 49:685–693

Hall BK (2000) Evo-devo or devo-evo – does it matter. Evol Dev 2:177–178

Hansen TF (2003) Is modularity necessary for evolvability? Remarks on the relationship between pleiotropy and evolvability. Biosystems 69:83–94

Hansen TF, Wagner GP (2001) Modeling genetic architecture: a multilinear theory of gene interaction. Theor Popul Biol 59:61–86

Hansen TF, Solvin TM, Pavlicev M (2019) Predicting evolutionary potential: a numerical test of evolvability measures. Evolution 73:689–703

Hendrikse JL, Parsons TE, Hallgrimsson B (2007) Evolvability as the proper focus of evolutionary developmental biology. Evol Dev 9:393–401

Jernvall J (2000) Linking development with generation of novelty in mammalian teeth. Proc Natl Acad Sci USA 97(6):2641–2645

Kacser H, Burns JA (1981) The molecular basis of dominance. Genetics 97:639–666

Keightley PD, Kacser H (1987) Dominance, pleiotropy and metabolic structure. Genetics 117:319–329

Lande R (1979) Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. Evolution 33:402–416

Mayer C, Hansen TF (2017) Evolvability and robustness: a paradox restored. J Theor Biol 430:78–85

Müller GB (2007) Evo–devo: extending the evolutionary synthesis. Nat Rev Genet 8:943–949

Pavlicev M, Hansen TF (2011) Genotype-phenotype map maximizing evolvability: modularity revisited. Evol Biol 38:371–389

Pavlicev M, Wagner GP (2012) A model of developmental evolution: selection, pleiotropy and compensation. Trends Ecol Evol 27:316–322

Pavlicev M, Widder S (2015) Wiring for independence: positive feedback motifs facilitate individuation of traits in development and evolution. J Exp Zool B Mol Dev Evol 324:104–113

Pavlicev M, Mitteroecker P, Gonzalez PM, Rolian C, Jamniczky H, Villena FP, Marcucio R, Spritz R, Hallgrimsson B (2016) Development shapes a consistent inbreeding effect in mouse crania of different line crosses. J Exp Zool B Mol Dev Evol 326:474–488

Riedl R (1975) Die Ordnung des Lebendigen: Die Systembedingungen der Evolution. Parey, Berlin
Szathmary E (1993) Do deleterious mutations act synergistically? Metabolic control theory provides a partial answer. Genetics 133:127–132
Wagner GP (2000) What is the promise of developmental evolution? Part I: why is developmental biology necessary to explain evolutionary innovations? J Exp Zool 288:95–98
Wagner A (2008) Robustness and evolvability: a paradox resolved. Proc Biol Sci 275:91–100
Wagner GP (2014) Homology, genes and evolutionary innovation. Princeton University Press, Princeton, New Jersey
Wagner GP, Altenberg L (1996) Perspective: complex adaptations and the evolution of Evolvability. Evolution 50:967–976
Wagner GP, Erkenbrack EM, Love AC (2019) Stress-induced evolutionary innovation: a mechanism for the origin of cell types. BioEssays 41:e1800188
Wright S (1934) The molecular and evolutionary theories of dominance. Am Nat 63:24–53
Zhu YO, Siegal ML, Hall DW, Petrov DA (2014) Precise estimates of mutation rate and spectrum in yeast. Proc Natl Acad Sci USA 111:E2310–E2318

Micro-Evo-Devo

David Houle and Luke T. Jones

Contents

Abstract

Micro-evo-devo is the study of genetic basis of developmentally mediated phenotypic variation, and the evolutionary forces that affect the fate of that variation within populations. Currently, there are few studies detailed enough to trace the short-term evolutionary events underlying changes in developmental processes, which leaves the general explanatory power of a micro-evo-devo approach unclear. One promising approach is to use artificial selection to directly cause evolution of developmentally mediated phenotypes and then infer the genetic and developmental underpinnings of that response. A second promising approach is to use a comparative approach in model systems such as sticklebacks that have repeatedly been challenged to adapt to similar environments in the recent past. Part of the reason that micro-evo-devo studies are still rare is an implicit assumption by some that the current variability of a population is irrelevant to long-term

D. Houle (✉) · L. T. Jones
Department of Biological Science, Florida State University, Tallahassee, FL, USA
e-mail: dhoule@bio.fsu.edu; ltjones@bio.fsu.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_189

patterns, and that the vast majority of short-term changes in development are essentially random with respect to long-term trends. On the other hand, all major evolutionary transitions or trends must consist of micro-evolutionary changes. Emerging evidence from a variety of systems shows that long-term patterns can sometimes be retrodicted from variability within extant populations. Further development of micro-evo-devo approaches is needed to enable us to determine the generality of these results.

Keywords

Genotype-phenotype-fitness map · Micro-evo-devo · Evolve and resequence · Variability · Quantitative genetics

Introduction

Understanding the evolution of phenotypic diversity is an enduring goal of biology. Two complementary approaches are necessary to realize that goal: comparative and process-based. The comparative approach describes biological diversity among taxa from the genomic to the phenotypic level, and places that diversity in a phylogenetic context. The process-based approach investigates how traits that exemplify that diversity actually evolve within populations (see chapter ▶ "A Process-Based Approach to the Study of Flower Morphological Variation").

The increasing importance of evolutionary developmental biology, or evo-devo, comes from the fact that much of that biological diversity is generated by developmental processes. The field of evo-devo arose from two nearly coincident developments in biology in the late 1970s and 1980s (Laubichler 2010). At this time, a number of evolutionary biologists reemphasized the explanation of the evolution of phenotypes rather than genes as their goal (see chapter ▶ "Evo-Devo's Contributions to the Extended Evolutionary Synthesis" for a different perspective on this shift). Nearly simultaneously, molecular developmental geneticists scored their first dramatic successes in identifying genes responsible for fundamental differences in development among taxa. Since then the core work in evo-devo has remained comparative, with a focus on identifying both the fundamental processes that underpin development in multiple taxa, and the underlying developmental and genetic basis of discrete differences among species. These approaches only indirectly inform us about the processes that generated developmental diversity.

This chapter briefly reviews the current status of the process-based approach in the evo-devo studies. Despite early calls for the process-based study of evo-devo (Stern 2000; Johnson and Porter 2001), such studies are still relatively uncommon. This leaves us uncertain whether the micro-level processes by which development must evolve influence the larger-scale events and patterns that can be studied using the comparative approach.

What Is Micro-Evo-Devo?

We define micro-evo-devo as the study of the nature and genetic basis of developmentally mediated phenotypic variation, and the evolutionary forces that affect the fate of that variation within populations. These twin emphases on the nature of phenotypic variation and on the interaction between that variation and natural selection, the genotype-phenotype-fitness map, are what make micro-evo-devo studies distinct from their parent disciplines, population, and quantitative genetics. The genotype-phenotype (GP) map is the biological machinery that turns a genotype into all the many phenotypes that an individual expresses during its lifetime (Lewontin 1974). By extending the map from phenotypes to fitness, one can in principle include all of the evolutionary forces that are hypothesized to give rise to the properties of the GP map that generate the evolutionary properties of developmental systems, including evolvability (see chapters ▶ "Evolvability" and ▶ "Variational Approaches to Evolvability: Short- and Long-Term Perspectives"), modularity, plasticity (see chapters ▶ "Developmental Plasticity and Evolution" and ▶ "Eco-Evo-Devo"), robustness, etc. Today micro-evo-devo encompasses studies of mutation and standing variation that affect development, the responses of morphology, and the developmental processes that underlie it to natural and artificial selection, and the causes of natural selection on morphological traits. These approaches are related to and complement both the "devo-evo" approach to the study of how developmental processes shape the evolutionary process (see chapter ▶ "Developmental Evolutionary Biology (Devo-Evo)") and comparative studies of recently diverged populations or species where contemporary data on variation and selection is relevant to the divergence under study.

Traditional evo-devo can address the nature of the genetic and developmental differences that have given rise to biological diversity, but cannot directly answer questions about why that particular variation has been recruited to evolutionary divergence. For example, a finding that a particular difference in morphology is the result of changes in gene regulation tells us what must have evolved – the cis-regulatory elements of genes involved in development – but it does not tell us how or why they evolved that way. Are there alternative paths that evolution might have taken? If so, why weren't they? Only at the population level can we hope to study all the evolutionarily relevant variation and its phenotypic effects.

While evolutionary biologists have long realized that the study of the evolution of development must ultimately encompass the origin and fixation of genetic variants affecting development, it took longer for the new molecular tools of developmental genetics to become sufficiently inexpensive for population-level studies. Explicit micro-scale studies of evo-devo are still relatively uncommon (Nunes et al. 2013). While some of this relative neglect is based on the historical origins of evo-devo outlined above, we suspect that it is also due to an implicit assumption among at least some evo-devo practitioners that what happens at the micro scale is not very relevant to the differences among species and higher taxa (e.g., Milocco and Salazar-Ciudad

2020). The use of the term "micro" implies an overlap with the more general micro- vs. macro-evolution distinction which concerns the relationship between evolution over short vs. long time scales or that within vs. among species. The skepticism that micro-evo-devo is relevant inherits some of aspects of the controversy over whether macro-evolution is just micro-evolution continued over a long time period (e.g., Gould 2002). Currently the connections between micro-evolution of development and the macro-pattern of diversity in development are unclear, as there are a limited number of relevant studies. The key challenge is to either establish the relevance of micro-evo-devo to the larger issues in evolutionary developmental biology, or, alternatively, explain how larger-scale or longer-term processes render micro-level changes irrelevant.

Micro-Evo-Devo in Action

To illustrate the scope of micro-evo-devo studies, let's consider example studies that span the diversity of approaches that are possible.

The most direct micro approach is to observe the response of a population to selection on a developmentally relevant phenotype. Selection can be used in several contexts. Natural populations can frequently experience selection, but it is rarely clear what the actual target of selection might be. In artificial selection the investigator measures and selects the individuals chosen for breeding. Artificial selection experiments are thus ideal for testing hypotheses about the nature of the developmental response when a particular phenotypic change is selected for.

For example, Marchini and Rolian (2018) investigated the mechanism by which a mouse population responded to selection for tibia length, one of the long-bones that has diversified among mammals. After only 14 generations of artificial selection for increased tibia length standardized by body mass, they had increased average length by 9% and 14% in 2 replicate populations. During bone development, a scaffolding of cartilage is produced by chondrocytes at each end of the developing bone, undergoes proliferation and enlargement, and then that scaffolding is filled in by the osteoblasts that form ossified, mineralized bone. Based on this knowledge, Marchini and Rolian (2018) could identify four potential mechanisms for an evolved increase in tibia length: prolongation of the period of bone growth, increases in the number of dividing chondrocytes, increases in chondrocyte size, and increases in the proliferation rate during ossification. Analysis of mice at the end of the experiment showed that the long-limbed treatments had increased the number of chondrocytes, while no differences were found in the other three characteristics. In this population, evolution did not proceed by all possible means, but was focused on just one process. This is in contrast with the differences in growth between limbs in bats, which have elongated forelimbs, and jerboas, which have elongated hindlimbs. In these taxa, increases in chondrocyte size are important in the elongated body parts. This difference in mechanism may reflect the fact that the experimental mice were selected to increase the length of one limb, but not to change the relative growth of their limbs.

Other micro-evo-devo studies investigate the genetic changes underlying changes in development, rather than directly studying development. In an evolve-and-resequence (E&R) experiment, next-generation sequencing of selected populations identifies the genomic changes that distinguish the populations after selection. Changes that cannot be explained by drift are inferred to be due to selection. For example, Turner et al. (2011) selected replicate populations of *Drosophila melanogaster* to have larger or smaller body size for 100 generations. The genomic response was traceable to a large number of genomic regions. The regions that responded to selection were far more likely to code for genes involved in the gene ontology categories anatomical development, cell number, and metamorphosis. This suggests that the changes in size in this experiment were accomplished by small alterations in a large number of developmental processes.

Studies of mice and flies like those just mentioned take advantage of the fact that these are premier model systems for the study of development, but are hampered by the difficulty of studying natural populations. In contrast, *Gasterosteus aculeatus*, the three-spine stickleback was chosen for micro-evo-devo studies due to a wealth of recently differentiated natural populations. This fish and its close relatives are found in near-shore marine environments in the Northern Hemisphere. They have repeatedly invaded freshwater habitats from the oceans, particularly as deglaciation proceeded after the last ice age, making every river and stream an independent evolutionary experiment with a geologic time signature. Sticklebacks have consequently been a favorite subject for studies of phenotypic evolution (Bell and Foster 1994), including the genetic basis of developmental changes. For micro-evo-devo, these natural experiments provide many of the advantages of artificial selection experiments, plus the fact that nature has done the work of selection over a much longer time period than is practical in the laboratory. For many traits the resulting differences in phenotypes between founder and derived populations are large enough to make it possible to use the comparative evo-devo toolkit to identify and test hypotheses about the basis of adaptation. This combination of features enables investigators to compare variation in the marine founder population to the products of evolution based on that variation, while investigating the cause of those differences.

For example, many freshwater populations undergo loss of spines and armor that help protect marine fish from predation. In freshwater these features actually increase predation by insect predators, and increase the demand for calcium, which is far more limited in freshwater habitats. In addition to contemporary studies documenting these selective forces, detailed fossil sequences show that the loss of spines and armor takes place gradually over a few 1000 generations (Hunt et al. 2008). Genetic changes that make large contributions to these repeated losses have been traced to cis-regulatory changes at two key developmental genes. The armor plates are greatly reduced by a mutation in the regulatory region of the *ectodysplasin* (*EDA*) gene (Colosimo et al. 2005; O'Brown et al. 2015), which plays an important role in cell-signaling during developmental of the neural crest and ectoderm. Remarkably, the same allele (haplotype) is involved in this adaptation throughout much of the range of the species. It is maintained at low frequency in the marine

population which founds each freshwater population by gene flow from the existing freshwater populations. This enables rapid adaptation upon invasion of a new drainage. In contrast, the genetic basis for loss of pelvic spines in some freshwater populations has been traced to deletions of an enhancer at the *Pituitary homeobox 1* (*Pitx1*) locus (Chan et al. 2010). In this case, the deletion present in each drainage was unique. Several lines of evidence suggest that this enhancer is in a region of the genome with a high mutation rate for deletions. Thus, in both of these cases of armor loss, the supply of the variation is enhanced, potentially explaining why these two regions are responsible for parallel adaptive events.

These dramatic cases of evolution due to fixation of large effect alleles do not explain all aspects of stickleback adaptation. Studies that map the genetic differences underlying many differentiated traits show that such changes typically involve a large number of genetic changes with a range of effect sizes (Peichel and Marques 2017). Even in the case of armor loss, many other genomic regions contribute to the multifarious differences among populations. For example *Eda* has a large effect on plate number, but minor effects on the size of the plates that are produced. A very useful heuristic model for adaptation is that of Fisher's geometric model (Orr 2005) of simultaneous adaptation of many traits. Fisher assumed that each trait has an optimal state at which fitness is maximized, and that the fitness of a phenotype is a smooth function of the distance to that optimum in the space of all possible phenotypes. He also assumed "universal pleiotropy," meaning that each mutation has some effect on all traits, although the combination of effects differs among mutations. When the population is not at the optimum, mutations will increase fitness when they move the phenotypic state closer to the optimum, and otherwise will decrease fitness. When the population is far from the optimum, mutations with large effects can be favored, but as the population approaches the optimum, variants with large effects are no longer favored, even if they would have provided a better initial step towards the optimum. Instead, a series of smaller effect mutations are fixed as the population approaches the optimum. The pattern of effects sizes in the adaptation of stickleback traits matches the expectation under Fisher's geometric model well (Peichel and Marques 2017).

Building Blocks of Micro-Evo-Devo

Many comparisons of evo-devo with traditional micro-evolutionary biology consider the latter to be synonymous with population genetics. The purview of population genetics is generation and inheritance of discrete inherited variants in their genetic context and the study of the forces affecting the fates of those genetic variants within populations. The phenotype plays no necessary role in population genetics once the relative fitness of genotypes is specified, although many specific population genetics models do include a connection between fitness and phenotype, albeit in a usually simplified and rather abstract form. This phenotype-blindness of population genetics has been the subject of scorn not only from practitioners of

evo-devo but also from biologists more concerned with the evolution of phenotypes than genes (Laubichler 2010).

There is, however, another tradition of micro-evolutionary studies based on quantitative traits that is complementary to that of population genetics. The field of quantitative genetics infers the inheritance of phenotypes from phenotypic data on related individuals without directly assuming anything about the underlying genetic variants. The nature of the phenotypic similarities allows the quantitative geneticist to predict the response of phenotypes to natural and artificial selection. Quantitative genetics is a natural way to evaluate important aspects of genetic architecture, such as evolvability (see chapters ▶ "Evolvability" and ▶ "Variational Approaches to Evolvability: Short- and Long-Term Perspectives"), modularity, and canalization (see chapter ▶ "Canalization: A Central But Controversial Concept in Evo-Devo"). The generation of new phenotypic variation by mutation or as a plastic response to the environment is also in the purview of quantitative genetics (e.g., Braendle et al. 2010; Houle and Fierst 2013; see chapter ▶ "Developmental Plasticity and Evolution").

An important aspect of this quantitative arm of micro-evolutionary biology is that the study of inheritance and selection readily generalizes to the study of complex phenotypes consisting of many potentially interrelated parts (Lande 1979; Lande and Arnold 1983). Almost any morphological phenotype is complex in this sense. Unfortunately, many studies of the evolution of development at all scales make the unrealistic but convenient assumption that phenotypes can be represented by a single measurement or as a set of discrete alternative states. The ability to measure complex phenotypes is increasing rapidly (see chapter ▶ "Phenotyping in Evo-Devo"), and incorporating these multivariate data is a challenge for all aspects of evo-devo studies (e.g., Pitchers et al. 2019).

Despite capturing largely complementary aspects of evolution, the population genetic and quantitative genetic approaches unfortunately cannot readily be combined into a single comprehensive approach to micro-evolution. They use completely dissimilar formalisms where the parameters in one tradition have no equivalents in the other tradition. For example, population genetics assign fitnesses to discrete genotypes, but discrete genotypes are not identified in the quantitative tradition. The fitness functions estimated in the quantitative tradition cannot be applied to genetic variants that lack phenotype information.

Building GP Maps

Population genetics and quantitative genetics operate on opposite sides of the GP map (Lewontin 1974). It has long been clear that what we need to fuse the complementary advantages of population and quantitative genetics is a GP map. Micro-evo-devo studies necessarily incorporate both population genetic and quantitative genetic concepts, connected through the concept of the GP map. Key advantages of a GP map-based approach include gaining a handle on the empirically challenging concept of pleiotropy (see chapter ▶ "Pleiotropy and Its Evolution:

Connecting Evo-Devo and Population Genetics"). Without an a priori notion of the map, the only way to study pleiotropy is to estimate effects of genetic variants on the prohibitively large universe of possible traits affected (Pitchers et al. 2019). Furthermore, the GP map concept enables study of how the map itself can evolve (see chapter ▶ "Epistasis"), potentially reshaping evolutionarily important parameters, including pleiotropy and evolvability.

The difficulty is that, with a few limited exceptions, GP maps are very poorly known. What knowledge evolutionary biologists have of GP maps usually comes from elsewhere in biology – from mutational screens, and from cell and developmental biology. Often such information initially is no more than a pointer for future work "Look in or around this gene for relevant genetic variation." In addition, there are many vaguer generalizations that we use as proxies for parts of the GP map, such as "premature stop codons have large phenotypic effects" and "synonymous amino acid substitutions will usually have no phenotypic effects."

Traditional developmental and evo-devo studies often build out parts of genotype-phenotype map as a byproduct of researching the function or evolution of genes or pathways. For example, the transcription factor *ovo* in *Drosophila melanogaster* and closely related species (reviewed in Chap. 6 of Wagner 2014) has 3 alternative promoters, 5 different transcripts with different functions and at least 13 enhancers. One transcript is necessary in primordial germ cells, while that and a second transcript act antagonistically to regulate gene expression in the ovary. A third transcript is involved in several aspects of cuticle differentiation, and three enhancers of this transcript generate spatially precise effects on the placement of trichomes (miniature protrusions on the cuticle) that vary among species (McGregor et al. 2007). Thus, work on this one gene has provided us with hypotheses about the function of regulatory and coding regions, some of which are expected to influence many phenotypes, some to have highly targeted phenotypic effects, while some may not have any phenotypic effects. However, note for all this effort, we are far from having any detailed predictions of, for example, what effects that substitution of specific base pairs will have on phenotypes.

A complementary tool that is now being exploited to build GP maps is the genome-wide association study (GWAS). In a GWAS, a set of genotypes are sequenced and their phenotypes are measured. When a statistically robust association between a pair of variants and a phenotype is discovered, we have measured the effect of those variants on a phenotype, one of the crucial parameters of both population and quantitative genetic models (Bastide et al. 2013). Ultimately merging the results of developmental genetic, evo-devo, and GWAS studies coupled with functional verification will allow us to make increasingly detailed GP maps to inform micro-evo-devo studies. An important challenge for GWAS studies is to incorporate pleiotropic effects (Pitchers et al. 2019).

Detailed models of development and the phenotypes it produces is a promising approach to building a hypothesis about GP maps (see chapters ▶ "Modeling Evolution of Developmental Gene Regulatory Networks" and ▶ "Computational Modeling at the Cell and Tissue Level in Evo-Devo"). For example, a detailed model of tooth development can reproduce the major features of tooth morphology and

provides hypotheses about which aspects of development will respond under simulated selective scenarios (Milocco and Salazar-Ciudad 2020). If such a model were coupled to experimental work in a model organism, this would be a powerful program to build a GP map, and then test its ability to predict micro-evolution.

What Can Micro-Evo-Devo Do for Evo-Devo?

Given the status of micro-evo-devo as a younger step-child of traditional, comparative evo-devo, an important question to ask is what micro-evo-devo can bring to the study of the evolution of development that is distinct from what can be learned using the comparative approach. One entrée to potential advantages of micro studies is to consider the limitations of traditional evo-devo studies.

The first such limitation is what we might call the "discreteness bias" of evo-devo studies. The chosen targets of evo-devo studies are overwhelmingly discrete differences among taxa – the transformation of wings to halteres in insects, the evolution of novel color patterns, the loss of limbs in snakes. The ability to investigate such discrete differences is a strength of evo-devo that only becomes a bias when there is a tendency to equate all interesting developmental changes with the causation of discrete differences. Questions about the evolution of important quantitative characteristics, such as differences in body size and the accompanying allometric changes in virtually every aspect of form are also interesting. Generalizations about the evolution of form are nevertheless being built on the catalog of causes of discrete differences among taxa (e.g., Carroll 2008).

Micro-evo-devo studies generally do not start out to explain discrete phenotypic differences, and thus help balance the scope of evo-devo. For example, Kamberov et al. (2013) studied an amino acid substitution in the human Ectodysplasin A (*Eda*) receptor (*EDAR*). This human polymorphism was targeted for study after population genetic studies revealed that the derived *EDAR* allele has been strongly favored by selection in East Asian and Native American populations over the past 30,000 years. Subsequent studies showed that it was associated with differences in scalp hair thickness and tooth shape in human populations. Kamberov et al. set out to verify that the coding variant was in fact responsible for these phenotypic associations by introducing the amino acid change into the mouse genome. The knock-in mice did, in fact, have 25% more hair follicles whose thickness was enhanced by one cell width, but also revealed 10–25% higher density of eccrine (sweat) glands, increases in branching in the mammary gland by about 25%, and 10%, and lower mammary fat pad size. Subsequent investigations in humans showed that individuals carrying the derived allele also had increased numbers of eccrine glands, as the mouse results predicted. While it remains unclear which of the phenotypic effects of the *EDAR* have been responsible for its spread in this human population, it is a revealing example of positive selection of a highly pleiotropic quantitative genetic variant.

Micro-evo-devo studies are well equipped to study the evolution of continuous traits rather than discrete differences. Patterns in continuous traits can be as relevant to larger scale evolutionary patterns as discrete differences. For example, there is a

long-standing controversy over how much allometry constrains evolutionary patterns. Bolstad et al. (2015) performed artificial selection on the slope of a conserved pattern of allometry for wing shape and size in *Drosophila melanogaster*. In just 26 generations the slopes evolved to be outside the range of slopes in the entire genus *Drosophila*, suggesting that allometry does not constrain wing shape. However, when artificial selection ceased, the slopes evolved back towards the original slope faster than they diverged under artificial selection. They also selected wing shape to evolve without altering allometric slope, but in those lines, there was little evidence of natural selection for the ancestral state. Consequently, Bolstad et al. interpreted this counter-selection as the result of a pleiotropic burden (see chapter ▶ "Concept of Burden in Evo-Devo") on allometry, rather than selection on wing shape per se. This suggests that this allometric relationship is constrained by pleiotropy with unknown aspects of development, not by a lack of genetic variation in slope.

It is more challenging to use the micro-evo-devo toolkit to study the opportunity for discrete changes in morphology, as, in many cases, discrete phenotypic variation is not present within populations. Studies of mutational effects can sometimes reveal discrete differences, such as the transformations of cell fate in the vulva of *Caenorhabditis elegans* (Braendle et al. 2010; see chapter ▶ "Devo-Evo of Cell Types"). In natural isolates, the pattern of cells is nearly invariant, but after mutations are allowed to accumulate for many generations, this pattern becomes more variable. Mutations in different genotypes generated different rates of transformations, and there were hints that the sorts of transformations that occur at the highest rate are more typical of those found in related species. In rare instances, populations contain individuals of two or more discrete morphological types, and these can be used to study the underlying developmental basis.

The second potential limitation is "endpoint bias." Having demonstrated the role of a particular gene in shaping phenotypic differences between taxa, it is natural to equate the evolution of the feature in question to the evolution of that gene. For example, Kopp et al. (2000) demonstrated that the gene *bric a brac* (*bab*) has evolved a novel, sex-specific regulatory role in the last two abdominal segments in some *Drosophila* species. This novel regulation enables male flies to evolve pigmentation and shape differences from female flies in those segments. Having demonstrated this, Kopp et al. then proposed a parsimonious selective scenario whereby selection on *bab* expression by sexual selection is responsible for the evolution of the novel male-specific features. However, an alternative hypothesis is that the initial evolution of sexual differentiation took place by some other mechanism, and the recruitment of *bab* into its current regulatory role took place later.

We do not criticize Kopp et al. for putting forward their parsimonious hypothesis, but there are theoretical and empirical grounds to suspect that the differences in development between species may be different from the mechanisms by which the phenotypes diverged in the first place. On the empirical side, developmental systems drift (DSD) is a well-known and common phenomenon whereby the developmental basis of traits diverges even though the trait has remained roughly constant since divergence from the common ancestor. One potential explanation for this

phenomenon is literally genetic drift – that there are completely equivalent ways to make the same phenotype, and this allows fixation of alleles that transition the system from one developmental solution to another (see chapter ▶ "Developmental System Drift"). Perhaps a more likely explanation for DSD is suggested by Fisher's geometric model (Orr 2005). When the population is far from the optimum, the variants with large effects initially fixed are likely to move some traits farther from their optimal state. Once such an imperfect mutation becomes fixed in the population, mutations fixed at later steps in the adaptive process will rectify the pleiotropic side effects of those early steps. The model predicts that adaptation will tend to zig-zag towards its final state, rather than proceeding by a single fixation event that achieves the optimal state. Thus, the developmental solution to an adaptive challenge may well shift once the population is near the optimum state. For example, a plausible scenario is that the *bab* enhancer now regulating coloration in male abdomens was recruited to correct the side effects of some earlier mechanism of achieving a similar phenotype. Now consider a second adaptive event that primarily involves different traits. The pleiotropic side-effects of that event may perturb development of our focal traits, again engendering evolution to restore that optimum phenotype (Pavlicev and Wagner 2012).

How these inferred shifts in the developmental basis of evolved states occur has not yet been addressed by micro-level experiments. Artificial selection and experimental evolution studies of developmentally interesting organisms have generated very few allelic fixation events due to their short duration, precluding examination of this issue. Natural experiments such as the repeated invasions of sticklebacks into freshwater habitats have a greater range of time depths and offer the opportunity to evaluate this possibility, although we know of no attempts to do so.

Do Micro Events Affect Long-Term Evolution?

There are many reasons to suspect that the micro-evolutionary events and processes that we can observe directly are not relevant to evolution above the species level and over long time periods (see chapter ▶ "Macroevolution"). For example, if the cis-regulatory changes of large effect in fact dominate long-term evolution (Carroll 2008), it is possible that the vast majority of the abundant polygenic variation within contemporary populations could have no long-term importance. Some paleontologists have long argued for such a discontinuity between micro-evolutionary processes and macro-evolutionary patterns on other grounds (Gould 2002). Even quantitative geneticists have generally doubted that the parameters measured within contemporary populations are stable enough to predict evolution over long time periods. Indeed a study that integrates data on evolutionary rates of body size evolution across time scales from generations to hundreds of millions of years suggests a discontinuity reminiscent of punctuated equilibrium (Uyeda et al. 2011). Up to a time depth of about one million years, body size of descendant populations or species always remains within a factor of about 1.5 of the ancestral value, but at longer time scales larger changes in body size become increasingly

likely. It is not clear what processes can explain this apparent discontinuity, but it certainly suggests that it is very unlikely that one of these rare large changes will be encountered in any particular population at the present. These doubts about relevance are likely an important factor in the relative paucity of micro-evo-devo studies.

The result, however, is that we have relatively little data we can use to empirically test whether micro- and macro-evolution are in fact related to each other. Recent studies suggest that in some taxa there may be a strong connection between micro processes and macro patterns, despite the many reasons there should not be. Perhaps the most striking example is the recent comparison of the diversification of wing size and shape in the dipteran family Drosophilidae (Houle et al. 2017). This contribution estimated the phenotypic variation produced by mutation in 21 aspects of wing size and shape (Houle and Fierst 2013) in *Drosophila melanogaster* and compared that variation to standing variation in the same species and to variation among over 100 Drosophilid species that diversified over at least 30 million years. Both mutational and standing variation within *D. melanogaster* were highly correlated with the rate of evolution in the family. Wing shape evolves slowly in this group, so it is possible that this strong relationship is a peculiarity of a suite of slow-evolving traits. However there are hints of similar patterns in traits that show higher rates of evolution as well, such as the blossoms of the plant genus *Dalechampia* (Bolstad et al. 2014). The relevance of micro-evo-devo studies to the broader field of evo-devo should not be lightly dismissed.

Cross-References

- ▶ A Process-Based Approach to the Study of Flower Morphological Variation
- ▶ Computational Modeling at the Cell and Tissue Level in Evo-Devo
- ▶ Concept of Burden in Evo-Devo
- ▶ Developmental Evolutionary Biology (Devo-Evo)
- ▶ Developmental Plasticity and Evolution
- ▶ Developmental System Drift
- ▶ Eco-Evo-Devo
- ▶ Epistasis
- ▶ Evo-Devo's Contributions to the Extended Evolutionary Synthesis
- ▶ Evolvability
- ▶ Macroevolution
- ▶ Modeling Evolution of Developmental Gene Regulatory Networks
- ▶ Phenotyping in Evo-Devo
- ▶ Variational Approaches to Evolvability: Short- and Long-Term Perspectives

Acknowledgments We thank Laura Nuño de la Rosa, Thomas F. Hansen, Mihaela Pavličev, Jacqueline Sztepanacz, Christophe Pélabon, and Sarah Kettelkamp for their comments. D. H. thanks Thomas Hansen and Christophe Pelabon for generating the project "Evolvability: A New and Unifying Concept in Evolutionary Biology?" which provided him an extended period at the Centre for Advanced Study at the Norwegian Academy of Science and Letters to work on this chapter.

References

Bastide H, Betancourt A, Nolte V, Tobler R, Stoebe P, Futschik A, Schloetterer C (2013) A genome-wide, fine-scale map of natural pigmentation variation in *Drosophila melanogaster*. PLoS Genet 9:e1003534

Bell MA, Foster SA (1994) The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford

Bolstad GH, Hansen TF, Pélabon C, Falahati-Anbaran M, Pérez-Barrales R, Armbruster WS (2014) Genetic constraints predict evolutionary divergence in *Dalechampia* blossoms. Philos Trans R Soc Lond Ser B Biol Sci 369:20130255

Bolstad GH, Cassara JA, Márquez E, Hansen TF, Van Der Linde K, Houle D, Pélabon C (2015) Complex constraints on allometry revealed by artificial selection on the wing of *Drosophila melanogaster*. Proc Natl Acad Sci 112:13284–13289

Braendle C, Baer CF, Felix MA (2010) Bias and evolution of the mutationally accessible phenotypic space in a developmental system. PLoS Genet 6:e1000877

Carroll SB (2008) Evo-devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. Cell 134:25–36

Chan YF, Marks ME, Jones FC, Villarreal G, Shapiro MD, Brady SD, Southwick AM et al (2010) Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a *Pitx1* enhancer. Science 327:302–305

Colosimo PF, Hosemann KE, Balabhadra S, Villarreal G Jr, Dickson M, Grimwood J, Schmutz J et al (2005) Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. Science 307:1928–1933

Gould SJ (2002) The structure of evolutionary theory. Belknap Press, Cambridge, MA

Houle D, Fierst J (2013) Properties of spontaneous mutational variance and covariance for wing size and shape in *Drosophila melanogaster*. Evolution 67:1116–1130

Houle D, Bolstad GH, Van Der Linde K, Hansen TF (2017) Mutation predicts 40 million years of fly wing evolution. Nature 548:447–450

Hunt G, Bell MA, Travis MP (2008) Evolution toward a new adaptive optimum: phenotypic evolution in a fossil stickleback lineage. Evolution 62:700–710

Johnson NA, Porter AH (2001) Toward a new synthesis: population genetics and evolutionary developmental biology. Genetica 112–113:45–58

Kamberov YG, Wang S, Tan J, Gerbault P, Wark A, Tan L, Yang Y et al (2013) Modeling recent human evolution in mice by expression of a selected EDAR variant. Cell 152:691–702

Kopp A, Duncan I, Carroll SB (2000) Genetic control and evolution of sexually dimorphic characters in Drosophila. Nature 408:553–559

Lande R (1979) Quantitative genetic analysis of multivariate evolution applied to brain: body size allometry. Evolution 33:402–416

Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. Evolution 37:1210–1226

Laubichler MD (2010) Evolutionary developmental biology offers a significant challenge to the neo-Darwinian paradigm. In: Ayala FJ, Arp R (eds) Contemporary debates in philosophy of biology. Wiley-Blackwell, Chichester, pp 199–212

Lewontin RC (1974) The genetic basis of evolutionary change. Columbia University Press, New York

Marchini M, Rolian C (2018) Artificial selection sheds light on developmental mechanisms of limb elongation. Evolution 72:825–837

McGregor AP, Orgogozo V, Delon I, Zanet J, Srinivasan DG, Payre F, Stern DL (2007) Morphological evolution through multiple cis-regulatory mutations at a single gene. Nature 448:587–590

Milocco L, Salazar-Ciudad I (2020) Is evolution predictable? Quantitative genetics under complex genotype–phenotype maps. Evolution 74:230–244

Nunes MD, Arif S, Schlötterer C, McGregor AP (2013) A perspective on micro-evo-devo: progress and potential. Genetics 195:625–634

O'Brown NM, Summers BR, Jones FC, Brady SD, Kingsley DM (2015) A recurrent regulatory change underlying altered expression and Wnt response of the stickleback armor plates gene EDA. eLife 4:e05290

Orr HA (2005) The genetic theory of adaptation: a brief history. Nat Rev Genet 6:119–127

Pavlicev M, Wagner GP (2012) A model of developmental evolution: selection, pleiotropy and compensation. Trends Ecol Evol 27:316–322

Peichel CL, Marques DA (2017) The genetic and molecular architecture of phenotypic diversity in sticklebacks. Philos Trans R Soc Lond Ser B Biol Sci 372:20150486

Pitchers W, Nye J, Márquez EJ, Kowalski A, Dworkin I, Houle D (2019) A multivariate genome-wide association study of wing shape in *Drosophila melanogaster*. Genetics 211:1429–1447

Stern DL (2000) Evolutionary developmental biology and the problem of variation. Evolution 54:1079–1091

Turner TL, Stewart AD, Fields AT, Rice WR, Tarone AM (2011) Population-based resequencing of experimentally evolved populations reveals the genetic basis of body size variation in *Drosophila melanogaster*. PLoS Genet 7(3):e1001336

Uyeda JC, Hansen TF, Arnold SJ, Pienaar J (2011) The million-year wait for macroevolutionary bursts. Proc Natl Acad Sci 108:15908–15913

Wagner GP (2014) Homology, genes, and evolutionary innovation. Princeton University Press, Princeton

Canalization: A Central but Controversial Concept in Evo-Devo

Paula N. Gonzalez and Jimena Barbeito-Andrés

Contents

Abstract

Canalization refers to, in a broader sense, the production of consistent phenotypic traits in the face of genetic and environmental variation. It is a key concept for evolutionary developmental biology because it is one of the properties of developmental systems that modulates the expression of phenotypic variation, influencing the potential for evolutionary change (i.e., evolvability). Although the relevance of canalization is widely accepted, many topics around this concept are still a matter of debate. In this chapter, we first summarize the views about canalization from developmental and genetic population biologists and then discuss the mechanisms, origin, and evolutionary role of canalization. Mechanisms proposed to explain how canalization is achieved range from

P. N. Gonzalez (✉) · J. Barbeito-Andrés
Institute for Studies in Neurosciences and Complex Systems (ENyS – Unidad Ejecutora de Estudios en Neurociencias y Sistemas Complejos) CONICET-UNAJ-HEC, Florencio Varela, Buenos Aires, Argentina
e-mail: pgonzalez@fcnym.unlp.edu.ar; paulan.gonza@gmail.com; barbeitoj@gmail.com; barbeito@fcnym.unlp.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_55

single molecular factors to complex interactions in the developmental-genetic architecture. Contrasting views also characterize explanations about the origin of canalization, with positions that favor an adaptive origin derived from selective forces and others that consider canalization as an emergent product of developmental processes. Independently of its origin, the role of canalization in the evolution of phenotypic traits is also controversial. Since canalized traits reduce the range of phenotypic outputs, canalization could be expected to reduce evolvability. However, it has been proposed that canalization allows the accumulation of new cryptic mutations that are maintained within populations and can be expressed in certain evolutionary contexts. In sum, we provide an updated discussion on this elusive but key concept, attempting to review contrasting and complementary positions.

Keywords

Phenotypic robustness · Developmental stability · Phenotypic landscape · Evolvability

Introduction

The vast phenotypic variation exhibited by extant and extinct organisms has long been a central theme for both developmental and evolutionary biologists. However, it was not until the emergence of evolutionary developmental biology (evo-devo) in the 1980s and 1990s that the production of phenotypic traits by developmental systems started to be integrated into evolutionary explanations. In this context, as important as variation are the properties of the genotype-phenotype map that affect the ability to produce and maintain variation. Among these properties, modularity, integration, canalization, and plasticity have attracted much attention because they are thought to have a central role in structuring and modulating the amount of phenotypic variation exposed to selection and, thus, can influence the potential for evolutionary change of phenotypic traits. Canalization, in particular, refers to the robustness of phenotypic traits to changes in genotype and environment and is therefore recognized as a property that affects traits' propensity to vary.

The term *canalization* was coined by Waddington to describe the ability of developmental systems to maintain the phenotypic traits invariant in the face of genetic and environmental disturbances (see chapter ► "Conrad Hal Waddington (1905–1975)"). Waddington's ideas about canalization are closely related to his view on the role of developmental processes in the genotype-phenotype map (G-P), which is represented by the metaphor of the epigenetic landscape (Fig. 1). The epigenetic landscape is depicted as a surface with valleys that represent different pathways favored by development and a rolling ball that in the original scheme represents an individual cell (the same metaphor has been later extended to the development of tissues, organs, and individuals, Jamniczky et al. 2010). The surface is sculpted by interactions between genes and their products, which overlap along ontogeny and

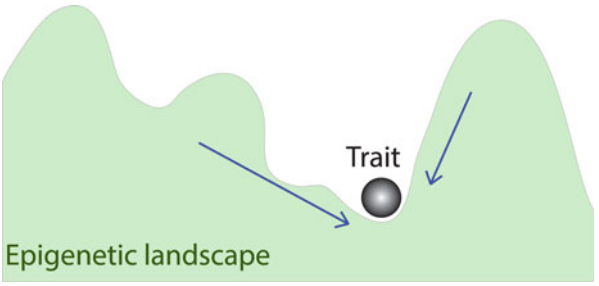


Fig. 1 Waddington's epigenetic landscape metaphor. Throughout the epigenetic landscapes, the localization of traits in deepest valleys is favored. Depths of valleys determine how much canalized a given trait is when it occupies this position

determine the depth of the valleys. Deeper valleys denote pathways more heavily buffered against external perturbation, which result in more canalized traits (Fig. 1). Waddington suggested that the shape of the landscape was modeled by natural selection. Based on the observation that wild-type specimens tend to be phenotypically less variable than mutants, he hypothesized that selection had stabilized development to produce canalized traits. The selective advantage attributed to canalization is that it ensures the production of the optimum phenotype under variable conditions. In a similar way, Schmalhausen proposed that "autoregulatory mechanisms" evolved as adaptive responses to environmental and genetic changes (see chapter ▶ "Ivan I. Schmalhausen (1884–1963)").

The concept of canalization has attracted much debate since it was formulated, and there is not a single and unified perspective regarding most of the aspects of canalization. In this chapter, we review the concept of canalization and attempt to provide the basis for understanding contrasting and complementary positions about the mechanisms involved in the production of robust phenotypes as well as the different views on the origin and evolutionary role of canalization.

Canalization: An Elusive Concept in Evolutionary Developmental Biology

The concept of canalization has been defined in different ways, and, to add more confusion, other terms such as phenotypic robustness, homeostasis, buffering, and developmental stability are often used as synonymous although they do not necessarily refer to the same phenomenon.

Wagner et al. (1997) pointed out the lack of a formal definition that incorporates canalization within a population genetic framework, which is necessary to study how and why phenotypic robustness originated in evolution. Canalization was thus defined taking into account the genetic variance of quantitative traits, which depends on the number of genes and their allelic frequency distribution, as well as the magnitude of effects of those genes on the phenotype. In this sense, canalization refers to reduced variability, defined as the tendency to vary, rather than to the actual level of variation found in a population. According to this view, research on canalization is the study of the evolution of those mechanisms that regulate the

evolvability of phenotypic traits. More canalized traits will exhibit a lower propensity to vary under the effect of new mutations and environmental perturbations than less canalized ones. This perspective focuses on the phenotypic variation introduced by the input of new mutations in each generation, i.e., the mutational variance (Gibson and Wagner 2000). As the capacity to generate heritable variation determines the potential to respond to selection, traits with higher mutational variance are expected to be more evolvable than canalized traits. However, as we discuss below, canalization can increase evolvability if it limits the expression rather than the occurrence of new mutations.

Phenotypic Robustness Against Genetic and Environmental Factors

Canalization is usually defined according to the source of perturbation against which the phenotype is buffered. Genetic canalization refers to the reduction of the effect of heritable perturbations on phenotypic traits, while environmental canalization limits the effect of any kind of nonheritable perturbations.

Genetic canalization is mainly related to the phenotypic robustness against new mutations, although in its broader sense also refers to robustness against epigenetic perturbations. It means that new alleles with potential effect on phenotypic traits are not expressed in some genetic backgrounds due to the action of buffering mechanisms. Given that the expression of an allele is affected by the presence of other alleles, genetic canalization is an epistatic phenomenon (see chapter ▶ "Epistasis").

For phenotypic robustness against environmental factors, a further distinction is made between the effects of macro- and microenvironmental variation. The first one includes external factors with effects on many specimens, such as temperature or nutrients, while microenvironment involves internal stochastic or indeterminate processes, such as random variation in the concentration or diffusion of molecules. In a broad sense, environmental canalization includes buffering against both macro- and microenvironmental effects although others reserve the term developmental stability to specifically refer to robustness against internal microenvironmental factors (de Visser et al. 2003). Canalization to macroenvironmental factors results in the production of similar phenotypic traits across different environments. Whether or not the same mechanisms account for these two types of canalization is still a matter of debate. Available evidence supports both a lack of concordance and significant associations between the measurements that describe phenotypic robustness against macro- and microenvironmental perturbations (de Visser et al. 2003). This suggests that they can be etiologically distinct.

As important as the phenotypic robustness against environmental variation is the capacity of genotypes to originate different phenotypes under different conditions, a phenomenon known as phenotypic plasticity (see chapter ▶ "Developmental Plasticity and Evolution"). Since its original formulation by Waddington, these properties have been considered as related because they reflect different "gene-by-environment interactions." More canalized traits are expected to exhibit relatively

flat reaction norms – which represent the interaction between genotypes and environments – while plastic traits display different values according to the external factors the organisms are exposed to. This apparent paradox is partially originated from the level of analysis, given that a specific feature of a system can be robust while their constituent components exhibit plasticity (Nijhout et al. 2017). This is the case, for example, of developmental plasticity when similar phenotypes are reached by changes in their trajectories of development. Therefore, the term "dynamically stable phenotypes" would be more appropriate to express the relationship between variation in the underlying processes and the observed insensitivity to macroenvironment perturbations.

Finally, microenvironmental canalization refers to stability of developmental systems in the face of internal perturbations, known as developmental noise, which contribute to developmental instability within a particular environment and genetic makeup. These perturbations are inherently stochastic at the molecular level and include, among others, the Brownian motion of molecules and fluctuations in the concentration of relevant gene products across morphogenetic gradients. In other words, canalization against microenvironmental variation is defined as the fidelity with which a genotype produces phenotypic characters within a particular individual in spite of random fluctuations in the internal environment.

Mechanisms of Genetic and Environmental Canalization

Explanations of how robustness is achieved range from those that focus on the molecular level to those that study canalization as an emergent property of the developmental-genetic architecture.

Mechanisms at the molecular level are found along the whole process that leads from the transcription of genes to the translation and assembly of proteins. One simple but effective strategy to buffer the effect of perturbations is to increase the amount of the outcome that is produced in each step (Siegal and Leu 2014). For instance, an increase in the promoter activation or even in the production of mRNA can reduce variation. However, this kind of solution is quite expensive, and more efficient alternatives that imply fine-tuning strategies should have evolved. In this sense, the role of chaperones – proteins that assist the folding and the assembly of other proteins – has received much attention. An important portion of proteins are prone to misfolding as they tend to produce interactions that are off-pathways. In these cases, molecular chaperones have a key role at maintaining proteins in their native states and assisting new folding events. When a protein underwent erroneous aggregation or misfolding, chaperones have the sufficient machinery to proceed by unfolding and disaggregating it to facilitate final proteolytic degradation (Salathia and Queitsch 2007; Kim et al. 2013). Due to this quality control role, chaperones have been postulated as candidates to explain how variation is buffered at this level.

Hsp90 is a well-known molecular chaperone that intervenes in signal transduction processes, and it has been found that alterations in its function lead to an increase in

morphological variation (Sato and Siomi 2010). Hsp90 has an important role in stabilizing its client proteins so when this function is altered, these client proteins become unstable (Salathia and Queitsch 2007). As a consequence, interference in the function of Hsp90 results in altered phenotypes and disrupted canalization, an effect that has been consistently shown not only in animals but also in plants (Rutherford and Lindquist 1998; Queitsch et al. 2002). In their pioneer work, Rutherford and Lindquist (1998) stated that Hsp90 under normal conditions buffers variation in morphogenetic pathways and tends to accumulate silent variation that becomes evident only in particular evolutionary contexts. Nowadays, although the idea of Hsp90 as a capacitor is widely accepted, new evidence has been added in relation to the complex functioning of this chaperone. Specchia et al. (2010) showed that Hsp90 is also implicated in the suppression of de novo mutations. In *Drosophila*, it was found that biogenesis of PIWI-interacting RNA (piRNA) relies on the activity of Hsp90. Since piRNA controls the expansion of transposons, Hsp90 indirectly modulates the transpositions produced by these elements.

More recently, the role of Hsp90 in buffering the effect of de novo mutations has been discussed. Geiler-Samerotte et al. (2016) found that the effect of this molecule depends on the selective pressures to which the populations have been exposed. When yeast cells experience reduced selection pressure, Hsp90 enhances the influence of spontaneous mutations on phenotypic traits, while in natural populations the effect is the opposite. In this line, claims have been made about the limits of approaches centered on punctual mechanisms to account for the robustness of more complex phenotypic traits. Instead of being the product of particular genes, the robustness of phenotypes emerges from the inner developmental-genetic architecture (Siegal and Bergman 2002; Green et al. 2017). In this view, properties such as redundancies among pathways, epistatic interactions, or the averaging effects of multiple independent sources of variation contribute to variation in canalization. Most of these explanations are based on the fact that genotypes often relate to phenotypes in a way that is not linear and, as a consequence, the amount of phenotypic variation that is produced in a given developmental scenario will depend on the complex relations of the G-P map. Although the experimental testing of these hypotheses is complex, recent work provides illustrative examples, such as the case of Fgf8, a key signaling factor that is involved in craniofacial development. It was found that a progressive reduction in the dosage of Fgf8 does not lead to a progressive change in craniofacial variation. In contrast, craniofacial morphology remains unchanged until a certain dosage threshold under which changes are really noticeable (Green et al. 2017). This suggests that the relation between Fgf8 expression and craniofacial phenotype is not linear. Moreover, below the critical Fgf8 threshold, morphological variance is larger (Green et al. 2017). This example supports the idea that more general mechanisms derived from the nonlinear architecture of development can explain the observed patterns of phenotypic robustness and also explains cryptic variation as a product of developmental nonlinearities.

How Do Biologists Measure Canalization?

In spite of being an elusive concept, there have been many attempts to obtain accurate measures of canalization. In general, canalization to macroenvironmental factors is evaluated as the precision with which consistent phenotypic traits are produced across different individuals in a population, while canalization to micro-environmental factors is commonly measured as the accuracy with which a given genotype produces symmetric characters within an individual (assessed by fluctuating asymmetry) or consistent phenotypes across different individuals that share the same external environment (Palmer 1994). Here, we summarize the main approaches used to measure phenotypic robustness through the assessment of morphological variation. The central idea is that canalization is expressed phenotypically as limited variation, while less canalized traits are characterized by large variation between individuals and between repeated parts within individuals.

The amount of variation among individuals in a given trait is considered as a proxy of its degree of canalization under the assumption that this measure represents how buffered is a trait against genetic and environmental perturbations. In fact, seminal experiments carried out by Waddington were based on this idea. After exposing developing pups of *Drosophila* to an extreme heat stress, Waddington (1957) described an increase in the among-individual phenotypic dispersion for a particular trait of the vasculature of the wings. From this simple observation, he concluded that in wild-type flies vein morphology was largely canalized and that phenotypes deviate from this developmental channel only under a severe stressful condition. Among-individual variation can be easily approached by analyzing simple statistical parameters that describe dispersion, such as variance and the related measure of standard deviation. More complex statistical analyses compare similarity of variances between groups in order to establish if there is a group (given, for instance, by a particular environmental condition) in which dispersion is increased or reduced.

A related measure is fluctuating asymmetry (FA), which is based on the idea that during development, corresponding parts of an individual (for instance, left and right sides of a bilateral structure) are expected to emerge as specular copies and that random deviations from this pattern can be interpreted as imprecisions in developmental processes (Klingenberg and Graham 2015). Since developmental noise does not affect a side preferentially, FA represents the random and nondirectional component of asymmetry. To calculate FA, first it is essential to have measures for both sides of the studied structure. The symmetric component of this structure is, therefore, the average of right and left measures across the sample. The asymmetric component can be divided into directional asymmetry (DA) that is the variation among right and left sides across the sample and FA that is defined by the differences between both sides for each individual. The statistical approach that has been used to separate and analyze these components is an adaptation of the Analysis of the Variance (ANOVA), with the individual and the side as factors. As FA usually has very low values, it is recommended to take repeated measures of the same variables to determine if the measurement error, which in an ANOVA model would be represented by the residual variance, is smaller than the magnitude of FA.

Evolution of Canalization: Adaptive, Congruent, or Intrinsic?

There are three main hypotheses for the evolutionary causes of genetic canalization that have been summarized as adaptive, congruent, and intrinsic (de Visser et al. 2003). The adaptive hypothesis proposes that genetic canalization evolved as the product of selection against deleterious effects of mutation and recombination. An alternative view is that the target of natural selection is the robustness against environmental perturbations and, thus, genetic canalization evolved as a sub-product of environmental canalization. Both hypotheses relate higher canalization to an increase in individual fitness (Fig. 2a). On the other hand, it has been suggested that instead of being the product of selection, genetic canalization is an intrinsic emergent property of complex genetic-developmental processes (Fig. 2b).

The evolution of genetic robustness to mutations is likely a prerequisite of complex life; otherwise organisms would likely not tolerate the observed levels of alleles with deleterious effects. Nevertheless, it is still poorly understood how the robustness to genetic variance evolved (Gibson and Wagner 2000). In its original formulation, it was thought to evolve under a particular mode of stabilizing selection toward the intermediate optimum. Waddington (1957) coined the term canalizing selection to refer to the process that stabilizes the population mean of a trait by selecting mechanisms that suppress the phenotypic expression of genetic variance (see also Wagner et al. 1997). The same reduction of phenotypic variance around the optimum can be achieved by the elimination or fixation of alleles within the population, although this does not represent the effect of genetic robustness. The long-term consequences of both modes are different because only the canalizing selection preserves the genetic variation and thus the potential for phenotypic change, as we will discuss in the next section. However, in most cases we cannot distinguish whether phenotypes exhibit decreased variation because genetic variance was eliminated or because the genes are not expressed as a consequence of (selected) buffering mechanisms. The selective hypothesis has attracted some debate as well, because canalization against mutations cannot proceed beyond a limit in which stabilizing selection eliminates the variation needed for selection for genetic canalization (Wagner et al. 1997).

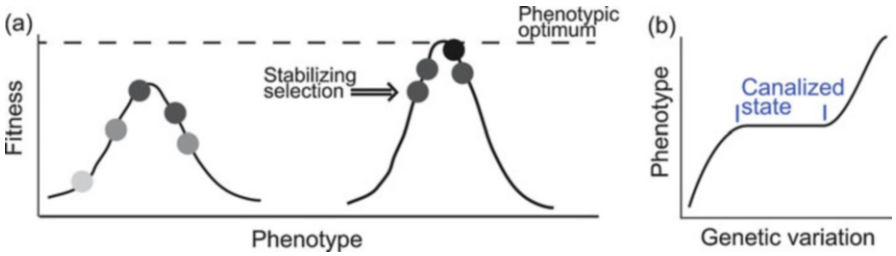


Fig. 2 (**a**) Genetic canalization evolves under the influence of stabilizing selection around the optimum phenotype; (**b**) Genetic canalization is an intrinsic property that results from the non-linearity of the Genotype-Phenotype map

Alternatively, the congruent origin suggests that genetic canalization derives from the selection of canalizing alleles that reduce the influence of environmental perturbations on phenotypic variation (Meiklejohn and Hartl 2002). In this sense, environmental canalization is thought to increase individual fitness because it favors the production of traits closer to the optimum phenotype. When the optimum phenotype remains the same across different environments, alleles that reduce phenotypic variance in a variable environment will be selected compared to non-canalizing alleles. In contrast, when the optimum phenotype changes, selection for phenotypic plasticity is expected. Implied in this hypothesis is that the same mechanisms are involved in both environmental and genetic canalization.

The central role of stabilizing selection in these explanations has been questioned by developmental systems approaches, which consider that robustness to genetic variance is an intrinsic property of nonlinear G-P maps (Nijhout et al. 2017). As a consequence of nonlinear relations, large perturbations in developmental processes might have no effect on phenotypic variation if they occur within a certain range of values in the phenotypic landscape. The curvatures in this landscape reflect the effect of genetic and epigenetic interactions on the phenotype: the steeper the curvatures, the larger the effect of the underlying factors on the phenotypic traits. Whether or not the shape of the G-P map evolved under the influence of selection can be debated. In particular, it can be argued that several nonlinear processes of pattern formation evolved via adaptation because they contribute to the stability of development, although the target of selection was not to reduce variation around the optimum phenotype. Within this framework, some studies have focused on the behavior of small gene expression networks under variable conditions. By using simulation tools to model the responses of small gene regulation networks facing different selective scenarios, it was shown that developmental processes are capable of attaining insensitivity to mutations even in the absence of stabilizing selection (Siegal and Bergman 2002). For instance, robustness to mutations can derive from simple properties of networks such as redundancy in connections: if two elements in the genetic regulatory network are linked by a large amount of connections, the network is more stable to mutations than in a scenario with few interactions. Examples of nonlinear processes that occur above the level of gene expression are relatively scarce because of their complexity, although, as we have seen above, recent studies show how the amount of phenotypic variance is modulated by intrinsic properties of the G-P map (Green et al. 2017).

Canalization and Evolvability of Phenotypic Traits

Canalization is relevant for evo-devo because it is one of the properties that modulate the production and expression of phenotypic variation that is exposed to natural selection (Hendrikse et al. 2007). On the one hand, more canalized traits are expected to display reduced potential to change under the influence of genetic and environmental effects. In comparison, genotypes with high variability (reduced state of canalization) change their phenotype much more readily than genotypes with low

variability (increased state of canalization) when faced the same mutational or environmental change. At a population level, therefore, any process that reduces the expressed variation of a trait reduces the capacity for evolution (or evolvability) of the trait (Wagner et al. 1997; see chapter ▶ "Variational Approaches to Evolvability: Short- and Long-Term Perspectives").

On the other hand, it has been argued that robustness to mutations and environmental changes actually contributes to evolvability (Masel and Trotter 2010). This is based on the assumption that canalization against genetic variation allows the accumulation of new mutations and existing alleles that are not phenotypically expressed but are maintained within populations in the form of cryptic genetic variation. As a consequence, the phenotypic space occupied under normal conditions is smaller than the genotypic space (Fig. 3a). Drastic changes in environmental conditions or any other perturbation can have a de-canalizing effect by revealing the cryptic genetic variation (Fig. 3b). Consequently, de-canalization results in the expression of new heritable phenotypic variation that is exposed to selection increasing the evolvability of organisms. This means that the ability to respond to new selective pressures, and thus the potential for evolutionary change, is increased in genotypes highly robust to the effect of mutations.

The release of cryptic genetic variation has been taken as the main evidence of the selective advantage of mechanisms that increase canalization (Masel and Trotter 2010). These contrasting effects of canalization result in an apparent paradox, as reduced variation at the phenotypic level is related to high levels of variation at the genetic level. Schmalhausen articulated this view when arguing that natural selection favors mechanisms that allow organisms to resist the effects of environmental insults and at the same time maintain the capacity to respond to environmental change. The term "evolutionary capacitance" was proposed to describe this ability to hide and release cryptic genetic variation.

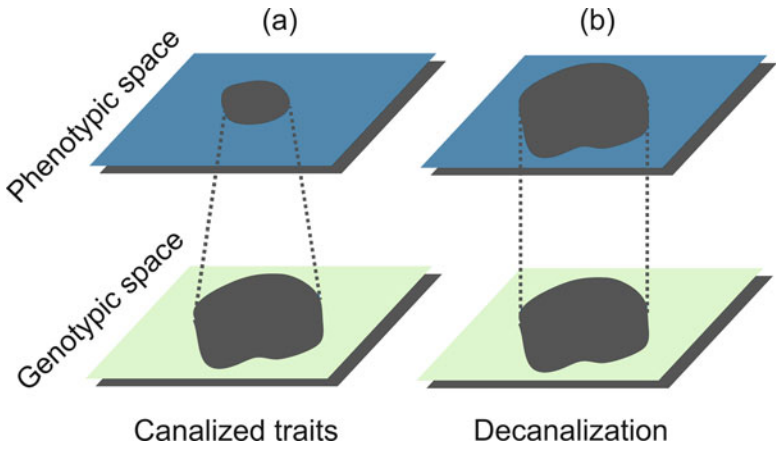


Fig. 3 (**a**) Canalized traits exhibit lower variation in the phenotypic than in the genotypic space; (**b**) After a genetic or an environmental perturbation, the accumulated cryptic genetic variation is released, increasing the variance in the phenotypic space

However, the role of cryptic variation in support of canalization has been questioned. Hermisson and Wagner (2004) showed that in any model with gene by gene (i.e., epistasis) and gene by environmental interactions, genes that have no phenotypic expression within a specific genetic background or environment can be expressed under different conditions even in the absence of canalization. This phenomenon is known as conditionally neutral variation, meaning that loci that are neutral in certain conditions can turn adaptive in a new genetic background or environment (Siegal and Leu 2014).

Future Directions

The study of canalization has been characterized by relatively opposite views about the mechanisms of phenotypic robustness and the evolutionary causes and implications of canalization in evolution. Future progress in this field might proceed by integrating in a more comprehensive agenda these apparent dichotomies. For instance, phenotypic robustness can be achieved by specific mechanisms at different levels, which are not mutually exclusive. As biological systems, organisms integrate a number of different mechanisms that stabilize the phenotype and operate at different levels, ranging from gene and cellular scales to larger networks that compromise the entire body. Instead of seeing different levels as separate and independent sources of explanation of canalization, interactions across these levels should be considered. A more realistic view emerges when the canalized phenotype is seen as the result of several underlying factors that dynamically interact to maintain a stable state (Nijhout et al. 2017).

Another topic in which contrasting views are found in literature is the evolutionary causes of canalization. Is it a product of selection for more adapted states? Or is it an intrinsic property of developmental systems? Again, alternative explanations could integrate, at least to some extent, these seemingly opposite hypotheses. Since characters are usually interconnected to other components of the system, they might be under the influence of indirect selection, which may not result, a priori, in an adaptation (Hansen 2011). How these nonadaptive and indirectly selected traits behave in the organism orchestra to produce a canalized phenotype is a matter of future study. However, it is increasingly evident that black-and-white approaches in this field should be overcome by broader and more comprehensive frameworks.

Cross-References

- ▶ Conrad Hal Waddington (1905–1975)
- ▶ Developmental Plasticity and Evolution
- ▶ Evolvability
- ▶ Epistasis
- ▶ Ivan I. Schmalhausen (1884–1963)
- ▶ Variational Approaches to Evolvability: Short- and Long-Term Perspectives

References

de Visser JAGM, Hermisson J, Wagner GP et al (2003) Perspective: evolution and detection of genetic robustness. Evolution 57:1959–1972

Geiler-Samerotte KA, Zhu YO, Goulet BE et al (2016) Selection transforms the landscape of genetic variation interacting with Hsp90. PLoS Biol 14:e2000465

Gibson G, Wagner G (2000) Canalization in evolutionary genetics: a stabilizing theory? BioEssays 22:372–380

Green RM, Fish JL, Young NM et al (2017) Developmental nonlinearity drives phenotypic robustness. Nat Commun 8:1970

Hansen TF (2011) Epigenetics: adaptation or contingency? In: Hallgrímsson B, Hall BK (eds) Epigenetics. Linking genotype and phenotype in development and evolution. University of California Press, California, pp 357–376

Hendrikse JL, Parsons TE, Hallgrímsson B (2007) Evolvability as the proper focus of evolutionary developmental biology. Evol Dev 9:393–401

Hermisson J, Wagner GP (2004) The population genetic theory of hidden variation and genetic robustness. Genetics 168:2271–2284

Jamniczky H, Boughner JC, Rolian C et al (2010) Rediscovering Waddington in the post-genomic age: operationalising Waddington's epigenetics reveals new ways to investigate the generation and modulation of phenotypic variation. BioEssays 32:553–558

Kim YE, Hipp MS, Bracher A et al (2013) Molecular chaperone functions in protein folding and proteostasis. Annu Rev Biochem 82:323–355

Klingenberg CP, Graham JH (2015) Analyzing fluctuating asymmetry with geometric morphometrics: concepts, methods, and applications. Symmetry (Basel) 7:843–934

Masel J, Trotter MV (2010) Robustness and evolvability. Trends Genet 26:406–414

Meiklejohn CD, Hartl DL (2002) A single mode of canalization. Trends Ecol Evol 17:468–473

Nijhout HF, Sadre-Marandi F, Best J, Reed MC (2017) Systems biology of phenotypic robustness and plasticity. Integr Comp Biol 57:171–184

Palmer A (1994) Fluctuating asymmetry analyses: a primer. In: Markow T (ed) Developmental instability: its origins and evolutionary implications. Contemporary issues in genetics and evolution. Springer, Dordrecht, pp 335–364

Queitsch C, Todd AS, Lindquist S (2002) Hsp90 as a capacitor of phenotypic variation. Nature 417: 618–624.

Rutherford SL, Lindquist S (1998) Hsp90 as a capacitor for morphological evolution. Nature 396:336–342.

Salathia N, Queitsch C (2007) Molecular mechanisms of canalization: Hsp90 and beyond. J Biosci 32:457–463

Sato K, Siomi H (2010) Is canalization more than just a beautiful idea? Genome Biol 11:109

Siegal ML, Bergman A (2002) Waddington's canalization revisited: developmental stability and evolution. Proc Natl Acad Sci USA 99:10528–10532

Siegal ML, Leu J-Y (2014) On the nature and evolutionary impact of phenotypic robustness mechanisms. Annu Rev Ecol Evol Syst 45:495–517

Specchia V, Piacentini L, Tritto P et al (2010) Hsp90 prevents phenotypic variation by suppressing the mutagenic activity of transposons. Nature 463:662–665

Waddington CH (1957) The strategies of the genes. Macmillan Publishers, New York

Wagner GP, Booth G, Bagheri-Chaichian H (1997) A population genetic theory of canalization. Evolution 51:329–347

Developmental Plasticity and Evolution

Annalise B. Paaby and Nicholas D. Testa

Contents

Abstract

The environment plays a crucial role in the developing organism, first in defining the developmental trajectory from genotype to phenotype, then by modifying that trajectory by natural selection. Nearly all traits exhibit some degree of phenotypic plasticity: the capacity to change, or to develop in response to, the environment. The plasticity of a trait can itself evolve, and some of the most specialized adaptations include evolved responses to environmental variation. Plasticity has long been theorized to potentiate adaptive evolution, by environmental induction of phenotypes that boosts the potential for subsequent genetic evolution or by revealing cryptic alleles in new environments that in turn generate new adaptive phenotypes. A plastic trait may vary continuously, which can be described by norms of reaction, or it may produce discrete types as a polyphenism, a codified adaptive response to specific environmental signals. The concept of plasticity can

A. B. Paaby (✉) · N. D. Testa
School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, USA
e-mail: paaby@gatech.edu; ntesta3@gatech.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_110

also be applied to variation in phenotype associated with a single genotype in a single environment. Such microenvironmental plasticity defines in part the robustness of a trait. In the evolution of complex traits, tension between plasticity and its opposite, canalization, may be crucial for rapid evolution, adaptation, and the emergence of novelty.

Keywords

Reaction norm · Polyphenism · GxE · Genetic accommodation · Genetic assimilation · Canalization · Evo-devo

Introduction

In natural systems, the environment plays two roles: first, it mediates how genotypes are translated into phenotypes; second, it imposes selection (West-Eberhard 2003; see chapter ▶ "Eco-Evo-Devo"). Together, these functions drive trait evolution, but the role of the environment in the first function can be overlooked even as its dominance is assumed in the second. This oversight may arise due to the explanatory power of genetics and its amenability to controlled laboratory experimentation. However, the ability of organisms to respond plastically to the environment is so ubiquitous that it is an essential component of the living world (Ehrenreich and Pfennig 2016). In many ways, we intuitively understand this: our expectation that the environment will impose influence is folded into many decisions we make, both scientifically and in our everyday lives. Nevertheless, in both mainstream culture and in scientific research, we often turn to genes first for biological explanations. In fact, genetic determinism – which does indeed play a profound role in human health, applied efforts in agriculture, and the evolution of natural populations – cannot be separated from environmental influence. The outcome of a genotype is undefined without specifying the environment. As genotype translates into phenotype, it travels along developmental trajectories that may be labile or robust and may be defined by generations of adaptive evolution or vulnerable to new influences. In turn, environmental selection pressures shape not only the phenotypes of an organism but the interactions between development and the environment that produces them.

Definitions and Related Concepts

Phenotypic plasticity is the ability of an individual to alter its phenotype in response to the environment, or the potential of an individual genotype to develop into alternative phenotypes in different environments (Fusco and Minelli 2010; Levis and Pfennig 2017). The first scenario can occur when a phenotype is labile over the course of an individual's lifetime: behavior, for example, or body size or composition, gene expression, or aspects of physiology. Even sex can change, in the case of

some trees, polychaetes, gastropods, and fish. The latter scenario occurs when a trait is expressed only once in a lifetime, like the age of reproductive maturity or the shape of a developmentally irreversible bony appendage. For such fixed traits, the critical aspect is that a single genotype holds the potential for different phenotypes, which are determined by the environment during development. One of the most well-studied examples is the formation of defensive "helmets" in *Daphnia* in the presence of predators (Agrawal et al. 1999).

When a plastic trait is discrete, the distinct phenotypes represent a *polyphenism* (Fig. 1a). Polyphenism is pervasive in eusocial insects as a mechanism for producing different castes, such as workers or queens, and may be controlled by diet, including feeding on royal jelly or pheromones (Simpson et al. 2011). Some moths and butterflies exhibit striking polyphenisms according to seasonal diet, including the development of spring *Nemoria arizonaria* caterpillars into morphs that mimic catkins, or oak tree flowers, versus leaf-eating summer caterpillars into mimics of oak twigs, and the development of prominent eyespots on the wings of adult *Bicyclus anynana* during the wet season versus the duller, camouflaged pattern during the dry season. Some locusts can develop into an antisocial "solitarious" phenotype or a swarming "gregarious" phenotype according to sight, smell, or tactile cues mediated by population density. Other insects exhibit polyphenic morphs with distinct dispersal abilities, such as long- or short-winged crickets and winged or wingless aphids, which are typically induced by signals of resource availability (Simpson et al. 2011). The plasticity of the *Daphnia* helmet (Agrawal et al. 1999) is a classic example of prey species polyphenism triggered by the presence of predators in the environment. This also occurs in barnacles, which grow hunched over in the presence of carnivorous snails, and bryozoans, which develop spines in the presence of predatory nudibranchs (Stearns 1989). Plants can also exhibit defensive plasticity, such as the increased production of mustard oil glycosides by the wild radish following damage by herbivorous caterpillars (Agrawal et al. 1999). Note, however, that plasticity is generally considered a polyphenism only when the phenotypes are discrete.

Most traits are not expressed as discrete types, but instead vary continuously (Fig. 1b). When plotted against variation in the environment, a continuous plastic trait can be represented by a *reaction norm* (Fig. 1c) (Stearns 1989). (It is worth noting that the distinction between a polyphenism and a continuously variable plastic trait can nevertheless be vague; the concept of a reaction norm was first introduced by Richard Woltereck while working in the early 1900s on helmet length in *Daphnia* (Simpson et al. 2011).) Plasticity is extremely pervasive, as the environment influences the expression of most traits. Most traits also exhibit nonzero heritability, indicating that phenotypes are almost always affected by genotype as well. Within populations, these two determinants – environment and genotype – influence trait expression such that different genotypes are likely to exhibit different reaction norms (Fig. 1d). When these functions have different slopes and intersect, we observe a *crossing of reaction norms*. Such nonparallelism is evidence of *gene-by-environment interaction* (GxE), in which the environment influences trait determination nonadditively across genotypes. For example, a genotype conferring slow metabolism might grow only slightly faster in a high-glucose environment compared

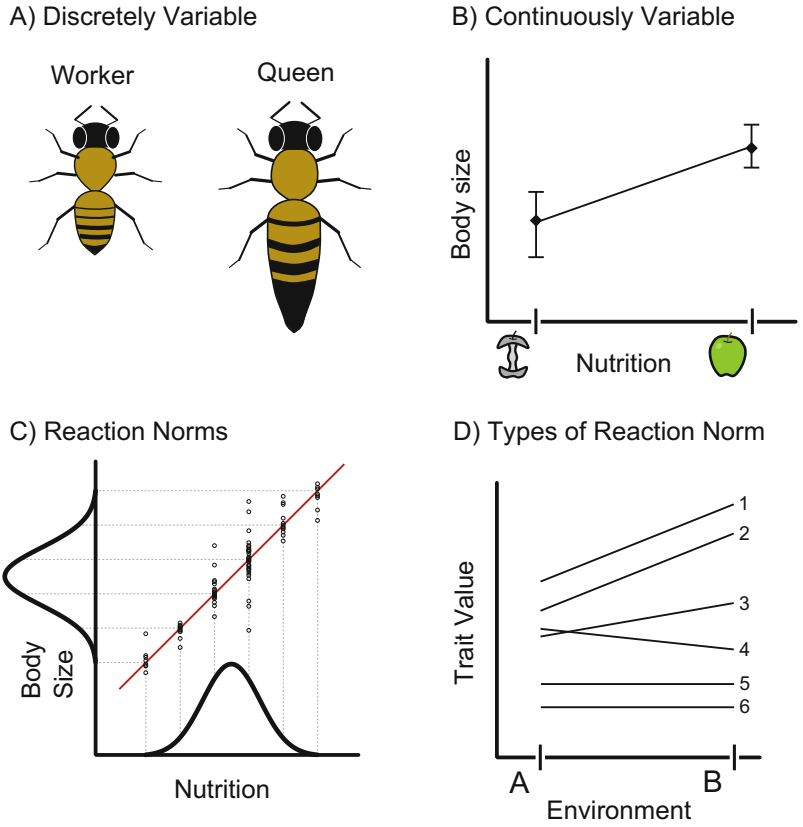


Fig. 1 A plastic trait that takes two or more discrete forms, like the castes of eusocial insects, is known as a polyphenism (**a**). Most traits vary continuously (**b**), and variation in the trait can be plotted against variation in the environment to produce a reaction norm (**c**). Typically, a reaction norm includes only individuals of the same genotype, to eliminate variation in phenotype contributed by the genetic component. Comparing the reaction norms of different genotypes captures the three most important components of phenotype variance: those arising from genetic effects, environmental effects, and the interaction between them (**d**). In this plot, reaction norms 1 and 2 both exhibit phenotypic plasticity, as genotypes 1 and 2 both produce different trait values in different environments. Here, genotype 1 has a higher average trait value than genotype 2, but the environment influences trait determination the same way in both genotypes. Norms 3 and 4 also exhibit plasticity, and the nonparallel slopes indicate a GxE interaction: the environment affects trait determination differently in genotype 3 than in genotype 4. In this comparison, the interaction is negative, as environment B raises the trait value of genotype 3 relative to environment A, but lowers it for genotype 4 (these two genotypes demonstrate interactions with every other genotype on the plot as well, though not all of these are negative). Norms 5 and 6 exhibit no plasticity, as the environment has no effect on trait value

to a low-glucose environment, whereas the difference might be dramatic for a genotype conferring fast metabolism. GxE is pervasive in natural populations (Morgante et al. 2015), and this is to be expected: if different genotypes produced parallel reaction norms, one should be fittest in all environments and eventually fix in

the population (Stearns 1989). Reaction norms themselves can evolve; this is discussed further in the section "Evolution of Plasticity."

The opposite of plasticity is *canalization* (see chapter ▶ "Canalization: A Central but Controversial Concept in Evo-Devo"), the production of an invariant phenotype even in a noisy environment (Stearns 1989). The term was coined by Waddington (1957), to illustrate the entrenched grooves or "canals" that developmental trajectories occupy in the formation of morphological features. Waddington conceived of canalization with regard to genetic variation, but now the term is used both ways, often specified as either "environmental canalization" or "genetic canalization" (Wagner et al. 1997). Although opposite in definition, the relationship between plasticity and canalization is intimate: a plastic trait can evolve into a canalized one via changes in the regulation of the trait's expression (Ehrenreich and Pfennig 2016), and plastic responses can themselves become canalized when responses to an environmental cue become stereotyped, as in a polyphenism. Trait lability and trait robustness may both evolve from the existence of conditionally functional variation, and a major motivation for studying plasticity and canalization is the hypothesis that dynamic tension between these phenomena might enable rapid evolution, adaptation, and the emergence of novel traits (Paaby and Gibson 2016).

The term *robustness* may be used as a synonym for canalization, though canalization is more often conceived in association with development, as in the ability of a developmental trajectory to withstand perturbations to produce an invariant phenotype (Masel and Siegal 2009). If the allelic or epigenetic state of a genic element is responsible for producing either an environmentally canalized phenotype or a plastic phenotype, then this element is considered a *phenotypic stabilizer* (Masel and Siegal 2009). A phenotypic stabilizer is analogous to a *phenotypic capacitor*, likewise a genic element with potential to store or release phenotypic variance, but in this case due to genetic, rather than environmental, variation. The switch of a phenotypic capacitor from one state to another, for example the disabling of the heat-shock protein HSP90, can lead to the release of *cryptic genetic variation* (Geiler-Samerotte et al. 2016; Rutherford and Lindquist 1998). Cryptic genetic variation is standing variation in a population that has little effect on phenotype until a perturbation induces expressivity, either through epistasis, like by the disruption of a phenotypic capacitor, or through GxE, if the perturbation is environmental (Paaby and Rockman 2014; see chapter ▶ "Epistasis").

In a seminal series of experiments, Waddington (1953) used heat shock to release cryptic genetic variation for, and promote eventual *genetic assimilation* of, a "crossveinless" wing morphology in wild-type strains of *Drosophila melanogaster*, kicking off decades of investigation into the potential role of this mechanism in adaptive evolution (see chapter ▶ "Conrad Hal Waddington (1905–1975)"). Genetic assimilation occurs when cryptic mutations, neutral under normal conditions, penetrate to phenotype in a new environment, and selection eventually fixes the trait such that the environmental stimulus is no longer required (Fig. 2). Constitutive expression is made possible by new combinations of alleles that underlie the evolution of the plasticity of the trait, which is known as *genetic accommodation*; genetic assimilation is a dramatic form of genetic accommodation, as plasticity is completely lost in the evolved population (Ehrenreich and Pfennig 2016;

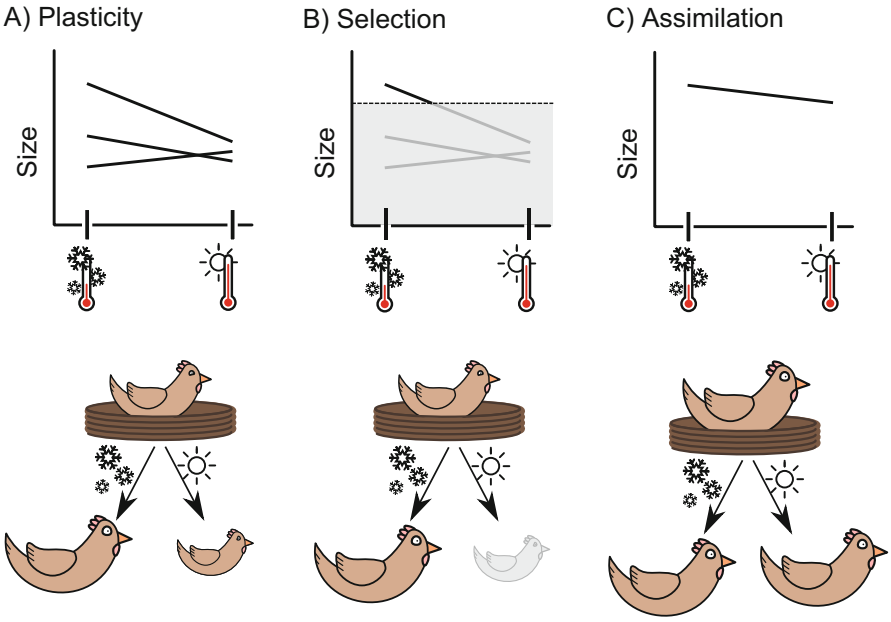


Fig. 2 Genetic assimilation can occur when phenotypic effects of genetic variation are revealed under a specific environment (**a**). The genetic variation is cryptic, such that the phenotype exhibits lower variance in the "normal" environment (here depicted as the warm condition) and higher variance in the new environment (the cold condition). Alleles that produce a novel phenotype (big birds) are now selected (**b**). In the beginning, the new phenotype is only produced in the new environment, but generations of selection produce allelic combinations that eventually fix trait expression in all environments (**c**). Most evidence for genetic assimilation comes from artificial selection experiments in the lab, so the extent of this phenomenon in natural systems is unknown

see chapter ▶ "Evo-Devo and Niche Construction" and the discussion of genetic accommodation therein). The case for genetic accommodation in trait evolution, including observations of plasticity in natural systems, has been comprehensively addressed in West-Eberhard's book on the topic (West-Eberhard 2003). However, Waddington's original experiments demonstrating genetic assimilation, and the contemporary investigations that succeeded them, provide proofs of principle but do not as yet clarify the extent to which plasticity, GxE, cryptic genetic variation, and canalization govern the evolution of natural populations.

Plasticity-First Evolution

A major question regarding plasticity in evolutionary biology is the relative importance of a "plasticity-first" mode of trait evolution, in which a plastic response to environmental change precedes and enables adaptive change, compared to a genes-first mode, in which selection acts on new genetic variants first and changes in plasticity are secondary. In other words, since the first step toward a new adaptive

phenotype involves the production of a new developmental variant, the question is whether trait evolution more often starts with an environmentally induced variant or a variant induced by mutation (Levis and Pfennig 2016). Three factors support a plasticity-first mechanism: first, environmental change is likely to affect many or all individuals in a population, whereas de novo mutation, by definition, occurs in only one individual; second, since plastic responses are always linked to the environments that induce them, they may be primed to increase fitness; and third, environmental plasticity can promote the accumulation of cryptic alleles, a store of heritable variation that might potentiate rapid genetic adaptation (Levis and Pfennig 2016).

A crucial mechanism by which plasticity probably facilitates trait evolution relies on accumulation of cryptic genetic variation on which selection may eventually act, as exampled by Waddington's (1953) evolution of the *Drosophila* crossveinless wing phenotype. Accumulation of cryptic variation is an emergent property of any genetic system with epistasis or GxE, though it may also be facilitated by the evolved stability of developmental trajectories, which can shelter cryptic alleles (Hermisson and Wagner 2004). Once a sufficiently destabilizing environment reveals cryptic variation, selection can target the focal trait but also the environmental dependence of its expression, such that plasticity itself evolves, in the process of genetic accommodation. Waddington's genetic assimilation experiments demonstrate a complete loss of plasticity because the trait became constitutively expressed, but selection can also promote developmental sensitivity to environmental signals. The most extreme form of sensitization results in the evolution of polyphenism (Levis and Pfennig 2016). Evidence for genetic accommodation in natural systems is mostly indirect – since testing trait expression in a true ancestor is typically impossible – but observations of physiological and behavioral traits in house finches, behavior and morphology in stickleback fish, and melanin production in *Daphnia* provide compelling support (Moczek et al. 2011). Trait plasticity can theoretically promote evolvability, because the location of a decision point along the developmental path can evolve just as the terminal phenotype of the plastic trait is adaptively refined (see chapters ▶ "Evolvability" and ▶ "Variational Approaches to Evolvability: Short- and Long-Term Perspectives"). This potentially dramatic lability of ontogenetic specification can increase the "evolutionary degrees of freedom" of the system (Moczek et al. 2011).

Despite the still-growing accumulation of observations elucidating how plasticity influences the evolution of natural systems (Levis and Pfennig 2017; West-Eberhard 2003), whether plasticity tends to jump-start evolutionary innovation remains an open question (Moczek et al. 2011). The best case study for this phenomenon involves investigation of spadefoot toads in the genus *Spea*, by Pfennig and colleagues. *Spea* tadpoles develop into either small-jawed omnivores or large-jawed carnivores, depending on diet. Examination of *Scaphiopus couchii*, an omnivorous species used as a proxy for the non-plastic *Spea* ancestor, revealed slow growth and a release of cryptic genetic variation for size, development, and gut length when exposed to carnivorous conditions. Two *Spea* species also show evidence of genetic assimilation. Both exhibit intermediate ecomorph frequencies when they live alone in a single-species "ancestral" condition, but they show near fixation of one or the

other ecomorph, independent of dietary resources, when they co-occur (Levis and Pfennig 2016). A macroevolutionary analysis of nematode evolution also provides evidence for the plasticity-first hypothesis. Here, plasticity appears to have promoted the diversification of mouthpart feeding mechanisms, including the evolution of a predatory and sometimes polyphenic morph with moveable teeth. The comparative analysis of 90 species showed that the historical appearance of mouthpart plasticity is associated with faster evolution, increased diversification, and subsequent independent losses of plasticity (Susoy et al. 2015). On the other hand, one analysis of gene expression and molecular evolution in the spadefoot toad system suggests that plasticity could be a consequence, rather than a cause, of rapid evolution. Genes with differential expression between the omnivore and carnivore ecomorphs were compared to a set of unbiased genes, both in the plastic *Spea* species and in four species that diverged before the evolution of plasticity. The biased genes, those presumed to be associated with plasticity, showed higher variance in expression and faster evolution than the unbiased genes, but the elevated rates predate the evolution of plasticity. With these findings, the authors speculate that plasticity may emerge when fast-evolving, dispensable genes become available for environment-dependent adaptation (Leichty et al. 2012).

Evolution of Plasticity

Given its inherent physical and chemical properties, by default development should respond plastically to environmental influence (Nijhout 2003). Evolved modifications to these biophysical responses to the environment include both the dampening of plasticity, via canalization, and codification of plasticity into a polyphenism. Under this expectation, Nijhout (2003) classifies plasticity two ways. Type 1 emerges as a function of the physical and chemical interface between the developing organism and the environment and is unlikely to increase fitness. Type 2 is an evolved adaptation to a particular environment. For continuous traits, evolution of plasticity means the evolution of reaction norms. Greater environmental heterogeneity speeds reaction norm evolution, though the extent to which environments are novel or rare may matter; cryptic genetic variation released in new environments will expose new reaction norms (Schlichting and Pigliucci 1998). The shape of reaction norms can vary dramatically by trait. Thermal traits often exhibit convex norms, with the lowest values at the temperature extremes; threshold-mediated traits are likely to be logistic; and morphological traits show a diversity of reaction norm shapes, including in different environments (Schlichting and Pigliucci 1998). As for possible genetic mechanisms that permit plasticity, three have been proposed: pleiotropy, epistasis, and overdominance. The pleiotropic model states that one gene can pleiotropically affect fitness in different environments; the epistatic model expects the interaction of two or more genes, some affecting the height of the reaction norm and some

determining its shape (see chapters ▶ "Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics" and ▶ "Epistasis"). The overdominance model predicts that plasticity should increase with homozygosity, as heterozygous loci buffer phenotypes from environmental perturbations (Pigliucci 2005).

Although polyphenisms are discrete, some may be the product of a discontinuous environment acting on the norm of reaction of what is otherwise a continuous trait (Nijhout 2003). Others are mediated by developmental switches, but even those may have evolved to direct the development of what was an ancestrally continuous trait. Hormone regulation, especially in insects, is a pervasive mechanism of polyphenism, wherein changes to hormone secretions or hormone sensitivity act on the process of metamorphosis. Notably, the environment that induces a polyphenic trait is often not the environment that imposes selection. For example, seasonal polymorphisms that promote fitness in either warm or cold environments often anticipate those conditions by photoperiod (Nijhout 2003).

In an unpredictable world, the ideal organism would have total plasticity: the ability to form or reform to every situation with maximum fitness. Obviously, total plasticity is not possible, and both costs and limits probably constrain its evolution (Murren et al. 2015. Costs to plasticity can be inferred from the evolution of specialist species; if a generalist could maximize fitness in multiple environments, then we would not expect specialists, with little ability to thrive outside their niches, to evolve in any of those environments (Kawecki 1994). And yet they do, across broad taxonomic and ecological ranges.

However, empirical studies often fail to detect any fitness costs to generalists. Identifying costs of plasticity can be tricky because they should not be confused with costs of the phenotypes themselves. If a generalist and a specialist both produce adaptive traits in a specific environment, but the traits are unequal and the specialist is fitter, this is not a cost of plasticity but a cost of phenotype. A cost of plasticity is the universal cost incurred to the generalist in all environments, such as the expense of larger genome to house additional genetic machinery. It may be that such universal costs are not so important in limiting the reach of plasticity and that instead insufficient selection is a more important constraint for the evolution of generalists (Murren et al. 2015). For example, generalist species may experience weaker selection against deleterious mutations that erode the fitness of environment-specific traits. Unlike specialists, in which fitness-compromising alleles will be exposed in all members of the population, in generalist populations only the subset of individuals within the specific environment will be subject to selection (Kawecki 1994). Variation in selection also affects the evolution of plasticity. Fluctuations in selection arising from environmental heterogeneity favor plasticity, over timescales occurring within individual lifespans as well as those spanning generations, especially when environmental cues are reliable. Limited genetic variation for plasticity has also been proposed as a constraint for its evolution, though recent analyses have estimated greater evolution of norms of reaction than of the traits themselves, suggesting that this may not be an important limitation (Murren et al. 2015).

Micro-plasticity and the Quantitative Genetics Perspective

This chapter, and the literature on plasticity in general, has been focused at the level of the organism. However, plasticity can be observed and quantified at the population level, and indeed this is an important consideration in the evolution of populations. From a quantitative genetics perspective, if the mean of a trait within a population changes when the environment changes, the trait is plastic (Pigliucci 2005). The plasticity may include GxE (and it usually does), but it need not, if the environment pushes trait expression in the same direction and with the same magnitude for every genotype. Technically, plasticity may occur even if the mean and variance of the population remain unchanged; consider crossing reaction norms, as in Fig. 1d, but in a perfectly symmetrical hourglass arrangement with a balanced number of individuals for each genotype. In this scenario, plasticity is only at the level of the organism and not at the level of the population, so the plastic response does not change the ability of the population to evolve if a new phenotype is favored. However, population-level plasticity can occur without a change in trait mean so long as the variance changes. Here, the plastic response could enable faster phenotypic evolution, as outliers become targets of selection. This is the premise behind the theory that cryptic genetic variation can potentiate adaptive evolution (Paaby and Rockman 2014). Or, if stabilizing selection disfavors phenotypic outliers, evolution of allele frequencies can occur without phenotypic evolution. Both of these scenarios require that the higher trait variance is associated with the new environment.

Usually, plasticity is discussed in terms of defined differences in the environment. However, even within controlled or static environments, individuals of the same genotype do not produce identical phenotypes. This variance in phenotype, due to unknown and uncontrolled variations in the developmental or external environment, is microenvironmental plasticity. This phenomenon has also been understood as developmental noise, stochastic or residual variation, and environmental sensitivity (Morgante et al. 2015).

The existence of microenvironmental plasticity implies that any individual genotype (reared in a constant environment) is associated not with one specific phenotype but with a distribution of possible phenotypes (Fig. 3). And just as genotype, the environment, or an interaction between them determines a phenotypic mean, so too will these factors influence phenotypic variance or the range of microenvironmental plasticity. The influence of genotype on microenvironmental plasticity can be strong, and sometimes its heritability is as large or larger than that of the trait mean. Consequently, microenvironmental plasticity itself can be a target of selection. For quantitative traits under stabilizing selection, microenvironmental plasticity in the trait should evolve toward zero, as the mean centers around the fitness optimum. Within fluctuating environments, selection should favor nonzero microenvironmental plasticity, as a bet-hedging strategy increases the probability that some individuals in the next generation will be fit in their environment (Morgante et al. 2015).

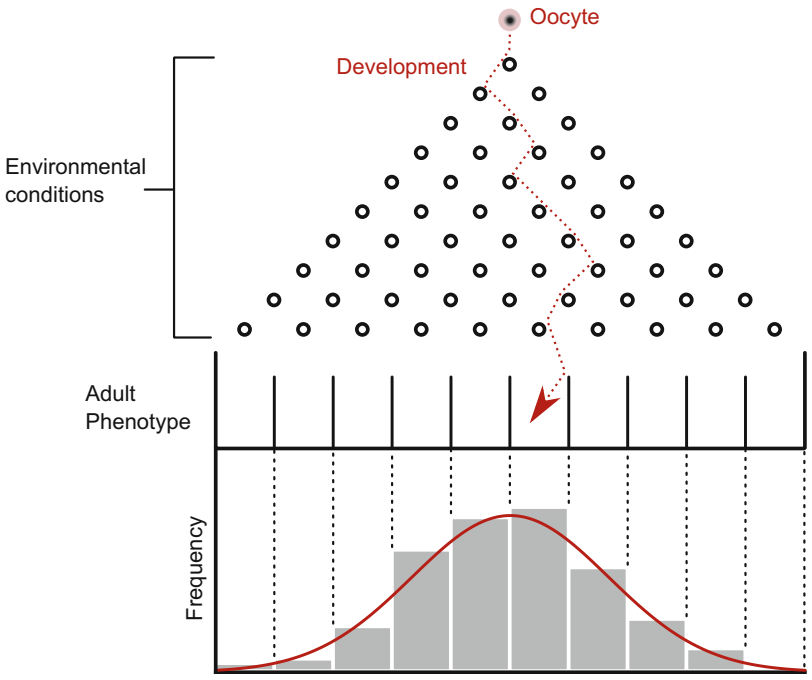


Fig. 3 The phenotype of any individual organism falls along a distribution of possible phenotypes, which is governed by both the individual's genotype and the environment. Plasticity occurs when different environments induce different distributions, but within a distribution, the different possible phenotypes may be considered products of "micro-plasticity" because they arise from minute and unknown variations in an otherwise controlled or consistent background. In this figure, we appropriate the concept of a "Galton board," wherein the pins represent micro-variations in the environment that, either stochastically or by unknown determinants, govern the developmental trajectory of the individual from oocyte to adult. In contrast to Waddington's canonical "epigenetic landscape" (Waddington 1957), here the ultimate phenotypic products are not discrete but continuous and are determined not by canalized developmental trajectories but assume, if we stick with the original conception by Galton, that each pin imposes a 50-50 left-right outcome for the falling ball and that the resulting distribution is approximately normal (Galton 1894). However, we make no assumptions that environmental micro-variations influence developmental outcomes with binomial probability or that phenotypic distributions are always normal

Exploiting Plasticity in the Laboratory

Research into the evolution of development can take advantage of dramatic plastic responses to environmental perturbation. Related taxa often exhibit conservation of developmental processes at the morphological level but divergence in the genetic mechanisms that govern them, a phenomenon called developmental system drift (True and Haag 2001; see chapter ▶ "Developmental System Drift"). Analyses of molecular evolution and comparative genomics can provide insight, but functional

dissection of developmental processes is especially hard in non-model systems and systems that cannot produce viable interspecies crosses. However, environmental perturbations can decanalize development and induce morphological aberrations that may in turn provide clues to the cellular processes that connect the diverged genes to the conserved phenotype. This is analogous to using genetic perturbations to reveal hidden but functional differences in the developmental mechanisms that vary cryptically within populations (Paaby et al. 2015) or have diverged across species (Verster et al. 2014).

Unlike a gene-based perturbation, an environment-based perturbation might not provide a hypothesis-testing framework with mechanistic specificity regarding developmental variation across lineages. However, this may be compensated by the relative ease and consistency that an environment-based perturbation affords. For example, temperature stress, chemical exposure, and nutrition are easily controlled in the lab. There is no expectation that an environmental stress will necessarily reveal functional differences connected to the stress itself, for example, through a history of selection. Rather, the idea is that the environmental perturbation will destabilize developmental trajectories to reveal cellular or genetic differences between the tested lineages. For example, polymorphism in the candidate gene *Ultrabithorax* was shown to underlie variation of expression in, and eventual genetic assimilation of, the bithorax phenotype in *D. melanogaster* lineages exposed to ether (Gibson and Hogness 1996).

Like those that induced the bithorax (Gibson and Hogness 1996) and crossveinless (Waddington 1953) phenotypes, an informative perturbation is one that is sufficient to deform the developmental trajectory but not so effective that it kills the organism outright. Temperature stress has been used in multiple systems to induce heritable, intraspecific phenotypic variation, some of which recalls other naturally evolved phenotypes (Moczek et al. 2011). Across lineages, divergence in function but also the expression of recurrent phenotypes arises from the twin aspects of lability and robustness that characterize developmental processes (Paaby and Gibson 2016). For traits undergoing developmental system drift, natural selection will favor changes that stabilize the phenotype around the canonical type when de novo mutations or new environments cause deformations to the developmental trajectory. Consequently, a perturbation that unveils glimpses of these deformations will not be one that evokes type 2 plasticity, an evolved adaptation to a known environment, but one that evokes type 1, a physical and chemical response (Nijhout 2003) that can exaggerate differences in mechanism.

Cross-References

- ▶ A Macroevolutionary Perspective on Developmental Constraints in Animals
- ▶ Canalization: A Central but Controversial Concept in Evo-Devo
- ▶ Conrad Hal Waddington (1905–1975)
- ▶ Developmental Evolutionary Biology (Devo-Evo)
- ▶ Developmental System Drift

- ▶ Eco-Evo-Devo
- ▶ Epistasis
- ▶ Evo-Devo and Niche Construction
- ▶ Evolvability
- ▶ Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics
- ▶ Variational Approaches to Evolvability: Short- and Long-Term Perspectives

References

Agrawal AA, Laforsch C, Tollrian R (1999) Transgenerational induction of defences in animals and plants. Nature 401:60–63

Ehrenreich IM, Pfennig DW (2016) Genetic assimilation: a review of its potential proximate causes and evolutionary consequences. Ann Bot 117:769–779

Fusco G, Minelli A (2010) Phenotypic plasticity in development and evolution: facts and concepts. Philos Trans R Soc Lond Ser B Biol Sci 365:547–556

Galton F (1894) Natural inheritance. Macmillan and Company, New York

Geiler-Samerotte KA, Zhu YO, Goulet BE, Hall DW, Siegal ML (2016) Selection transforms the landscape of genetic variation interacting with Hsp90. PLoS Biol 14:e2000465

Gibson G, Hogness DS (1996) Effect of polymorphism in the *Drosophila* regulatory gene Ultra-bithorax on homeotic stability. Science 271:200–203

Hermisson J, Wagner GP (2004) The population genetic theory of hidden variation and genetic robustness. Genetics 168:2271–2284

Kawecki TJ (1994) Accumulation of deleterious mutations and the evolutionary cost of being a generalist. Am Nat 144:833–838

Leichty AR, Pfennig DW, Jones CD, Pfennig KS (2012) Relaxed genetic constraint is ancestral to the evolution of phenotypic plasticity. Integr Comp Biol 52:16–30

Levis NA, Pfennig DW (2016) Evaluating 'plasticity-first' evolution in nature: key criteria and empirical approaches. Trends Ecol Evol 31:563–574

Levis NA, Pfennig DW (2017) Phenotypic plasticity. In: Pfennig K (ed) Oxford bibliographies in evolutionary biology. Oxford University Press, New York

Masel J, Siegal ML (2009) Robustness: mechanisms and consequences. Trends Genet 25:395–403

Moczek AP et al (2011) The role of developmental plasticity in evolutionary innovation. Proc Biol Sci 278:2705–2713

Morgante F, Sorensen P, Sorensen DA, Maltecca C, Mackay TF (2015) Genetic architecture of micro-environmental plasticity in *Drosophila melanogaster*. Sci Rep 5:09785

Murren CJ et al (2015) Constraints on the evolution of phenotypic plasticity: limits and costs of phenotype and plasticity. Heredity 115:293–301

Nijhout HF (2003) Development and evolution of adaptive polyphenisms. Evol Dev 5:9–18

Paaby AB, Gibson G (2016) Cryptic genetic variation in evolutionary developmental genetics. Biology 5(2):28

Paaby AB, Rockman MV (2014) Cryptic genetic variation: evolution's hidden substrate. Nat Rev Genet 15:247–258

Paaby AB, White AG, Riccardi DD, Gunsalus KC, Piano F, Rockman MV (2015) Wild worm embryogenesis harbors ubiquitous polygenic modifier variation. eLife 4:e09178

Pigliucci M (2005) Evolution of phenotypic plasticity: where are we going now? Trends Ecol Evol 20:481–486

Rutherford SL, Lindquist S (1998) Hsp90 as a capacitor for morphological evolution. Nature 396:336–342

Schlichting CD, Pigliucci M (1998) Phenotypic evolution: a reaction norm perspective. Sinauer Associates Incorporated, Sunderland

Simpson SJ, Sword GA, Lo N (2011) Polyphenism in insects. Curr Biol 21:R738–R749

Stearns SC (1989) The evolutionary significance of phenotypic plasticity. Bioscience 39:436–445
Susoy V, Ragsdale EJ, Kanzaki N, Sommer RJ (2015) Rapid diversification associated with a macroevolutionary pulse of developmental plasticity. eLife 4:e05463
True JR, Haag ES (2001) Developmental system drift and flexibility in evolutionary trajectories. Evol Dev 3:109–119
Verster AJ, Ramani AK, McKay SJ, Fraser AG (2014) Comparative RNAi screens in *C. elegans* and *C. briggsae* reveal the impact of developmental system drift on gene function. PLoS Genet 10(2):e1004077
Waddington CH (1953) Genetic assimilation of an acquired character. Evolution 7:118–126
Waddington CH (1957) The strategy of the genes. George Allen and Unwin, London
Wagner GP, Booth G, Bagheri-Chaichian H (1997) A population genetic theory of canalization. Evolution 51:329–347
West-Eberhard MJ (2003) Developmental plasticity and evolution. Oxford University Press, Oxford

Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics

Mihaela Pavličev

Contents

Abstract

Pleiotropy, the involvement of a gene in development and variation of multiple traits, is a concept considerable appreciation in developmentally as well as statistically oriented fields of evolutionary biology. Here I argue that this feature makes pleiotropy a particularly suitable guiding topic for connecting the two branches of evolutionary biology, and integrate evolutionary descriptions from the molecular, over developmental, to populational mechanisms. I first describe some of the challenges in defining pleiotropy, and then focus on evolution of

M. Pavličev (✉)
Department of Evolutionary Biology, Unit for Theoretical Biology, University of Vienna, Vienna, Austria
e-mail: mihaela.pavlicev@univie.ac.at

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_52

pleiotropic constraints, which I suggest is the centerpiece of the connection. Finally, I address some of the future challenges.

Keywords

Pleiotropy · Modularity · Epistasis · Evolvability

Introduction

Pleiotropy is a concept deeply rooted both in mechanistically oriented developmental genetics, as well as in statistical approaches of population genetics. Pleiotropy not only reflects developmental genetic structure but also importantly mediates the effect of developmental structure on the future response to selection. The question to what extent pleiotropy constrains long-term response to selection is particularly important for the role of pleiotropy in evo-devo. In the past, the focus on microevolution invited model assumptions, which limited the ability to study long-term dynamics of pleiotropy and of the genotype-to-phenotype map in general (such as lack of context dependency of genetic effects; see chapter ▶ "Epistasis"). Here, some of the general challenges of studying pleiotropy will be discussed first, followed by the focus on the ability of pleiotropy and associated constraint, to evolve. By having roots in mechanistic and variational traditions, with much accumulated knowledge in both, pleiotropy is a particularly central concept when unifying evolutionary biology.

General Definition

In most general terms, pleiotropy refers to a situation in which mutation in a single gene causes phenotypic changes in multiple distinct traits. These can be large phenotypic changes, often deleterious in individuals, such as those observed in pathological syndromes (e.g., phenylketonuria) in which pleiotropic effects were first recognized, or they can be minute effects on multiple traits which, when studied across a population of individuals, manifest as covariation between traits. The pattern of mutational effects on the phenotypic traits can reveal the developmental genetic structure, as simultaneous changes in different traits suggest that a portion of the genetic-developmental basis between the traits is shared.

Even though pleiotropic effects were recognized earlier in medical syndromes and correlated effects have been acknowledged by Mendel and Darwin, the term pleiotropy was first coined by developmental geneticist Ludwig Plate in 1910, whose interest was in the intersection of evolution and development. Many of the early studies of pleiotropy attempted to explain the physiological basis of pleiotropy (Stearns 2010). Yet, it is the work on the phenotypic correlations and their consequences for evolutionary change that arguably provided the strongest impulse for the surge of studies of pleiotropy in evolutionary biology, such as culminated in morphological integration. The study of phenotypic correlations and covariances

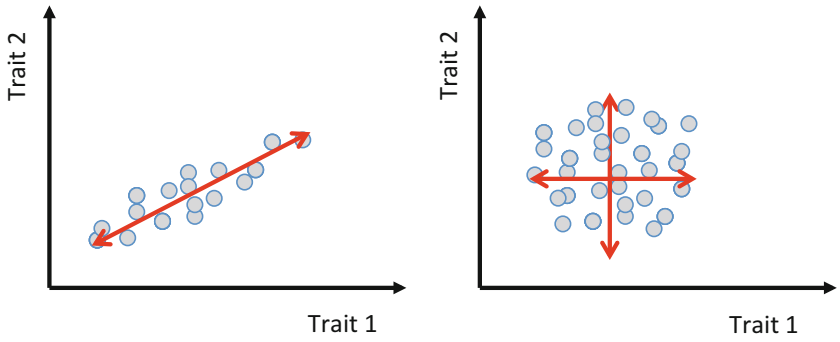


Fig. 1 The distribution of variation in two traits in (**a**) the presence of strong pleiotropy and (**b**) in the absence of pleiotropy when the variation in the two traits is independent from each other. In the first case, the direction of most variation is shown to be the direction along which both traits change simultaneously. In (**b**), any phenotypic direction has equal amount of variation

motivated the quantitative genetic study of underlying genetic covariances and their evolutionary consequences (Lande 1979), effects of pleiotropy on standing variation (Turelli 1985), as well as the relationship between the genetic and phenotypic correlations (Cheverud 1988).

The main evolutionary consequence of pleiotropy is its directing of genetic variation along particular phenotypic directions and preventing others (Fig. 1). Evolutionary change is biased toward those phenotypic directions, which exhibit the greatest heritable phenotypic variance (Schluter 1996). With respect to any particular trait, variation shared with other traits can either prevent or diminish the response to selection, or it can cause correlated response in other traits. This is because when mutation at a locus affects more than one trait, but only the change in a single trait is advantageous, it depends on the selection regime acting on all affected traits, whether such variation can be selected upon. When such correlated effects are mainly deleterious, response in a population will likely not realize, in spite of apparently sufficient variation in the selected trait (Hansen et al. 2003). This situation is referred to as evolutionary or pleiotropic constraint. If the correlated mutational effects on other traits are selectively neutral, the advantageous mutation can spread in a population; however, it will simultaneously change traits that have not been directly selected. One of the most prominent examples using such pleiotropic side effects is the model by George C. Williams (1957) that senescence is due to *antagonistic* pleiotropy. According to this model, the beneficial effects on individuals early in life that contribute to individual's reproductive success and are thus selected for, are associated with the negative pleiotropic effect on late life stages (i.e., senescence). As there is a strong selection on reproductive life period, but not on life period after reproduction, such effects persist in the population (the gain of reproductive fitness early in life outweighs its cost). More recently, deleterious side effects have been proposed to themselves become a source of selective pressure to become compensated by secondary mutations. A good example here are side effects of mutations conferring drug resistance in some conditions but are fitness reducing in

others (Lenski 1988 and many later). This model suggests enrichment of compensatory mutations in traits, which are themselves under stabilizing selection but share genes with traits under directional selection. The compensatory mutations allow the traits' basis to avoid the correlated response, hence, *not* to adopt a new phenotype, but to maintain a phenotype. Such phenomenon could underlie developmental systems drift (True and Haag 2001) – a situation in which developmental processes underlying the same trait in different taxa are highly modified and appear as if they are drifting while the end phenotype is under stabilizing selection (Johnson and Porter 2007; Pavlicev and Wagner 2012) (see chapter ▶ "Developmental System Drift").

Challenges in Determining Pleiotropy

Many aspects of pleiotropy that are important for its influence on short- and long-term evolutionary change are being revisited. In the following sections, I will first explain some of the challenges in defining pleiotropy; next, I will focus on the evolution of pleiotropy itself; and finally, I will conclude with the open challenges that need to be addressed in the future to gain full appreciation of the role of pleiotropy in evolutionary change.

What Is a Trait?

One of the most fundamental problems in determining pleiotropy of a mutation is in defining the traits, in order to count them (Zhang and Wagner 2013). Whereas infinitely many measurements can be taken, not every measurement that can be taken on an organism describes a biologically distinct trait. Different approaches have been taken to tackle this problem. One is based on a biological process, often growth. Separate processes are thereby considered to characterize separate traits. For example, the long bones (the bones in arms and legs) have a clear direction of growth in the length, due to the growth plates from which the cell proliferation occurs. This makes long bone length a meaningful biological trait. But such trait choice may be harder when we talk about the skull, where traits are highly interdependent and biological dimensions are harder to define. In the skull, traits are often defined as the directions of the variation, using ordination techniques (e.g., such as PCA) to determine the number of independent directions in space that together capture most of the variation between the individuals. Single directions thereby consist of contribution of multiple measurements. While this approach is extremely useful for the analysis of dimensionality of phenotypic variation, i.e., in how many independent directions a population phenotype may respond to selection, such statistical dimensions (in particular later PCs) may encompass more than a single biological trait and may therefore cause difficulties when such phenotypic variation is being associated with underlying genetic sequence variation (Cheverud 2007). The approach thus has advantages but also disadvantages in particular when used to

define pleiotropy. For example, the highly correlated traits such as fore and hind limbs, may appear as a single trait axis in some species, and the interesting biological problem of their overcoming pleiotropic constraint and obtain the ability to produce independent variation, would be lost. The field has not reached a general consensus on the definition of trait that would be equally satisfactory in all its uses.

The Many Mechanisms of Pleiotropy

Pleiotropy describes a variational phenomenon, in which differences in genetic sequence are associated with differences in multiple phenotypic traits. This association can arise by many different mechanisms. Already early on, it was recognized that single or different gene products from the same locus may underlie pleiotropic effects on different traits and that the respective gene may play a direct role in the development or physiology of the particular trait or it may affect a trait through a cascade of other developmental processes and traits. The classical example is the joint between two bones: even if only one of the bones is affected by a genetic mutation, the effect will be reflected by the adjacent bone, because its growth is regulated in part by the physical presence of the joint. Mutation in this case has an indirect pleiotropic effect via the interactions between traits. With more detailed insight into gene function, it became recognized that a product of a single gene may become modified in many ways (splicing, posttranscriptional modifications, epigenetic states, differential effect of environment, etc.) and finally that various functions of a multifunctional gene may be performed by different functional domains, allowing for separate evolution. Thus, a question arises whether pleiotropy is a property of a gene or of a mutation within a gene (Stern and Orgogozo 2008). Not every mutation within a gene affects all functions of a gene. Rather, different mutations in the same gene may have different degrees of pleiotropy, some affecting single traits, some all traits that gene is involved in the development of. The pleiotropy at the level of a gene is thus rather a propensity, a number of traits the mutations in this gene *can* affect, whereas the realized pleiotropy refers to the specific mutation.

How Many Traits? Pleiotropy and the Evolution of Complexity

One of the long-standing but often not explicit discussions about pleiotropy is about its overall effect across the genome and phenome and therefore its prevalence. While geneticists agree that most genes are to some degree pleiotropic (i.e., pleiotropy is *ubiquitous*), there is a disagreement in the models as to whether all genes affect, directly or indirectly, all traits (pleiotropy is *universal*). The implicit assumption of universal pleiotropy, for example, underlies many models, including the suggestion that as the organisms become more complex, their potential for successful adaptation decreases (*the cost of complexity* (Orr 2000)). This extrapolation is based on the idea that the increase in organismal complexity involves an increase in the number of traits. Assuming universal pleiotropy, it follows that each gene affects an increasing

number of traits as the complexity increases. Fisher had previously argued that in the phenotypic space with two traits, the proportion of advantageous mutations, out of all possible mutations, decreases when the size of mutation increases (a.k.a. Fisher's geometric model (Fisher 1930)). Smaller mutations are more likely to be advantageous. Orr (2000) has shown that the more traits are affected by the mutation, the more this relationship is exacerbated. Thus, the advantageous mutational steps diminish in size as the complexity increases, given that increase in complexity also means increase in the pleiotropy of the mutation.

Contrasting universal pleiotropy is the model of restricted pleiotropy, or variational modularity, which asserts that the gene pleiotropy is restricted to the traits with common development or function. These traits likely frequently experience simultaneous selection, whereas reducing pleiotropic association with other traits reduces interference between traits that are under conflicting selection pressure. So far, empirical work established good evidence for restricted pleiotropy of gene effects in genetic and phenotypic variational patterns (e.g., Mezey et al. 2000; Su et al. 2010). In addition, the absence of negative effect of pleiotropy on effect size, and even its potential enhancement, has also been shown (Wagner et al. 2008; Wang et al. 2010).

An important aspect of restricted pleiotropy has been noted by Hansen (2003). Hansen pointed out that, given a certain number of genes in the genome, reducing the interference due to pleiotropic genes comes at the price of reducing the number of genes affecting each trait. As smaller genetic basis offers lesser mutational potential for a trait, there is therefore a trade-off between restricting interference and reducing the potential to generate mutation in the first place. Hansen thus proposed that most evolvable genotype-phenotype maps would be those with intermediate levels of pleiotropy.

Phenotypic Traits Versus Fitness

The organisms, which are most suitable for the study of evolution because of their rapid reproduction, namely, microbes, often do not offer appropriate individually measurable phenotypic traits. Instead, the effects of mutations are measured directly on fitness of a clone, as a rate of reproduction. Fitness is only a single variable, and therefore pleiotropy in this case has been conceptualized differently, namely, as the effect of mutation on fitness measured in multiple environments or in multiple genetic backgrounds. This concept of pleiotropy converges with gene-by-environment interaction, and it should be noted that pleiotropy and gene-by-environment (or gene-by-gene) interaction indeed are mathematically equivalent (Falconer 1952; Pavlicev and Cheverud 2015).

Evolution of Pleiotropy

While it is interesting to understand how the above aspects of pleiotropy affect its role in response to selection, another important characteristic of pleiotropy in the

context of evo-devo is its own *evolvability*. By funneling genetic variation along certain directions of phenotypic space, pleiotropy conserves developmental structure and plays a role in maintaining structural properties such as a *bauplans*. This aspect of pleiotropy is crucial in explaining phenotypic inertia and discrete organismal diversity, rather than continuous distribution of phenotypic traits across species. Evolutionary developmental biologists have made an important point by emphasizing the role of constraint (as opposed to ubiquitous variation) in shaping the future potential to evolve. However, this view should not be taken to mean that pleiotropic constraints are absolute constraints, to which no change can be introduced. The most convincing evidence that pleiotropy evolves is the evolutionary divergence of repeated, or serially homologous, elements, such as segments in arthropods, limbs in quadrupedal vertebrates, digits, vertebrae, petals of the flowering plants, etc. These organismal parts share genetic basis, i.e., the same core genes regulate the development of each repeated part. Yet we know that the degree to which the repeated elements have diverged in some species is enormous, for example, the divergence of the forelimb and hind limb in apes and humans, or in birds and bats, or segments in arthropods such as bees in which some segments retained legs, some have wings, and yet others are fused into the thorax, head, or abdomen (Fig. 2). The implication of these observations is that the correlated response due to shared genes must have been alleviated in these traits, in order for them to individualize and diverge from each other in an organism. To demonstrate this change, many studies have focused on comparison of phenotypic intertrait correlations between species (Marroig and Cheverud 2001). While it appears that for some trait complexes, these are fairly stable and conserved across many species, other trait covariances have changed considerably over time, such as the forelimb and hind limb during the transition from quadrupedal to bipedal primates (Young et al. 2010) or different functional modules in the skulls of *Anolis* lizards (Sanger et al. 2012). Yet this interspecific change itself also has its origin in a population. How does this happen?

One of the mechanisms by which pleiotropic effects can change has been obscured by our own assumptions about the constancy of genetic effects. At the level of population, heritable phenotypic diversity can be fairly well described by considering the effects of substitutions at single loci to be independent of each other and consequently that they can be added up across the genome. This implicitly assumes that the effects of gene substitutions are a property of the particular locus at which they occur, and it follows that the phenotypic changes of a population occur by the change of frequency of the alleles at these loci. It has been long known that the effect of a substitution can change when the genetic background changes, that is, when substitution takes place in the context of a different genotype at another locus and this can have an effect even within a population. Yet these effects are often small in interbreeding populations, and thus the short-term additive approximation is fairly good. But these "interactions" between loci (▶ "Epistasis"), or also across environmental conditions, can contribute the kind of variation in gene effects necessary to select for the effect sizes in different backgrounds. Important for the evolution of pleiotropy is that such variability of genetic effect is not limited to the effect size, e.g., whether the substitution increases a trait only slightly or considerably. Rather, the

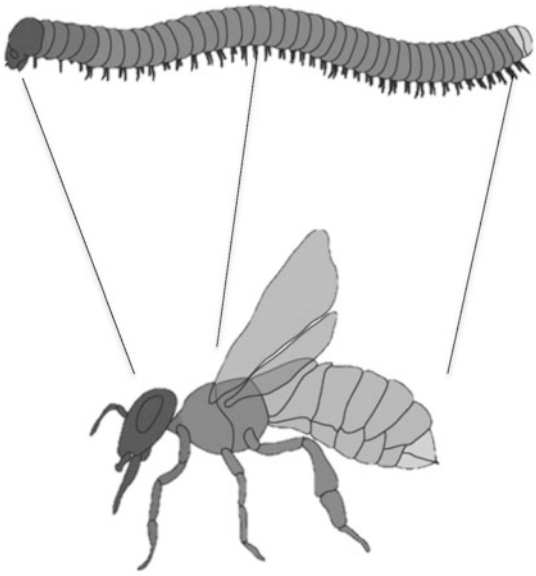


Fig. 2 Individualization of the body segments (Figure adapted after: Genetic Science Learning Center. "Homeotic Genes and Body Patterns." Learn. Genetics. March 1, 2016. Accessed September 2, 2016. http://learn.genetics.utah.edu/content/basics/hoxgenes/.). In the millipede above, most of the segments have uniform structure, whereas in the bee, the thorax segment are integrated and highly differentiated from the abdominal segments

pleiotropy of a mutation has been also shown to differ across genetic and environmental backgrounds, e.g., whether one or two traits are affected by the mutation, or whether they are both affected in the same or different ways. This variation in pleiotropy introduces the potential for pleiotropy to evolve and with it the degree to which pleiotropy constraints the evolution of traits. Genetic background-dependent variation in pleiotropic effects has been detected (Leamy et al. 2009; Pavlicev et al. 2008) and modeled to show that it can indeed modify the trait correlations (Pavlicev et al. 2011; Watson et al. 2014).

How can this play out in the change of correlation between traits that are affected in their development by the same gene, such as the serially homologous forelimb and hind limb?

A pleiotropic gene interacts during development of any particular trait with other local genes that are also involved in building this trait, and these local genes differ across traits the pleiotropic gene is involved in, e.g., between the forelimb and the hind limb. The different effects of pleiotropic genes can thus be thought as interactions with different (internal) environments, to stay with limb example; that of the forelimb and that of the hind limb. As these "environments" change under divergent selection, the effects of pleiotropic genes on the forelimb and hind limb diverge.

Taking the potential effect of the context into consideration thus enables us to extend the small-scale dynamics, taking place within the relatively invariant context of a homogeneous population (microevolutionary level), to larger-scale evolution at the interspecific level (macroevolution). While the apparent disparity between the two levels has caused substantial disputes over what processes are important in evolutionary change, at least some of that void between the intra- and interspecific

change can be bridged by including the context dependency of genetic substitutions into the models.

Future Challenges

The genotype-to-phenotype map, of which pleiotropy is an important aspect, is an abstraction of developmental mechanisms that translate genetic variation into phenotypes. So far, it has played a coarse role in the study of heritable phenotypic pattern and its effects on the short-term response to selection. Future challenges are twofold. One is the understanding of various mechanisms that generate similar pleiotropic patterns and determining to what extent the longer-term responses are influenced by the specific mechanism involved (e.g., whether pleiotropy is due to the same or different gene products). The other challenge is the evolution of these mechanisms and pleiotropy themselves. This would essentially confront the molecular and developmental mechanisms with population dynamics, as much as provide population dynamics with the much needed molecular and developmental basis. Pleiotropy is not the only aspect of genotype-phenotype map that can provide this bridge. The reason that pleiotropy is particularly attractive in this respect is that it is a central concept in both fields of evolutionary biology and has a rich history of research in both.

Cross-References

- ▶ A Macroevolutionary Perspective on Developmental Constraints in Animals
- ▶ Developmental System Drift
- ▶ Epistasis
- ▶ Evolvability
- ▶ Evolution of Complexity
- ▶ Variational Approaches to Evolvability: Short- and Long-Term Perspectives

References

Cheverud JM (1988) A comparison of genetic and phenotypic correlations. Evol Int J Organ Evol 42:958–968

Cheverud JM (2007) The dangers of diagonalization. J Evol Biol 20:15–16; discussion: 39–44

Falconer DS (1952) The problem of environment and selection. Am Nat 86:293–298

Fisher RA (1930) The genetical theory of natural selection. Oxford University Press, Oxford

Hansen TF (2003) Is modularity necessary for evolvability? Remarks on the relationship between pleiotropy and evolvability. Biosystems 69:83–94

Hansen TF, Armbruster WS, Carlson ML, Pelabon EC (2003) Evolvability and genetic constraint in *Dalechampia blossoms*: genetic correlations and conditional evolvability. J Exp Zool B Mol Dev Evol 296(1):23–39

Johnson NA, Porter AH (2007) Evolution of branched regulatory genetic pathways: directional selection on pleiotropic loci accelerates developmental system drift. Genetica 129:57–70

Lande R (1979) Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. Evol Int J Organ Evol 33:402–416
Leamy LJ, Pomp D, Lightfoot JT (2009) Genetic variation in the pleiotropic association between physical activity and body weight in mice. Genet Sel Evol 41:41
Lenski RE (1988) Experimental studies of pleiotropy and epistasis in *Escherichia coli*. II. Compensation for maladaptive effects associated with resistance to virus T4. Evolution 42:433–440
Marroig G, Cheverud JM (2001) A comparison of phenotypic variation and covariation patterns and the role of phylogeny, ecology, and ontogeny during cranial evolution of new world monkeys. Evol Int J Organ Evol 55:2576–2600
Mezey JG, Cheverud JM, Wagner GP (2000) Is the genotype-phenotype map modular? A statistical approach using mouse quantitative trait loci data. Genetics 156:305–311
Orr HA (2000) Adaptation and the cost of complexity. Evol Int J Organ Evol 54:13–20
Pavlicev M, Cheverud JM (2015) Constraints evolve: context dependency of genetic effects allows evolution of pleiotropy. Annu Rev Ecol Evol Syst 46:413–434
Pavlicev M, Wagner GP (2012) A model of developmental evolution: selection, pleiotropy and compensation. Trends Ecol Evol 27:316–322
Pavlicev M, Kenney-Hunt JP, Norgard EA, Roseman CC, Wolf JB, Cheverud JM (2008) Genetic variation in pleiotropy: differential epistasis as a source of variation in the allometric relationship between long bone lengths and body weight. Evol Int J Organ Evol 62:199–213
Pavlicev M, Cheverud JM, Wagner GP (2011) Evolution of adaptive phenotypic variation patterns by direct selection for evolvability. Proceed Biol Sci/R Soc 278:1903–1912
Sanger TJ, Mahler DL, Abzhanov A, Losos JB (2012) Roles for modularity and constraint in the evolution of cranial diversity among Anolis lizards. Evol Int J Organ Evol 66:1525–1542
Schluter D (1996) Adaptive radiation along genetic lines of least resistance. Evol Int J Organ Evol 50:1766–1774
Stearns FW (2010) One hundred years of pleiotropy: a retrospective. Genetics 186:767–773
Stern DL, Orgogozo V (2008) The loci of evolution: how predictable is genetic evolution? Evolution Int J Organ Evol 62:2155–2177
Su Z, Zeng Y, Gu X (2010) A preliminary analysis of gene pleiotropy estimated from protein sequences. J Exp Zool B Mol Dev Evol 314:115–122
True JR, Haag ES (2001) Developmental system drift and flexibility in evolutionary trajectories. Evol Dev 3:109–119
Turelli M (1985) Effects of pleiotropy on predictions concerning mutation-selection balance for polygenic traits. Genetics 111:165–195
Wagner GP, Kenney-Hunt JP, Pavlicev M, Peck JR, Waxman D, Cheverud JM (2008) Pleiotropic scaling of gene effects and the 'cost of complexity'. Nature 452:470–472
Wang Z, Liao BY, Zhang J (2010) Genomic patterns of pleiotropy and the evolution of complexity. Proc Natl Acad Sci U S A 107:18034–18039
Watson RA, Wagner GP, Pavlicev M, Weinreich DM, Mills R (2014) The evolution of phenotypic correlations and "developmental memory". Evol Int J Organ Evol 68:1124–1138
Williams GC (1957) Pleiotropy, natural selection, and the evolution of senescence. Evol Int J Organ Evol 11:398–411
Young NM, Wagner GP, Hallgrimsson B (2010) Development and the evolvability of human limbs. Proc Natl Acad Sci U S A 107:3400–3405
Zhang J, Wagner GP (2013) On the definition and measurement of pleiotropy. Trends Genet 29:383–384

Epistasis

Thomas F. Hansen

Contents

Abstract

Epistasis is broadly synonymous with gene interaction, referring to cases in which the effects of changing a gene depend on the state of other genes. Beyond this, the term has acquired a number of different technical and nontechnical meanings, which has led to confusion and misunderstanding in communication across disciplines. Clear communication about epistasis is particularly pertinent in evolutionary developmental biology both because of the relevance of epistasis to some of its key research questions such as the evolution of evolvability and canalization, and because evo-devo acts as a trading zone for cross-disciplinary communication.

T. F. Hansen (✉)
Department of Biology, CEES and EVOGENE, University of Oslo, Oslo, Norway
e-mail: thomas.hansen@bio.uio.no

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_53

Keywords

Gene interaction · Quantitative genetics · Genotype-phenotype map · Evolvability · Canalization

Introduction

In genetics, the term "epistatic" was introduced by Bateson and Punnett to describe deviations from the expected 9:3:3:1 ratio of two independently segregating Mendelian pairs with dominance. In his influential 1909 book on Mendelian inheritance, Bateson used the terms "epistatic" and "hypostatic" to refer to cases in which one factor, the epistatic one, conceals the effects of another, hypostatic, one. Hence, his choice of the Greek term epistatic with the meaning of "upon" "standing" or "stopping." This terminology was in analogy with the contemporary use of the terms dominant and recessive, when one dominant allelomorph (allele) conceals the effects of another recessive one on the same pair (locus). Bateson saw the need for different terms to describe the analogous relationship between alleles at different loci. Bateson did not seem to intend this strictly. Throughout his book he stressed that dominance is not a principle but a matter of degree, and this extends to epistasis. Later, different forms of deviations from the 9:3:3:1 ratio gave name to different types of epistasis such as dominance, recessive, and compositional epistasis.

Bateson's usage was soon supplemented by another concept of gene interaction. In the key 1918 paper unifying Mendelian segregation with the biometric laws of heredity, Fisher noted that the effects of independently segregating factors need not add up in a linear manner, and he coined the term "epistacy" for deviations from statistical additivity. With a century of hindsight it is easy to think that Fisher chose a slightly different term to underline the difference between his statistical and Bateson's biological notion of gene interaction, but Fisher provided no discussion of the matter, and did not make the same terminological distinction with regard to dominance. In any case, Fisher's term epistacy eventually slid out of usage and was replaced with epistasis.

This terminological conflation of statistical and biological epistasis has been an obstacle in cross-disciplinary, even within-disciplinary, communication about gene interaction. While biological measures of gene effects are defined as differences between specific genotypes without regard to their relative occurrence, the statistical measures are defined as average deviations of the genotype effects from population averages over all genotypes in a population. The latter makes statistical gene effects and epistasis dependent on the composition of a population, so that common genotypes, for example, tend to have smaller effects than rare genotypes. Within the field of quantitative genetics the statistical definitions of gene effects proved convenient in terms of describing similarities among relatives and predicting the short-term response to artificial selection, but the statistical description of epistasis as a residual from additive effects averaged out the effects of biological epistasis and led to the notion that epistasis was uncommon, inert, and inconsequential for

selection dynamics at least. This clashed with the intuitions of systems-oriented biologists that (biological) epistasis was ubiquitous and essentially important for organismal function and evolution.

Population genetics used a notion of epistasis that is closer to the biological concept than to the statistical concept of quantitative genetics. In theoretical population genetics, the effects of genotypes on fitness are stipulated in advance and not as statistical averages. This is the basis of most of the standard insights on the effects of epistasis on evolution as in Wright's shifting-balance theory, the Bateson-Dobzhansky-Muller model for the evolution of reproductive isolation, coadapted gene complexes, and the evolution of sex and recombination.

Molecular genetics stuck to Bateson's narrow definition of epistasis as a mutation that masks the effect of another mutation on another gene. This was linked to the idea that an epistatic mutation would be in a gene that acted downstream to an hypostatic mutation and that epistasis therefore could be used as a tool to infer position of genes in genetic pathways.

These different notions of epistasis lived side by side during the development of the modern synthesis but came in closer contact in the 1980s. The emergence of an evolutionary quantitative genetics brought the methods and theory of quantitative genetics into evolutionary biology, and the different notions of epistasis and ideas about its importance came in conflict. Evolutionary developmental biology accentuated this with its focus on how the genotype-phenotype map affects evolution. Epistasis is a property of the genotype-phenotype map and plays a crucial role in key research questions of evodevo such as the evolution of evolvability and canalization. The interest in gene regulation and gene networks in evodevo and systems biology also brought the molecular genetics view of epistasis in contact with the epistasis concepts of evolutionary biology.

Epistasis as a Property of the Genotype-Phenotype Map

Gene products function in complex biochemical pathways and are thus embedded in networks of molecular interaction. The epistasis concept is not used to describe interactions at this level. Instead it describes interactions between the *phenotypic* effects of genetic *changes*, i.e., allele substitutions including mutations. Epistasis is not a property of the gene but a property of two or more gene *substitutions* that may be epistatic in relation to each other. This makes epistasis an aspect of the genotype-phenotype map. The mapping from genotypes to phenotypes is an abstract description of how phenotypic changes relate to genotypic changes. An additive genotype-phenotype map means that any specific substitution of alleles will have the same phenotypic effect regardless of the state of other genes (i.e., regardless of the position in genotype space), so that the cumulative phenotypic effect of several substitutions equals the sum of their individual phenotypic effects. Every deviation from this pattern may be termed gene interaction and again divided into dominance and epistasis depending on whether the composite changes happen at the same or

different loci, although an interaction between two subsequent changes of the same allele is sometimes called intralocus epistasis.

The strategy of modeling the dynamics of single alleles one-by-one was successful in demonstrating the power of natural selection and in elucidating fundamental principles of microevolution, but it has been less helpful in understanding macroevolution, because the additive summation of effects becomes increasingly unrealistic with larger changes. In a sense, additivity is a constant-evolvability assumption that allows little room for genetic constraints to affect evolution.

Epistasis can be conceptualized as nonlinearities in the genotype-phenotype map (e.g., Rice 1998). As shown in Fig. 1, the same genetic change can have different phenotypic effects depending on position in the genotype-phenotype map. Moving from position A to position B, the convexity of the map leads to an increased phenotypic effect. This is called positive epistasis. Moving from position B to position C, the concavity of the map leads to a decreased phenotypic effect. This is called negative epistasis. Moving into the flat areas of the map, genotypic changes are still possible, but their phenotypic effects vanish. This is called canalization (e.g., Flatt 2005). With the map in Fig. 1, the evolvability is high in the middle region, but moving from position B out towards the edges shows how negative epistasis leads to canalization and reduced evolvability. This constitutes an epistatic constraint on evolution, because it is not possible to change the phenotype beyond the limits of the map.

Real genotype-phenotype maps need not be shaped as in Fig. 1. The degree and sign of curvature and the existence and position of absolute limits to phenotypic

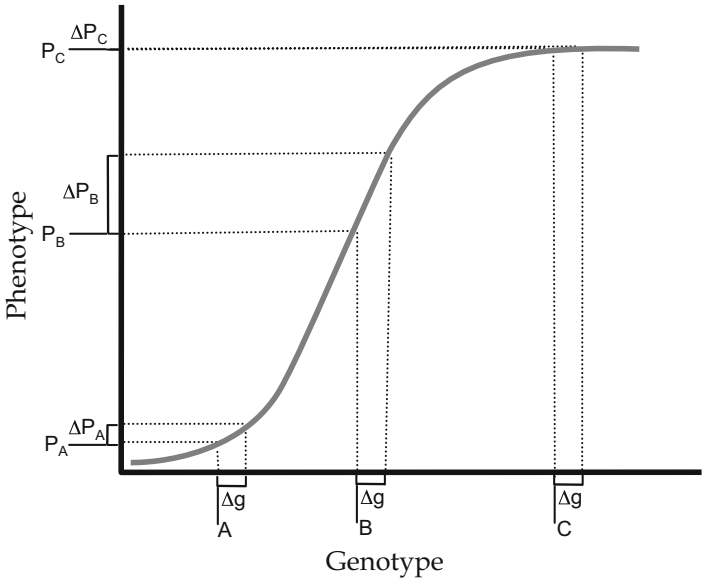


Fig. 1 A nonlinear genotype-phenotype map. The same genetic change, Δg, will have different phenotypic effects, ΔP, depending on the genetic background (positions *A*, *B,* or *C*) in which it happens (Modified from Hansen (2015))

change are empirical questions. The figure illustrates how epistasis allows the evolution of evolvability, and how this depends not on epistasis in general but on particular systematic patterns of epistasis. Positive epistasis in the direction of selection leads to evolution of increased evolvability, while negative epistasis leads to the evolution of decreased evolvability (canalization).

Statistical Epistasis

The Statistical Genotype-Phenotype Map of Quantitative Genetics

The statistical model of the genotype-phenotype map initiated by Fisher is at the core of quantitative genetics. Here genetic effects are defined as statistical deviations from an average. In its modern form the model starts with defining the average effect of an allele as the average deviation of its carriers from the population mean (technically an average excess; the difference between average excess and average effect will be ignored for simplicity). The additive effect (breeding value) of an individual is the sum of these effects for all the alleles it carries. The actual phenotype of the individual may deviate from the breeding value both because of environmental effects and because its genetic component may deviate from the additive sum due to dominance or epistasis. For example, the average deviation of individuals carrying two specific alleles at the same locus may not equal the sum of the average effects of these two alleles. The average deviation from the sum is then the statistical dominance effect of these two alleles. Similarly, the average deviation of individuals carrying two specific alleles at different loci may differ from the sum of the average effects of the alleles, and this difference is a (statistical) epistatic deviation. In general the epistatic effect of any set of alleles is defined as the average deviation of the carriers of this set from the prediction given by taking the sum of all the lower-order effects of these alleles, i.e., the sum of their average effects, dominance effects, and lower-order epistatic effects (Lynch and Walsh 1998).

One may think of the statistical genotype-phenotype map as a multiple regression of individual phenotypes on the presence/absence of alleles and sets of alleles. Dominance and epistasis are interaction effects in this model. The variance explained by the sum of the average effects (i.e., first-order effects) is the additive (A) genetic variance, and the variance explained by the interactions between alleles at the same locus is the dominance (D) variance. There are many different epistatic variances. The variance explained by interactions between two alleles at different loci is the additive-by-additive (AA) epistatic variance, the variance explained by interactions among two alleles at one locus and one at another locus is the additive-by-dominance (AD) epistatic variance, the variance explained by interactions among four alleles at two loci is the dominance-by-dominance (DD) epistatic variance, the variance explained by interactions among three alleles at three different loci is the additive-by-additive-by-additive (AAA) epistatic variance, etc. The sum of all these variances is the total genetic variance.

This decomposition is useful in describing inheritance and similarity between relatives. The covariance between phenotypes of two related individuals is a sum of contributions of all these variance components, each weighted with the probability that the two relatives share the allele sets in question (Lynch and Walsh 1998). For example, full sibs share half the additive effects and thus half the additive variance; they further share one quarter of the dominance effects, one quarter of the AA epistatic effects, and smaller fractions of higher-order epistasis. Offspring and a parent share half the additive variance, none of the dominance variance, one quarter of the AA epistatic variance, and smaller fractions of higher-order AAA types of epistatic variance.

Significantly, because all the alleles carried by an individual are inherited from its two parents, all the additive variance in a generation has been inherited from the previous generation. In contrast, none of the dominance variance and only fractions of the epistatic variances are normally inherited from the previous generation. This is because sets of alleles are broken up and recombined into new combinations each generation.

Epistasis, Inheritance, and Selection

From these considerations, it is clear why the additive effects and the additive variance play central roles in inheritance and selection. Natural selection acts on variation, and the additive variance is the heritable component of the phenotypic variance in a population. Natural selection does not see the difference between components of variance, but only the effects on the additive component are transferred to the next generation and contribute to evolution by natural selection. The smaller fractions of epistatic variance that are inherited, most significantly the one quarter of the AA epistatic variance, can yield a minor evolutionary effect, but this effect is transient because the selected allele combinations are continuously being broken down by recombination. If selection ceases, the gain achieved by selection on epistatic variance is removed at a geometric rate by recombination.

This has served as a theoretical justification for the focus on additive variance in quantitative genetics and for the single-gene perspective of population genetics and most other fields of evolutionary biology. Fisher's average effect is an elegant device for capturing the dynamics of individual alleles without in fact assuming that their effects are biologically additive. In a large population, a specific allele will find itself in myriads of different combinations with other alleles. The effects of selection on the allele will depend on its phenotypic effect averaged over all these combinations, and this is precisely what the average effect is measuring. The definition of statistical epistasis ensures that the epistatic deviations must sum to zero, and hence that they do not affect the dynamics of individual allele frequencies. Hence, the focus on statistical additivity in quantitative genetics is not based on an assumption of biological additivity but on an identification of the statistical averages that govern the dynamics of individual alleles in complex systems of biological interaction.

Statistical and Biological Epistasis

Even though statistical epistasis and epistatic variances are largely inconsequential for evolutionary dynamics, this does not extend to biological epistasis. As the additive effects are averages over genotypes in a population, they will change when the genetic background is changing, and this change is determined by biological epistasis. In Fig. 2, distributions of "molecular" genetic variation on the x-axis are mapped into distributions of phenotypically expressed genetic variation on the y-axis. At each point, A, B, and C, the molecular variation is the same, but due to the epistasis the distributions of phenotypically expressed genetic variation are different. Over the range of variation at each point, the map is approximately linear, and fitting a statistical regression would support an approximately additive model at each point, so that the variation mapped to the phenotype axis would be additive genetic variation. Moving from point A to point B, the positive epistasis increases the additive variance, and moving on towards point C, the negative epistasis in this region would reduce the additive variance, and evolvability would disappear as complete canalization is approached. At each point during this trajectory the phenotypic response to selection could be predicted from the additive genetic variances, but the long-term dynamics would be determined by the effects of epistasis on the dynamics of the additive variance.

Even if the range of variation was sufficient to cover nonlinearities as in Fig. 3, the statistical epistasis would be estimated as deviations from the best-fitting linear

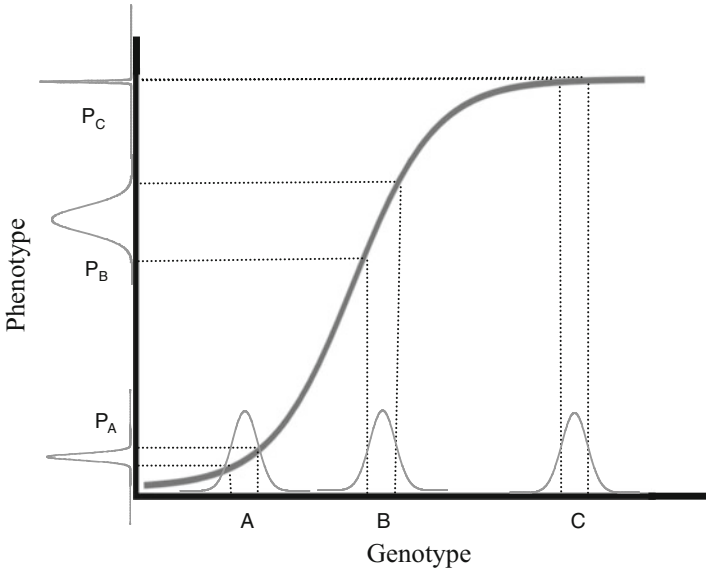


Fig. 2 The same levels of molecular genetic variation will generate different levels of variation in the phenotype depending on the genetic background (positions *A*, *B,* or *C*) (Modified from Hansen (2015))

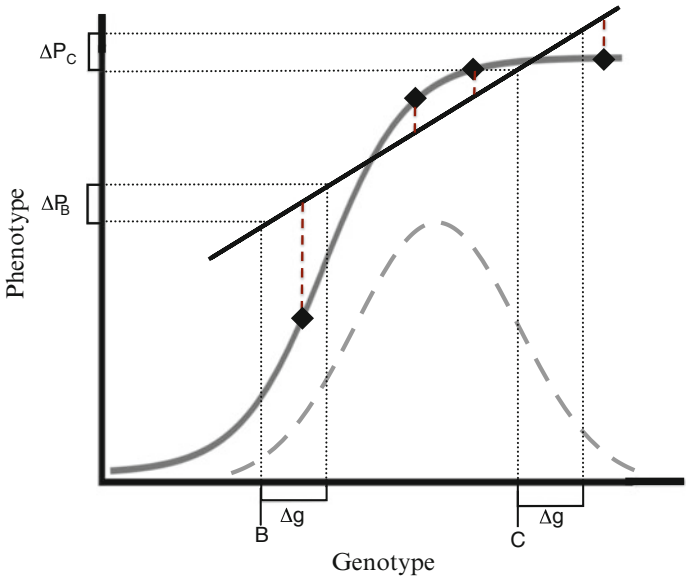


Fig. 3 Fitting an additive model (*straight black line*) over a range of genetic variation (*dashed-line* distribution along x-axis) captures the average effect, ΔP, of an allele substitution, Δg, over the range but also constrains the average effects to be constant so that $\Delta P_B = \Delta P_C$. Epistasis causes residual deviations from the linear model (*diamonds*), but their variance does not indicate specific patterns in the map

approximation and fail to describe the specific nonlinearities in the map. Epistatic variance could be detected, but it would be similar regardless of whether the biological epistasis was positive, negative, or simply random. The model would predict constant additive effects and evolvability over the range of the map.

A clear conceptual distinction between biological and statistical epistasis emerged gradually in the 1990s. In a key paper, Cheverud and Routman (1995) introduced the concept of "physiological" (= biological) epistasis and showed that it can influence the additive genetic variance. Hansen and Wagner (2001) developed this further and showed how "functional" (= biological) epistasis could be represented in a quantitative genetics framework. Carter et al. (2005) used Hansen and Wagner's multilinear representation of epistasis to formally describe the effects of biological epistasis on selection dynamics. In particular, they described how positive directional epistasis leads to the evolution of increasing additive variance and evolvability, while negative directional epistasis has the opposite effect. If the epistasis is nondirectional without any systematic patterns, the dynamics are almost indistinguishable from an additive model.

Such systematic effects of biological epistasis on the selection response have nothing to do with selection on epistatic variance. Selection on the epistatic variance leads to a buildup of linkage disequilibrium that is transient in the sense that it is rapidly broken down by recombination. In contrast, the effects of directional

epistasis are permanent, because they are mediated through changes in the genetic background that modify the biological effects of subsequent allele substitutions. If selection increases the frequency of alleles that, say, increase a trait, and these alleles have an average positive epistatic interaction with other alleles that have a positive effect on the trait, then these other alleles will more often find themselves in genetic backgrounds that elevate their effects. These elevated effects are permanent in the same sense as changes of allele frequencies are permanent.

Permanent effects of epistasis on the selection response were not captured by quantitative-genetics theory, because the statistical representation of epistasis as residuals from a regression constrained it to be nondirectional. The missing conceptual distinction between statistical and biological epistasis then led many to the inference that epistasis in general was unimportant (reviewed in Hansen 2013).

The NOIA model of Álvarez-Castro and Carlborg (2007) provides a general framework for representing most forms of functional (biological) and statistical epistasis and for translating between them.

Estimating Epistasis

In classical quantitative genetics, epistasis is estimated either as epistatic variance components inferred from patterns of resemblance between relatives or from line-cross analyses (Lynch and Walsh 1998). Line-cross analyses are based on regressions of the mean phenotypes of different crosses ("line-cross derivates") on the fraction of genes they have from each parental line and on their level of heterozygosity. For example, a back cross between the F1 and a parental is predicted to have 75% of its genes from this parental and 25% from the other and to be 50% heterozygotic. This allows the fitting of crude models of interaction between genes from the two parental lines. In principle, nonlinearities of the form illustrated in Fig. 1 can be inferred from such data, but classical line-cross analysis has yielded few insights due to its focus on significance testing rather than estimation and on the distinction between AA, AD, and DD types of epistasis. In any case, this method is now largely superseded by marker-assisted approaches.

Quantitative-trait locus (QTL) and genome-wide association studies (GWAS) use molecular markers to identify positions in the genome with effects on phenotypic traits. These approaches have been focused on identifying genes and estimating their individual effects, but it is possible to fit regression models with interactions that can identify epistasis (Lynch and Walsh 1998; Malmberg and Mauricio 2005). The detection of epistasis is made difficult by the large number of potential interactions and the use of significance thresholds to detect individual effects. Strong and systematic patterns of epistasis may go undetected, because they are spread over many interactions with individually small effects and there is a danger that significant interactions may be extremes that are atypical of the general patterns. Evidence for epistasis often comes from variants of these models in which larger ranges of phenotypes are studied (e. g., Huang et al. 2013).

The empirical study of epistasis has suffered from a lack of connection between statistical methods and theoretical relevance (Hansen 2015). The classical epistatic variance components have little evolutionary relevance, and the marker-based estimates are typically constrained to be nondirectional by the use of the standard statistical regression model. Le Rouzic (2014) reviews modifications and methods for detecting directional patterns of epistasis. There is also a tradition for studying theory-relevant patterns of epistasis on fitness, for example, by regressing fitness against correlates of accumulated mutations to estimate levels of synergistic epistasis among deleterious mutants. More recently, systematic studies of interactions between induced mutations on fitness and life-history traits in yeast and bacteria have been used to elucidate the role of epistasis in adaptation (e.g., Perfeito et al. 2014).

Epistasis Analysis in Molecular Genetics

In molecular genetics, epistatic interactions between, usually loss-of-function, mutations are used to infer the position of genes in a pathway. Following Bateson an epistatic mutation is a mutation that masks the effect of another (hypostatic) mutation, and this relationship is taken as evidence that the gene with the epistatic mutation is coming after the other in a pathway. The validity of this inference requires a number of auxiliary assumptions including the two mutations being the only factors affecting the phenotype. Drees et al. (2005) give a general overview of epistasis analysis.

More generally, the relationship between epistasis and the underlying structure of metabolic pathways, gene-regulatory networks, or physiological/developmental interactions is a topic of research in systems biology.

The Importance of Epistasis

The main relevance of epistasis for evodevo, at least, comes from its connection to the evolution of evolvability and canalization. It has only recently been recognized that this depends on systematic patterns of gene interaction that are not identifiable within the models of statistical genetics. Consequently, there is only scattered work to identify and formally describe how the many possible patterns of interaction and nonlinearity of the genotype-phenotype map may influence evolution. Beyond the identification of directional epistasis and convexity as key elements in the evolution of evolvability (e.g., Rice 1998; Carter et al. 2005), there is a body of work on how canalization may hide genetic variation that can subsequently be released in an evolutionary capacitance mechanism (e.g., Hermisson and Wagner 2004).

More generally, epistasis is related to the complexity of the genotype-phenotype map. It is here useful to distinguish between magnitude and sign epistasis. While sign epistasis refers to cases where a change in the genetic background would change the order of the effects of genotypes at a locus, magnitude (or order-preserving)

epistasis refers to cases where only the magnitude and not the order of effects are changed. Specifically, sign epistasis has been defined as a change in the ordering of fitness values, and this sets up the possibility of complex dynamics with the possibility of internal equilibria and multistability that may act as strong constraints on evolution (Weinreich et al. 2005). The existence of complex epistasis creating multipeaked genotype-fitness relations was a premise of Wright's view of evolution as expressed in his shifting-balance theory and contrasts with the Fisherian view of smooth additive landscapes (e.g., Whitlock et al. 1995). For Wright, evolution consisted in jumps between such peaks mediated by genetic drift in small subpopulations. A general model of the interaction between genetic drift and epistasis can be found in Barton and Turelli (2004).

One important question is whether patterns of epistasis may reflect limits to evolution. If a trait is selected up towards a limit, we may expect a pattern of negative epistasis where allele substitutions that increase the trait towards the limit show increasing canalization or even reversals of effect when the trait approaches the limit. Such epistatic constraints can in principle be investigated by studying the relationship between phenotypic trait values and the effects of allele substitutions, but this has of yet not received systematic attention. On the other hand, the existence of epistasis may also provide the possibility of breaking constraints by allowing pleiotropic effects to evolve (Pavlicev and Cheverud 2015).

The influence of epistasis increases with increasing distance in genotype space, and this makes it important in macroevolution and speciation. This is illustrated by the Bateson-Dobzhansky-Muller model for the evolution of postzygotic reproductive isolation. Even without differences in selection regime, isolated populations will experience different genetic changes due to genetic drift (e.g., systems drift). Such changes must be compatible with the genetic background in their own population, but there is no selection for compatibility with the genetic background of a different population, and hybridization will then generate individuals with untested gene combinations. Such combinations with deleterious effects on fitness are called Bateson-Dobzhansky-Muller incompatibilities. These will accumulate at an accelerating pace with increasing genetic difference between populations, and virtually guarantee that complete reproductive isolation will eventually arise as genetic distance is increasing.

Epistasis is a factor in the evolution of recombination and sexual reproduction. The costs and benefits of breaking up old and creating new allele combinations depend on the patterns of epistatic interaction among the alleles. While the breakup of coadapted gene complexes is unfavorable, it can be favorable to create offspring with diverse gene combinations to increase the probability that some of them are well adapted or free from combinations of deleterious alleles. If adaptation requires individually nonfavorable mutations in several genes, the rate of adaptation may be greatly elevated by sexual recombination. According to the deterministic-mutation hypothesis, sex is maintained as an adaptation to reduce the mutation load, but this works only in the presence of relatively strong *synergistic* epistasis where the fitness effects of several deleterious mutations are more severe than the (multiplicative) effects of the mutations in isolation.

Summary of Epistasis Terminology

The key distinction in epistasis terminology is between statistical epistasis, Fisher's epistacy, on one side, and what has variously been called biological, functional, or physiological epistasis on the other.

Statistical epistasis refers to the interaction terms in a least-squares regression on the presence of alleles. It can be divided into pairwise additive-by-additive (AA) and higher-order interactions. The variances explained by these interaction terms are the additive-by-additive epistatic variance, etc.

Hansen and Wagner (2001) defined functional epistasis as a dependency of the effects of a genetic substitution (on one or multiple loci) on the genetic background (i.e., the state of other loci in the genotype). This is the essence of the biological epistasis concepts including Cheverud and Routman's (1995) physiological epistasis, which was defined as a dependence of the difference in genotypic values at one locus on the state of another locus. The idea behind these concepts was to formally define epistatic effects independently of the composition of a population. They are still relative to a reference genotype, however, and specification of the reference genotype remains essential in all modeling of epistasis. Estimation and modeling of epistasis may be misleading if implicitly assumed reference genotypes are not made clear. Tools for translating between different reference genotypes and for relating biological and statistical epistasis are provided in Hansen and Wagner (2001), Barton and Turelli (2004), and Álvarez-Castro and Carlborg (2007).

Positive and negative epistasis refer to interactions for which the composite effect of two or more substitutions are elevated above or depressed below the sum of their individual effects. This requires a scale, and positive epistasis in one direction equals negative epistasis in the other. Systematic positive or negative interactions in one direction are called directional epistasis, while cases in which positive and negative interactions cancels out are called nondirectional epistasis. Magnitude epistasis or order-preserving epistasis is used when changes in the genetic background only cause changes in the magnitude of effects, while sign epistasis or order-breaking epistasis refer to cases in which the order of effects of the genotypes at a locus are changed. Multilinear epistasis refers to a pattern in which sets of genotypic effects are proportionally modified by changes in the genetic background.

The terminology for fitness epistasis is convoluted with positive and negative epistasis sometimes referring to interactions between beneficial (fitness-increasing) mutations and sometimes to interactions between deleterious (fitness-decreasing) mutations. In addition, terms such as synergistic, antagonistic, and diminishing-returns epistasis are used for positive or negative fitness interactions in either direction. It is also essential to distinguish between Wrightian fitness where epistasis is usually defined as deviations on a multiplicative scale and Malthusian fitness where it is usually defined as deviations on an arithmetic scale (Wagner 2010). Fitness epistasis may also differ depending on whether the reference genotype is one with maximal or average fitness. Furthermore, epistasis for fitness must be distinguished from epistasis in the traits underlying fitness. Unless the fitness function is

linear, these will differ, and with a nonlinear (e.g., stabilizing) fitness function, an additive genetic architecture in the trait will generate systematic epistasis for fitness.

The widespread relevance of gene interaction has given rise to many context-dependent terminologies including the Bateson-Dobzhansky-Muller incompatibilities for deleterious fitness interactions between alleles from different populations, the concept of a modifier where one gene is assumed to change the effect of another without itself having an effect on the trait, the concept of differential epistasis when pleiotropic effects are differentially modified by a change in the genetic background, and the concept of compensatory change where the effect of one substitution is nullified by another.

Cross-References

- ▶ Canalization: A Central but Controversial Concept in Evo-Devo
- ▶ Evolvability
- ▶ Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics
- ▶ Variational Approaches to Evolvability: Short- and Long-Term Perspectives

References

Álvarez-Castro JM, Carlborg Ö (2007) A unified model for functional and statistical epistasis and its application in quantitative trait loci analysis. Genetics 176:1151–1167

Barton NH, Turelli M (2004) Effects of genetic drift on variance components under a general model of epistasis. Evolution 58:2111–2132

Carter AJR, Hermisson J, Hansen TF (2005) The role of epistatic gene interactions in the response to selection and the evolution of evolvability. Theor Popul Biol 68:179–196

Cheverud JM, Routman EJ (1995) Epistasis and its contribution to genetic variance components. Genetics 139:1455–1461

Drees BL, Thorsson V, Carter GW, Rives AW, Raymond MZ, Avila-Campillo I, Shannon P, Galitski T (2005) Derivation of genetic interaction networks from quantitative phenotype data. Genome Biol 6:R38

Flatt T (2005) The evolutionary genetics of canalization. Q Rev Biol 80:287–316

Hansen TF (2013) Why epistasis is important for selection and adaptation. Evolution 67:3501–3511

Hansen TF (2015) Measuring gene interaction. In: Moore JH, Williams S (eds) Epistasis: methods and protocols. Methods in molecular biology. Springer, New York, pp 115–142

Hansen TF, Wagner GP (2001) Modeling genetic architecture: a multilinear theory of gene interaction. Theor Popul Biol 59:61–86

Hermisson J, Wagner GP (2004) The population genetic theory of hidden variation and genetic robustness. Genetics 168:2271–2284

Huang W, Richards S, Carbone MA, Zhu D, Anholt RRH, Ayroles JF, Duncan L, Jordan KW, Lawrence F, Magwire MM, Warner CB, Blankenburg K, Han Y, Javaid M, Jayaseelan J, Jhangiani SN, Muzny D, Ongeri F, Perales L, Wu Y-Q, Zhang Y, Zou X, Stone EA, Gibbs RA, Mackay TFC (2013) Epistasis dominates the genetic architecture of *Drosophila* quantitative traits. Proc Natl Acad Sci U S A 109:15553–15559

Le Rouzic A (2014) Estimating directional epistasis. Front Genet 5:198

Lynch M, Walsh B (1998) Genetics and analysis of quantitative characters. Sinauer, Sunderland

Malmberg RL, Mauricio R (2005) QTL-based evidence for the role of epistasis in evolution. Genet Res 86:89–95

Pavlicev M, Cheverud JM (2015) Constraints evolve: context dependency of gene effects allows evolution of pleiotropy. Annu Rev Ecol Evol Syst 46:413–434

Perfeito L, Sousa A, Bataillon T, Gordo I (2014) Rates of fitness decline and rebound suggest pervasive epistasis. Evolution 68:150–163

Rice SH (1998) The evolution of canalization and the breaking of von Baer's laws: modeling the evolution of development with epistasis. Evolution 52:647–656

Wagner GP (2010) The measurement theory of fitness. Evolution 64:1358–1376

Weinreich DM, Watson RA, Chao L (2005) Sign epistasis and genetic constraint on evolutionary trajectories. Evolution 59:1165–1174

Whitlock MC, Phillips PC, Moore FB-G, Tonsor SJ (1995) Multiple fitness peaks and epistasis. Annu Rev Ecol Syst 26:601–629

Variational Approaches to Evolvability: Short- and Long-Term Perspectives

Arthur Porto

Contents

Abstract

Evolvability, the ability of a biological system to respond to selection, has recently become a key concept in evolutionary developmental biology and an integral part of the vocabulary of a budding extended evolutionary synthesis. While some of the theoretical principles behind the evolvability of complex organisms have been established, there are also several aspects of it that remain controversial. How does evolvability itself evolve? Is evolvability constrained by mutation? Can current definitions account for evolutionary innovations?

A. Porto (✉)
Centre for Ecological and Evolutionary Synthesis, University of Oslo, Oslo, Norway
e-mail: a.g.c.porto@ibv.uio.no

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_114

Here, I will describe some of the research programs dedicated to the study of evolvability of complex organisms. I will then establish its relationship with modularity and robustness and conclude with questions about the nature of evolvability that remain unresolved. My aim is to show that research in evolvability has become integrative in nature and that this change has been aided by an increasing incorporation of the genotype-to-phenotype map into the variation-based evolutionary theory.

Keywords

Development · Evo-devo · Robustness · Constraints · Evolutionary synthesis · Modularity

Introduction

Evolvability has become a central concept in evolutionary developmental biology. A biological system is considered evolvable if it can produce and maintain adaptive genetic variation over evolutionary timescales (Hansen 2006). While the idea behind evolvability is much older, the term itself has emerged relatively late in evolutionary thinking (Nuño de la Rosa 2017). It is not clear who was the first to use it with its modern meaning, but by the late 1980s, the term was frequently used in computer science research devoted to virtually evolving systems. At its core, evolvability was used to describe the observation that certain virtual species responded more readily to selection than others and, therefore, had a high chance of surviving long-term (Dawkins 1988). This observation was important because it directly suggested that certain properties of living organisms made them more likely to successfully respond to environmental challenges, i.e., certain properties influenced their long-term adaptive dynamics. Such properties were often counterintuitive. For example, researchers quickly noticed that imposing certain constraints on the kind of variation generated during development was actually beneficial to a virtual organism's evolvability (e.g., Rasmussen et al. 1990). Similarly, evolvability could not be taken for granted. While a few virtual species thrived, many failed to cope with selective challenges and simply went "extinct." The concept had immediate appeal to evolutionary biologists who were critics of the adaptationist program and who were interested in the role of developmental constraints in evolution (e.g., Gould 1966).

Biologists had been experiencing difficulties when trying to explain the evolution of complex traits purely in terms of random genetic changes. Evolution of complex structures, like the vertebrate eye, depends on so many coordinated changes, all of which do not lead to a viable structure when occurring independently. Evolvability depends, therefore, on the production of *structured* variation. In their view, development was the key aspect missing from the modern synthesis. Development is structured in such a way that it enhances the probability that mutations will improve performance (Wagner and Altenberg 1996).

Since then, the study of evolvability has become a special focus. Some authors have even suggested that evolvability is going to be a cornerstone of an extended evolutionary synthesis, which will be centered in developmental systems (Pigliucci 2008). According to them, this new synthesis will allow evolutionary biology to answer several questions that have not been properly addressed by the modern synthesis. For example, why do we see forms that remain unchanged at macroevolutionary timescales when we can experimentally show that evolution can proceed at a fast pace? How can we explain the evolution of complex morphological novelties in which intermediate forms are not viable? Are there limits to what genetic change can achieve? Or are we able to select for any form our minds can conceive?

Given these questions, and the assertion that evolvability may help answer them, it would be beneficial to discuss some of the research that is behind the term evolvability in more detail. Today, the term is used in several contexts in research programs in evo-devo, quantitative genetics, and other fields. For that reason, this chapter starts by exploring some definitions and follows it with a brief introduction to the different approaches used in this field.

Defining Evolvability

While most authors would agree with a theoretical definition of evolvability as the ability to produce and maintain adaptive genetic variation over evolutionary timescales, working definitions of evolvability are as diverse as the number of scientific disciplines that use them (Nuño de la Rosa 2017; Pigliucci 2008). For the purposes of this chapter, working definitions of evolvability that have emerged in the context of variational (population genetic) approaches will be used (Houle 1992; Wagner and Altenberg 1996). Within that context, evolvability can be divided into short- and long-term conceptualizations. This division is largely artificial, and recent works have emphasized the overlap between them (Houle et al. 2017), but they provide a natural starting point for understanding evolvability.

Short-Term Evolvability

At short timescales, the input of new mutations in a population will be relatively limited, so one can think of evolvability strictly at a population genetic level. If we assume that genetic variation provides the "fuel" that populations use to respond to selection, evolvability becomes intimately dependent on the availability of genetic variation. In this context, evolvability is highest in traits that present abundant standing additive genetic variation within populations and that are relatively autonomous from other traits under different selective regimes. This is the approach to evolvability in studies such as Houle (1992) and Hansen and Houle (2008).

Long-Term Evolvability

At long timescales, approaches equating evolvability to standing genetic variation become more controversial, since the input of new mutations and the evolution of evolvability itself start being relevant to the dynamics of adaptation. In other words, at these timescales, one must think about evolvability at the level of variability, instead of variation (Wagner and Altenberg 1996). This is because variability is defined as the range of phenotypes that a population is able to reach given a certain number of mutations. It measures the *propensity* to vary and depends on the input of new genetic variation. Variability is, therefore, a different property. This property depends on the genetic architecture of the organism and on the epigenetic interactions with the environment. In this sense, treating evolvability as variability represents a significant change to the concept of evolvability, since it then becomes dependent on properties of the genetic/developmental system and thus on developmental constraints (Wagner and Zhang 2011). Developmental constraints play a role during evolution and become a potential explanation for observed macroevolutionary patterns (Alberch 1991).

Perspectives on the Study of Evolvability

Research in evo-devo has come a long way toward understanding the factors that influence the evolvability of complex organisms. At this point, several theoretical principles have been established, but there are also several topics on which research is ongoing. This section will describe some of the research programs dedicated to the study of evolvability of complex organisms and conclude with open questions that still need to be addressed in the field. In general, the focus is going to be placed on approaches that allow for an integration between developmental (organismal) biology and the variation-based evolutionary theory (Pavlicev and Wagner 2012). For other works presenting different perspectives on this issue, see Gerhart and Kirschner (1997), Pigliucci (2010), and also the chapter on ▶ "Evolvability."

Quantitative Approaches: Evolvability at Short Timescales

Quantitative approaches to evolvability usually assume that it can be reasonably measured, at short timescales, based on genetic variation that is already present within populations. In this context, more standing genetic variation means more evolvability. Houle (1992) was the first to propose a measurement of evolvability in quantitative traits that can be directly compared across taxa. In his work, and work that followed it (Hansen and Houle 2008), evolvability became operationalized as the mean-scaled additive genetic variance, usually illustrated in the univariate case as

$$e = V_A/\bar{z}^2$$

where V_A refers to the additive genetic variance of trait z and $\bar{z}$ refers to the trait mean. The logic behind such formulation is simple. When defined this way, evolvability directly measures the response to selection that would be observed in a population/species given a standardized amount of directional selection. It is, therefore, a dimension-free and internally consistent measurement (Hansen and Houle 2008).

At this point, a large number of studies have reported standardized estimates of univariate evolvability (e.g., Garcia-Gonzalez et al. 2012). In general, authors are uniform in agreeing that there is abundant genetic variation for individual traits. While this observation has led some authors to conclude that one can indeed take evolvability for granted, caution is necessary when interpreting such results (Hansen and Houle 2008; Walsh and Blows 2009). Abundance of genetic variation in individual traits is misleading, as most traits are also genetically correlated with several other traits, a situation that leads to a considerably different perspective regarding the evolution of complex traits (Walsh and Blows 2009).

Quantitative Approaches: Multivariate Evolvability

Most traits that are of interest to evolutionary developmental biologists are genetically associated with other traits in the organism. After all, organisms are inherently multidimensional, being composed of suites of traits that arise from a common genome through shared developmental processes. Genetic associations among traits have two main consequences for evolvability. The first is that evolutionary change in individual traits is often not possible without change in other traits as well (Lande 1979). That leads to an interesting complication. How can adaptation in one trait proceed without affecting other traits that have already adapted? This question will be answered in the next section, when discussing the genotype-to-phenotype map.

The second main consequence of genetic association is that the distribution of genetic variation in multivariate space might be far from homogeneous, with some directions presenting much more variation than others (Fig. 1) (Schluter 1996; Walsh and Blows 2009). In other words, in a multivariate setting certain trait combinations are more easily changed than others.

This observation has led to the formation of an empirical research program dedicated to searching for signatures of evolutionary constraints by looking at dimensions that have either disproportionately abundant or diminished available genetic variation within populations. Work in this area usually falls into one of two main categories: (1) nearly null spaces and (2) lines of least resistance. Nearly null spaces refer to dimensions of the phenotypic space with little to no additive genetic variation. Those dimensions would have shaped phenotypic evolution due to their diminished evolvability (Blows and McGuigan 2015). Empirical works arguing for the existence of nearly null spaces are considerably more abundant than the evidence for their long-

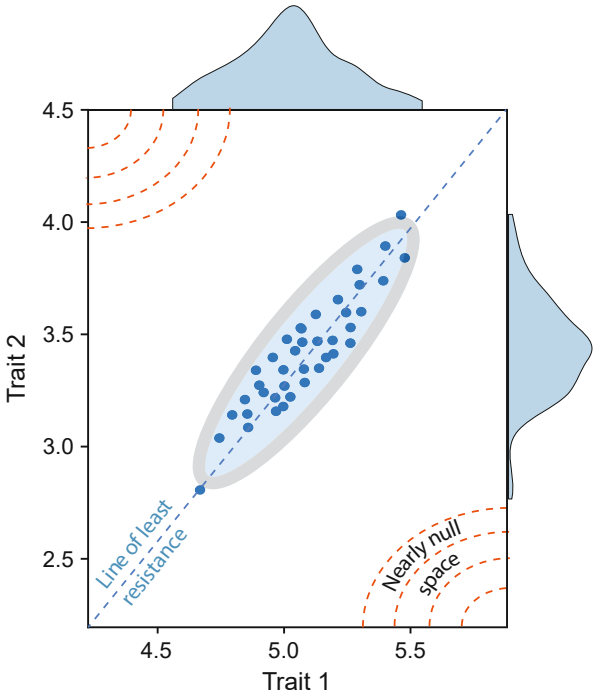


Fig. 1 From univariate to multivariate evolvabilities. In this example, the univariate and multivariate distributions of trait values in a population of individuals are shown for two hypothetical traits (1 and 2). Univariate trait distributions are illustrated as density plots at the edge of the main plot area. Note that both traits present similar amounts of variation. Variation for individual traits is also abundant, suggesting large univariate evolvabilities. However, when looking at the bivariate plot, one will notice that variation is heterogeneously distributed. A single axis (diagonal line) concentrates the majority of within-population variation. I refer to this axis as the line of least evolutionary resistance. Theoretically, evolvability should be highest along this axis. Evolvability along other dimensions is extremely limited. Consequently, it would be very difficult for this population to reach the area highlighted with dotted contour lines (i.e., nearly null space). This example illustrates that univariate evolvabilities are meaningless when traits are genetically correlated with other traits under different selection regimes

term effects on evolution. This can be expected for several reasons. First, the design of empirical studies often limits our ability to confidently estimate dimensions with little variation (Cheverud 1988). It is difficult to avoid some degree of sampling error, which impacts our confidence in these dimensions. Second, on macroevolutionary timescales, nearly null spaces can be temporally transient, since new genetic variation may emerge within the population through new mutational input.

Another approach has been to look at dimensions with disproportionately large amounts of genetic variation, since they influence evolutionary change by acting as directions of maximal evolvability. Schluter's (1996) work was the first to suggest that adaptive evolution might often proceed toward what he calls genetic line of least evolutionary resistance (LLER), or simply the direction of the phenotypic space that

concentrates the greatest part of the total additive genetic variation. New World Monkeys represent one of the clearest examples of how evolutionary processes may interact with the LLER. This group is one of the most morphologically diverse groups of primates. Species of New World monkeys occupy a wide array of ecosystems and exhibit stark differences in ecology and behavior. Marroig and Cheverud (2005) showed that the diversification of the group into diet-based adaptive zones was associated with changes in absolute size, which is the direction of maximal evolvability within species. Evolution also occurred away from size in some taxa, but morphological evolution along these dimensions was limited. When selection was aligned with size (positive interaction), evolution occurred rapidly, creating the radiation of adaptive forms that is characteristic of the group.

Qualitative Approaches: The Genotype-to-Phenotype Map

As seen above, the multivariate distribution of genetic variation is one of the key elements influencing the evolvability of biological systems. An understanding of how this variation is structured requires knowledge of the biological mechanisms that translate genetic change into phenotypic ones (Alberch 1991). Consequently, a considerable amount of research in evolvability is devoted to the structure of the genotype-to-phenotype map (GP map).

Alberch (1991) was the first to introduce the concept of a genotype-phenotype mapping function. In his view, much of the evolutionary biology research driving the modern synthesis ignored development and organismal form. He opposed the idea that genes provide a blueprint for the phenotype and, instead, saw the interactions at cellular and tissue levels as the essential components structuring the distribution of genetic variation in multivariate space. In his research, Alberch used experimental manipulations of limb development to show that induced malformations, even when they produce novel forms not observed in nature, are still limited by morphogenetic properties of the system. Therefore, development constrains the variation that is allowed to arise and later become fixed in evolution.

Since Alberch's work, the use of the GP map metaphor has taken hold within evo-devo, becoming an active area of research. To increase generality, GP maps are usually summarized in statistical terms rather than described at the developmental level (e.g., Pavlicev et al. 2008). For example, many research projects attempt to quantify how many genes underlie variation in complex traits, whether those genes affect one or multiple traits and what kind of effect size distribution gene variants have.

More central to the issue of evolvability, considerable effort has been devoted to understanding the properties of GP maps that increase the probability that a random mutation will lead to an increase in fitness – i.e., properties that increase the organisms' evolvability. Several properties of highly evolvable GP maps have been identified. Gerhart and Kirschner (1997) and Wagner and Zhang (2011) provide a comprehensive summary. For the purposes of this chapter, two main properties that have been widely discussed in the context of complex organisms will be highlighted. The first is the modularity of gene effects and the second is mutational robustness.

Modularity

With the observation that the GP map influences the evolvability in complex organisms, one of the questions that emerged early in the study of evolvability is what kind of mapping function would be more likely to increase an organism's capacity to respond to selection. If each mutation affected a large number of (functionally) unrelated traits, creating genetic covariation between them, beneficial changes in one trait would potentially lead to disadvantageous change in others. So, the likelihood that such architecture would be beneficial would be extremely low.

This conflicting dynamics of complex adaptation across traits is not present, however, when GP maps are modular (Wagner and Altenberg 1996). Modularity can be defined as a structure of the GP map in which related traits (i.e., share function or development) share a larger portion of their genes than unrelated traits (Fig. 2). The main consequence of such architecture is that mutations are more likely to lead to coordinated changes in traits that share a function and will generally not affect traits that are unrelated to it. Evolvability is, in this sense, much higher than in the first type of mapping function due to higher coordination among traits that perform the same function and lower interference among traits that are unrelated.

Modularity is presented in more details in other chapters of this volume, so this section will just briefly mention one line of evidence used to infer modularity in GP maps.

Modularity hypotheses are frequently tested through quantitative trait loci (QTL) studies. QTLs represent regions of the genome that affect quantitative traits. By comparing the distribution of QTL effects within and between hypothesized

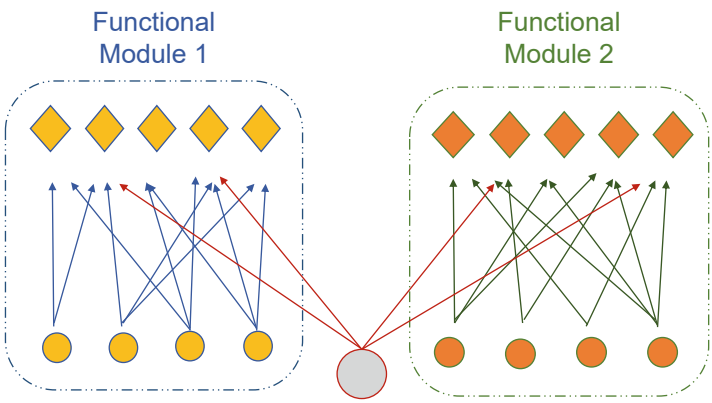


Fig. 2 Modularity in the genotype-to-phenotype map. Hypothetical map showing a modular relationship between genotype (circles) and phenotype (diamonds) that is mediated by developmental processes (arrows). Smaller circles represent modular genetic factors, and the large circle represents a genetic factor that affects traits in both modules. Note that most genetic factors are modular, that they affect multiple traits (pleiotropy), and that most traits are affected by multiple factors (polygeny)

modules, one can test whether gene effects are usually restricted to traits that share function or developmental origin. A classic example of modularity is the separation of the mammalian skull in two modules: the face and neurocranium (Leamy et al. 1999). These modules have for long been considered semiautonomous based on both developmental processes and for functional reasons. Several studies have found that the genetic basis for facial and neurocranial traits is semi-independent. Around 62% of all the genomic regions identified as underlying variation in these traits affect exclusively one region, and only 38% affect both regions simultaneously (Leamy et al. 1999). If we assume that gene effects are to a large extent modular and the last decades of QTL studies suggest this is a general pattern, what are the evolutionary consequences of such GP maps?

As seen above, traits belonging to the same module will evolve in a coordinate fashion, while traits in different modules will evolve semi-independently, favoring evolvability (Wagner and Altenberg 1996).

Robustness

One of the properties of the GP map that has a long history within evolutionary biology is robustness. A GP map can be considered robust if it produces a consistent phenotype even when faced with genetic or environmental perturbations. Robustness has been studied under the guise of canalization ever since Waddington (1942) noticed that wild-type organisms tend be less variable than their laboratory counterparts that carried specific mutations. While such phenomenon might, at first glance, seem to reduce evolvability in the short term by reducing the availability of heritable genetic differences within a population, this is not necessarily the case (Draghi et al. 2010). It is precisely because it hides genetic variation from selection that robustness can have a positive effect on evolvability. Since not all genetic variation is immediately exposed to selection, robust systems can accumulate cryptic genetic variation to be released posteriorly. In other words, robustness leads to the formation of evolutionary capacitors. Many different stimuli can lead to the release of genetic variation contained in these capacitors, fueling rapid evolutionary change in moments of increased environmental challenge. Genetic stimuli, for example, occur when a mutation somewhere else in the genome leads such capacitors to release heritable genetic variation in phenotypic traits (Pavličev and Cheverud 2015). This is a special case of epistasis, a term used in genetics to refer to cases in which the effect of an allele on the phenotype depends on genetic variation in other parts of the genome (see chapter ► "Epistasis"). Epistatic release of genetic variation by new mutations is particularly common in certain GP maps. Maps characterized by the presence of a few major genes with strong epistasis among themselves and strong pleiotropic effects are primed for evolutionary capacitance. The other indirect consequence of biological epistasis is that it provides the conditions necessary for the evolution of evolvability by directional selection (see the section "Evolution of Evolvability").

The classic example of robustness comes from the study of RNA secondary structures (Schuster et al. 1994). RNA is a single-stranded molecule that serves multiple roles within eukaryotic cells, such as serving as a template for the

translation of DNA into proteins or regulating gene expression. Proper folding of RNA molecules is essential for their functioning. When these molecules were studied in terms of their GP map, researchers noticed that there were few common secondary structures (phenotype) for RNA molecules. In some cases, different secondary structures could be produced in a single mutational step (network neighbors). In other cases, the exact same secondary structures could be produced by very different underlying sequences, often several mutational steps away from each other. In other words, many different DNA sequences led to very similar folding patterns, suggesting the existence of vast selectively neutral networks. The main implication of such a finding is that it suggests that populations could harbor cryptic genetic variation in the form of a neutral network of sequences, all of which generate similar phenotypes. This cryptic genetic variation, in turn, allows a population to be within a single mutational step to multiple different neighbors, therefore potentially increasing the evolvability of this population when faced with new environmental demands.

Future Challenges

Evolution of Evolvability

One of the challenges in the study of evolvability is understanding its own evolution (Pigliucci 2008). As mentioned above, modularity and robustness directly contribute to an organism's evolvability. Yet, these are properties of organisms that do not directly contribute to the fitness of individuals in the same way that individual traits do. As such, evolvability is not a traditional target of selection, and its evolution is the source of some controversy within the field. Comparative and experimental data have both established that evolvability can evolve (see Pavličev and Cheverud 2015). However, we currently lack a consensus on how changes in evolvability are achieved, as well as how quickly these changes occur. While some authors argue that evolvability can be achieved through neutral processes (True and Haag 2001), other highlight the necessity of selection (Pavličev and Cheverud 2015), and some even argue that evolvability is a group- or species-level adaptation (Gerhart and Kirschner 1997). Due to the nature of this chapter, we will here concentrate on a promising model for the evolution of evolvability under directional selection, which is the model involving the modification of gene effects (Pavlicev et al. 2010). In this model, the effect of a gene is genetically variable due to epistatic interactions with other loci. As such, a gene's effect can evolve as a consequence of allele frequency changes in other parts of the genome. Natural selection can, therefore, lead to an increase in a trait's evolvability as a by-product of the evolution of a gene's effect on the trait being selected. It can also decrease the correlation between those and other nonselected (or contrarily) selected traits. This, in turn, will lead to a change in the variational properties of the developmental system (Pavličev and Cheverud 2015).

Mutation

On macroevolutionary timescales, evolvability depends critically on the input of new mutations. If no new mutations appeared within populations, organisms would quickly run out of heritable genetic variation, as beneficial alleles segregating within populations would rapidly become fixed. For this reason, an entire research program is dedicated to the study of mutational effects on living organisms (e.g., Houle et al. 2017). This program is devoted to answering the question of whether mutational patterns and rates influence evolvability. Theoretical works suggest that mutational rates may indeed influence the rate of adaptation, therefore limiting evolvability (Hill 1982). However, accurate estimates of the input of variation by new mutations are especially difficult to obtain. Sample sizes required to estimate such parameters are often unrealistic, given species' life cycle durations. For this reason, most of our knowledge comes from a few model species. Empirical results obtained so far suggest that patterns of mutational effects are often aligned with patterns of evolutionary divergence (e.g., Houle et al. 2017). While this could be seen as giving support to the idea that the availability of mutational variation is a key factor in determining the organisms' evolvability, some authors have recently pointed out that observed rates of evolution are often much slower than what mutations could give rise to (Houle et al. 2017). In their view, patterns of mutational variation are evolvable and could be aligned with the direction of evolutionary divergence simply because they are responding to the same selective pressures. In any case, understanding the influence of new mutational input on evolvability is going to be a major challenge moving forward.

Morphological Novelty

One of the biggest challenges to research in evolvability is explaining the appearance of morphological novelties. A morphological novelty is a novel body part that cannot be considered homologous to body parts of the ancestral lineage (Wagner and Lynch 2010 ▶ "Developmental Innovation and Phenotypic Novelty"). For a part to be considered as novel, it must arise at a certain point in development, and, from that point on, its development is due to the interactions nearly exclusively between cells within that structure. For example, the bird wing would not be considered a morphological novelty, because it is homologous to the forelimbs of tetrapods. Only feathers would be considered as such. There are two main mechanisms according to which morphological novelties can arise: (1) differentiation among serially repeated elements or (2) de novo origination (Wagner and Lynch 2010). The first mechanism involves a structure that is present in multiple copies within an organism and that has some of its copies further modified and individualized into a novel structure. The mammary glands (derived from accessory glands) would be an example of this process. In placental mammals, the mammary gland has acquired developmental independence from hair, developing without the formation of a hair follicle. Because of this significant change in development, it became a derived individualized body part. The second process (de novo) involves the appearance of a novel structure to compensate for the changes or loss of a

previous structure without which the organism is not capable of surviving. The syndesmosis tibiofibularis in birds is an example of such structures. It emerged at the heels of a considerable reduction in hind limb size in birds relative to their ancestors. This reduction caused the fibula to lose its connection with the tarsal joint, and this connection was replaced in birds by the syndesmosis tibiofibularis (Müller and Streicher 1989).

One of the consequences of this conceptualization of morphological innovation is that it implies that the evolution of a morphological novelty requires the evolution of a new gene regulatory network. This is a property of evolvability of which we have a very limited understanding and empirical work is still in its infancy. Empirical evidence for the evolution of gene regulatory networks mainly comes from studies of transposable element-driven network innovation (Lynch et al. 2011). The basic idea is that transposable elements (TEs) often carry with them transcription factor binding sites that can become alternative promoters, enhancers, and so on. In this sense, they can promote extensive network rewiring, opening up the possibility of formation of novel networks. It remains an open empirical question whether TE-mediated rewiring of gene networks is the prevalent mechanism for evolutionary innovations. The second major idea is becoming formulated at the level of novel cell types, as a basic paradigm of novel entities. The idea is that the core regulatory networks underlying new cell types (see chapter ▶ "Devo-Evo of Cell Types") evolve by introducing new protein interactions between the transcription factors (reviewed in Arendt et al. 2016).

Conclusions

One of the central goals of evolutionary developmental biology is to understand the extent to which developmental processes have shaped the diversity of living species. The interest in evolvability has, therefore, greatly increased in recent years. Research in evolvability is largely integrative in nature. Understanding the evolvability of complex organisms requires integration of population genetic mechanisms and developmental mechanisms, through the use of the genotype-to-phenotype map metaphor. Evolvability lies, therefore, at the core of evo-devo. In this chapter, I discussed some of the challenges in defining evolvability and its relationship with modularity and robustness and concluded with open questions that still need to be addressed in the field. Ultimately, the usefulness of the concept of evolvability depends on a good understanding of its own evolution. While both empirical and theoretical works suggest that it can evolve, we still need to understand the conditions in which these changes take place and also the speed at which it changes.

Cross-References

▶ A Macroevolutionary Perspective on Developmental Constraints in Animals
▶ Devo-Evo of Cell Types
▶ Developmental Innovation and Phenotypic Novelty

▶ Dispositional Properties in Evo-Devo
▶ Evo-Devo's Contributions to the Extended Evolutionary Synthesis
▶ Evolvability

References

Alberch P (1991) From genes to phenotype: dynamical systems and evolvability. Genetica 84(1):5–11
Arendt D, Musser JM, Baker CV, Bergman A, Cepko C, Erwin DH, Pavlicev M, Schlosser G, Widder S, Laubichler MD, Wagner GP (2016) The origin and evolution of cell types. Nat Rev Genet 17(12):744–757
Blows MW, McGuigan K (2015) The distribution of genetic variance across phenotypic space and the response to selection. Mol Ecol 24(9):2056–2072
Cheverud JM (1988) A comparison of genetic and phenotypic correlations. Evolution 42(5):958–968
Dawkins R (1988) The evolution of evolvability. In: Langton C (ed) Artificial life: the proceedings of an interdiciplinary workshop on the synthesis and simulation of living systems. Addison Wesley, Santa Fe, pp 202–220
Draghi JA, Parsons TL, Wagner GP, Plotkin JB (2010) Mutational robustness can facilitate adaptation. Nature 463(7279):353–355
Garcia-Gonzalez F, Simmons LW, Tomkins JL, Kotiaho JS, Evans JP (2012) Comparing evolvabilities: common errors surrounding the calculation and use of coefficients of additive genetic variation. Evolution 66(8):2341–2349
Gerhart J, Kirschner M (1997) Cells, embryos, and evolution: toward a cellular and developmental understanding of phenotypic variation and evolutionary adaptability, vol 575.21 GER. Blackwell Science, Malden
Gould SJ (1966) Allometry and size in ontogeny and phylogeny. Biol Rev 41(4):587–638
Hansen TF (2006) The evolution of genetic architecture. Annu Rev Ecol Evol S 37:123–157. https://doi.org/10.1146/annurev.ecolsys.37.091305.110224
Hansen TF, Houle D (2008) Measuring and comparing evolvability and constraint in multivariate characters. J Evol Biol 21(5):1201–1219
Hill WG (1982) Rates of change in quantitative traits from fixation of new mutations. Proc Natl Acad Sci 79(1):142–145
Houle D (1992) Comparing evolvability and variability of quantitative traits. Genetics 130(1):195–204
Houle D, Bolstad GH, van der Linde K, Hansen TF (2017) Mutation predicts 40 million years of fly wing evolution. Nature 548(7668):447–450
Lande R (1979) Quantitative genetic-analysis of multivariate evolution, applied to brain – body size allometry. Evolution 33(1):402–416
Leamy LJ, Routman EJ, Cheverud JM (1999) Quantitative trait loci for early-and late-developing skull characters in mice: a test of the genetic independence model of morphological integration. Am Nat 153(2):201–214
Lynch VJ, Leclerc RD, May G, Wagner GP (2011) Transposon-mediated rewiring of gene regulatory networks contributed to the evolution of pregnancy in mammals. Nat Genet 43(11):1154–1159
Marroig G, Cheverud JM (2005) Size as a line of least evolutionary resistance: diet and adaptive morphological radiation in new world monkeys. Evolution 59(5):1128–1142
Müller GB, Streicher J (1989) Ontogeny of the syndesmosis tibiofibularis and the evolution of the bird hindlimb: a caenogenetic feature triggers phenotypic novelty. Anat Embryol 179(4):327–339
Nuño de la Rosa L (2017) Computing the extended synthesis: mapping the dynamics and conceptual structure of the evolvability research front. J Exp Zool Part B Mol Dev Evol 328:395–411

Pavličev M, Cheverud JM (2015) Constraints evolve: context dependency of gene effects allows evolution of pleiotropy. Annu Rev Ecol Evol Syst 46(1):413–434. https://doi.org/10.1146/annurev-ecolsys-120213-091721

Pavlicev M, Wagner GP (2012) Coming to grips with evolvability. Evol Educ Outreach 5(2):231–244

Pavlicev M, Kenney-Hunt JP, Norgard EA, Roseman CC, Wolf JB, Cheverud JM (2008) Genetic variation in pleiotropy: differential epistasis as a source of variation in the allometric relationship between long bone lengths and body weight. Evolution 62(1):199–213

Pavlicev M, Cheverud JM, Wagner GP (2010) Evolution of adaptive phenotypic variation patterns by direct selection for evolvability. Proc R Soc Lond B Biol Sci 278:1903–1912. https://doi.org/10.1098/rspb.2010.2113

Pigliucci M (2008) Is evolvability evolvable? Nat Rev Genet 9(1):75–82

Pigliucci M, Muller GB (2010) Evolution-the extended synthesis. MIT Press, Cambridge, Massachusetts, vol 576.82 E9.

Rasmussen S, Knudsen C, Feldberg R, Hindsholm M (1990) The coreworld: emergence and evolution of cooperative structures in a computational chemistry. Physica D 42(1–3):111–134

Schluter D (1996) Adaptive radiation along genetic lines of least resistance. Evolution 50 (5):1766–1774

Schuster P, Fontana W, Stadler PF, Hofacker IL (1994) From sequences to shapes and back: a case study in RNA secondary structures. Proc R Soc Lond B Biol Sci 255(1344):279–284

True JR, Haag ES (2001) Developmental system drift and flexibility in evolutionary trajectories. Evol Dev 3(2):109–119

Waddington CH (1942) Canalization of development and the inheritance of acquired characters. Nature 150(3811):563–565

Wagner GP, Altenberg L (1996) Perspective: complex adaptations and the evolution of evolvability. Evolution 50(3):967–976

Wagner GP, Lynch VJ (2010) Evolutionary novelties. Curr Biol 20(2):R48–R52

Wagner GP, Zhang J (2011) The pleiotropic structure of the genotype–phenotype map: the evolvability of complex organisms. Nat Rev Genet 12(3):204–213

Walsh B, Blows MW (2009) Abundant genetic variation+ strong selection= multivariate genetic constraints: a geometric view of adaptation. Annu Rev Ecol Evol Syst 40:41–59

Part XI

Extensions of Evo-Devo

Evo-Devo's Contributions to the Extended Evolutionary Synthesis

Gerd B. Müller

Contents

Abstract

Evo-devo is not only a field of empirical research but its results also have significant consequences for evolutionary theory. Predominantly, these address the evolution of the phenotype, a topic sidelined by population theoretical accounts of evolution. Original contributions to this domain concern the generative principles of phenotypic variation, the development-environment interaction, the construction of organismal complexity, and the origination of novelty. The theoretical corollaries of evo-devo have become part of an ongoing reform project in evolutionary theory, a particular version of which is termed the "extended evolutionary synthesis." The logical structure of this revised evolutionary framework differs from the classical population-theoretical account, opening up new kinds of questions and establishing a distinct set of empirically testable predictions. Beyond improving our understanding of evolvability, evo-devo's contribution to the

G. B. Müller (✉)
Department of Theoretical Biology, University of Vienna, Vienna, Austria

Konrad Lorenz Institute for Evolution and Cognition Research, KLI Institute, Klosterneuburg, Austria
e-mail: gerhard.mueller@univie.ac.at

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_39

extended synthesis can be epitomized in the principle of inherency, the notion that physical development, both in its material properties and its dynamical processes, guides phenotypic evolution.

Keywords

Evolution · Development · Evolutionary theory · Evo-devo · Modern Synthesis · Extended synthesis · Inherency

Introduction

Since its inception in the early 1980s, evo-devo has brought new questions, new goals, and new methodologies to the study of evolution, complementing the hitherto prevailing population theoretical approach with an organismal component. The processes underlying the evolving interactions between genes, cells, and tissue masses, which shape and modify multicellular organisms over time, were opened up to experimental and quantitative study. The innovative thrust generated by the combined examination of developmental and evolutionary questions led to the foundation of a distinct field of research, including scientific journals, professional societies, dedicated meetings, and other forms of disciplinary institutionalization such as research units and academic positions. Thus, evo-devo has triggered a fundamental transformation of the field of evolutionary biology by introducing a new research agenda, and it continues to grow as a discipline that is predominantly concerned with understanding how form-generating systems arise and how evolving developmental processes are regulated. Without doubt, this branch of evo-devo will lead to further elucidations of the empirical details of the evolution of development.

Yet there is another side to evo-devo, equally crucial for its establishment and its continuing success, namely the conceptual advancement it is expected to provide for evolutionary theory. Indeed, many of the foundational publications advocated this critical role of evo-devo, but the sheer bulk of empirical results oftentimes distracts from paying closer attention to the novel theoretical insights gained over the last decades. The present Reference Guide emphasizes that theoretical agenda by providing entries on many of the key conceptual themes of evo-devo, such as constraint, plasticity, evolvability, inherency, modularity, heterochrony, and many more. Some of these concepts have become quite elaborate and formalized, producing new models of the genotype-phenotype relation and of evolving developmental processes, whereas others have remained more tentative in nature. This chapter examines how evo-devo's theoretical outcomes affect evolutionary theory in general.

Developmental biology is not the only domain from which new evolutionary concepts have emerged in recent years. Genomics, behavioral ecology, inheritance research, systems biology, and other fields were equally influential in triggering theoretical progress, both on topics that were and were not part of the standard evolutionary explanation. With the mounting requirement for integration of these new findings into a formal theory structure, several proposals for a modified

evolutionary framework have been put forward. The most elaborated of these has been called the "extended evolutionary synthesis" (EES) (Pigliucci and Müller 2010; Laland et al. 2015), a project in-the-making that has attracted both support and criticism. Here, I will discuss the ways in which the theoretical advances resulting from evo-devo contribute to the EES and, in fact, represent one of its pillars. I will concentrate on those conceptual domains in which the contrast with assumptions of the standard theory is most apparent, such as with the mechanisms that generate selectable variation, the interface of development with the environment, and the origins of organismal complexity. For other concepts of evo-devo, which are equally important but represent less of a divergence from the received theory, I refer to the relevant chapters in this Reference Guide.

Extended Evolutionary Synthesis

Evolutionary theory emerged from pre-evolutionary conceptions, such as recapitulation, gradualism, and population growth, and underwent several transformations after Darwin's first major synthesis in the mid-nineteenth century, which established the fundamental relation between variation in populations, inheritance, and differential reproduction. During the 1930s to 1950s, the neo-Darwinian reformulation of Darwin's variation-and-natural-selection principle together with its unification with Mendelian and population genetics gave rise to what has been called the Modern Synthesis (MS). Despite the significant progress in empirical research since, the MS has remained the standard theoretical paradigm of evolutionary biology for nearly a century, still represented as such in most biology textbooks today. Although several additions were made even during that period of theoretical stabilization, such as gene flow, neutrality, and inclusive fitness, these amendments all belong to the population realm of evolution that is at the core of the MS and thus allowed for relatively smooth integration. But the radically new findings of recent decades that come from genomics, systems biology, epigenetics, behavioral ecology, and evo-devo were not as easily reconciled with the standard model and prompted several scenarios for theory expansion (e.g., West-Eberhard 2003; Jablonka and Lamb 2005; Koonin 2011), among them also the EES.

The EES is a new and comprehensive proposal, which consists partly of revised elements of the standard theory and partly of new elements. As explained elsewhere in more detail (Laland et al. 2015; Müller 2017), the extended framework recognizes the core principles of variation in populations, genetic inheritance, and natural selection, but reinterprets the role of some of these parameters and lessens their exclusiveness for explaining the evolutionary process. In addition to adapted classical factors, the EES incorporates elements of inclusive inheritance theory, plasticity theory, niche construction theory, and evo-devo theory. Those inclusions significantly modify the logic of the evolutionary argument (Müller 2017). In contrast to the MS, the EES contends – to mention only the most essential points – that (a) selectable phenotypic variation is governed not simply by genetic variation but by the properties of evolving developmental systems that include a multitude of

nongenetic determinants and process dynamics; (b) development is evolutionarily modified not merely by natural selection but also by direct environmental induction of developmental variation, which is only secondarily exposed to selection; (c) genetic evolution is not the sole driver of phenotypic change and serves to stabilize morphogenetic solutions that are inherent to the developmental generation of phenotypic structure; (d) populations of organisms are not only passively exposed to natural selection but actively create the environment that will be selective for future generations; and (e) inheritance between generations is not by genes alone but also by several non-DNA-based mechanisms such as epigenetic, behavioral, material, and cultural processes. These arguments derive from evidence established over the past decades of empirical research, as provided in the original EES literature (Pigliucci and Müller 2010; Laland et al. 2015) and many of the chapters in this collection.

Two major features characterize the distinctiveness of the EES. One is *causal reciprocity*. This term denotes the ubiquitous feedback relations between (a) organismal activity and the environment, which is constantly modified by the actions of organisms that thus create the environment for future generations and (b) developmental systems and their plastic interrelations with external and internal sources. Niche construction theory and plasticity theory capture these two domains in great detail (Odling-Smee et al. 2003; West-Eberhard 2003). The second distinctive feature is *constructive development*. This refers to the reliance of evolution on the mechanistic properties that define development and that determine the structural solutions that can be attained. On this principle, phenotype construction is not the readout of a genetic program but the result of continuing and systemic feedback interactions between cell behaviors, gene activity, and tissue properties that provide the constructional templates on which organismal bodies are built. Here belong the concepts developed by evo-devo that will be discussed below.

Evo-Devo's Distinctive Concepts

The constructive component of the EES consists of several theoretical generalizations that were derived from empirical evo-devo. The best known is *developmental bias*. It refers to the fact that not all variants in a population arise with equal probabilities. Rather, some phenotypes are more readily generated than others, because the production of variation is both facilitated and constrained by the developmental system that has come to characterize a given taxon. Developmental constraint has been one of the foundational topics of evo-devo (Alberch 1982), and examples include a large variety of systems, such as the evolution of ammonite shells, beak diversity in songbirds, bilateral flower symmetry, vertebrate phalanges, and many more. Some of these constraints are rooted in developmental dynamics and others in the genetics of development (see chapter ▶ "A Macroevolutionary Perspective on Developmental Constraints in Animals"). It is important to note that developmental constraints act not only through limitations to potentially unbounded

genetic variation, for example due to deleterious pleiotropic effects, but are also responsible for creating preferred formative outcomes in phenotypic variation.

One of the best studied cases of constrained variation concerns the digits of vertebrates, which, despite varying substantially in number and phalangeal composition, leave many possibilities of the potential morphospace unrealized. For instance, the pentadactyl limb pattern of extant vertebrates is known to undergo numerous types of digit loss, such as in Australian skinks and lizards, but the individual loss of one of the central digits never occurs. The same is true for a number of other possible patterns of digit reduction. On the other hand, additions of digits can be generated in multiple species but occur only at specific locations and patterns (Lange et al. 2014). Even though the polydactylous forms rarely go to fixation (although *Acanthostega* might be a case), the tendencies towards polydactyly can be increased in certain species such as the Maine Coon cat. The patterns of digit loss and digit addition that can be produced experimentally also indicate that not all variants can be generated by the limb system, and the phenotypic outcomes of experimental or natural genetic variation, such as mutations that interfere with sonic hedgehog expression, are nonrandom (Lange et al. 2014). Such nonrandom components in the production of phenotypic variation channel the constructional solutions established in evolution. They are equally determined by the facilitating properties of development, which could also be called positive or enabling constraints.

Another source of evolutionarily constructive development lies in the *emergent quality* of developmental processes. Since these processes include multilevel feedback interactions between genes, gene products, cells, and multicell composites, each with its own physical and functional properties, changes at one level – initiated by mutation, selection, or environmental induction – can have nontargeted effects on the behavior of entities at another level. For example, changes in the surface tension of cells, caused by proteins and polypeptides of the cell surface, determine the shapes that spontaneously form upon cell aggregation (Foty et al. 1996), or altered rates of morphogen diffusion result in emergent cellular patterns (Kondo and Miura 2010). Many of these effects have been experimentally studied and modelled, highlighting nonlinear behaviors and threshold effects that characterize development (Lange et al. 2018).

A major cause of the emergent behaviors of developmental systems is the mesoscale physics of cells and soft tissues that are mobilized by evolutionary changes at the molecular level and that affect, for instance, differential adhesion, cell-cell signaling, morphogen diffusion, or oscillatory properties. The phenotypic outcomes of such modifications to key dynamical patterning modules (Newman and Bhat 2008) include the spontaneous formation of specific types of cell assemblies, such as tissue layering, cavity formation, or repetitive arrangements. Developmental and external context strongly influence these formative processes. All such autoregulatory and selforganizing features of cell-based development result in emergent effects in the construction of complex multicellular organisms and can drive major transitions in evolution (Newman 2016). Thus, constructive development, leading to the generation of particular selectable variants in phenotypic evolution, is profoundly determined by autonomous and emergent cell behaviors. Indeed, the link

between emergent variation and evolutionary change is one of the critical questions in evolutionary biology (Badyaev 2011).

Third, constructive development is a main driver of the evolution of *complex phenotypic organizations* seen in organismal bodyplans and organ systems. The fact that we observe only a limited set of basic body designs in nature, of which all species are modifications, points to a process of progressive locking in of specific developmental solutions. This order-on-order effect, which has variably been associated with the concepts of generative entrenchment, constructive neutral evolution, or burden (see chapter ▶ "Concept of Burden in Evo-Devo"), is based on the further elaboration of the kinds of generic multicellular motifs described above and the continuous increase in developmental interdependencies created by cell communication, tissue induction, and other forms of interacting processes. Stabilized networks of developmental interdependency are responsible for the conservation of developmental pathways and structural designs. Evo-devo models of pattern formation and morphogenesis demonstrate how exploratory morphodynamic networks, characterized by a prevalence of emergent processes, can be progressively substituted by more hierarchically organized morphostatic networks (see chapter ▶ "Mechanisms of Pattern Formation, Morphogenesis, and Evolution").

The stabilized morphological motifs shared by taxonomic groups have traditionally been called homologues. This fundamental principle of phenotypic organization, sidelined by population dynamical theory, is addressed by evo-devo based homology concepts that emphasize the role of shared developmental pathways and the modular configuration of development (see chapter ▶ "Developmental Homology"). Another perspective highlights the mobilization of physical forces involved in the formation of morphological templates that represent basic organizing themes in plants (Niklas 2000) and animals (Newman and Bhat 2008). In addition, increasingly hierarchized gene regulatory systems serve to reproduce and fixate such structural themes, leading to the closer mapping between genotypes and morphological phenotypes observed in extant organisms. Hence, evo-devo mechanisms are not only responsible for the generation of phenotypic complexity but, via the stabilization of heritable regulatory mechanisms, also organize the evolution of genetic interaction.

Consequences for Evolutionary Theory

Evo-devo is often seen as a program merely devoted to understanding how development evolves, complementing evolutionary theory but not requiring any adjustments of its basic logic or structure (Laland et al. 2014). By contrast, among the many theoretical contributions of evo-devo, the concepts of bias, emergence, and organization address features of evolution that were not part of the traditional explanatory canon. Thus, evo-devo adds new components to the evolutionary framework, but it also challenges some of its received tenets (Walsh and Huneman 2017).

One domain in which a major conceptual innovation arises is the generation of *phenotypic variation*. Obviously, heritable phenotypic variation is a prerequisite for

natural selection to become effective. Evo-devo demonstrates that phenotypic variation is not a straightforward consequence of genetic variation, as prescribed by the standard account, nor is it always imperceptibly small, continuous, and incremental. Instead, the variation produced by any given developmental system will be biased, both through its canalized dynamics and its inherent physical properties, and can sometimes be abrupt. Variational bias invalidates the role of natural selection as the singular directional factor in organismal change. Furthermore, the implicit bistabilities and threshold behaviors that characterize all complex interactive systems, such as development, challenge the gradualistic prerequisite of the classical approach as they lead to discontinuities in phenotypic variation. Unlike in a population-based account, which rests on statistical correlations between genetic and phenotypic variation, empirical evo-devo can explore the variational dimensions of any given developmental system and can be predictive about its behaviors under conditions of evolutionary change (see chapter ▶ "Computational Modeling at the Cell and Tissue Level in Evo-Devo").

Furthermore, the concepts of evo-devo capture the *process interface between development and the environment*. Whereas the classical notion of phenotypic plasticity addressed this issue at an abstract level, using standard tools of population theory, such as the reaction norm concept, evo-devo, and in particular the subdomain of eco-evo-devo (see chapter ▶ "Eco-Evo-Devo"), examine developmental plasticity and relate direct environmental influences on developmental variation to evolutionary outcomes (see chapter ▶ "Developmental Plasticity and Evolution"). Research in this area is making substantial progress, highlighting – among other processes – the influences of microbiome, epigenetics, and small RNA. Plasticity theory adds a new factor, environmental induction (West-Eberhard 2003), to the suite of evolutionarily effective components in the standard framework and is a challenge to the received notion that only natural selection is able to modify developmental parameters with transgenerational effects. One of the consequences of accepting the plasticity mode of evolution is that phenotypic accommodation and genetic assimilation need to be taken seriously, and the concept of "phenotype-first," or "genes-as-followers" (Jablonka 2006), requires a proper place in evolutionary theory.

A third domain of evolutionary theory to which evo-devo makes a distinctive contribution is the origin of *phenotypic novelties*. Whereas the standard approach either sidestepped the problem of how the characters whose variation it explains originate, or attributed it simply to additive genetic variation and subsequent function shifts (Mayr 1960), evo-devo concentrates on developmental causation. Depending on the chosen perspectives and definitions (Peterson and Müller 2016), it is thought that this can happen either by environmental or genetic perturbations that exceed the buffering capacity of development (Moczek 2008), or by a transition between adaptive fitness peaks through the overcoming of developmental constraints (Hallgrímsson et al. 2012), by large-scale rewirings of regulatory networks based on transposable element invasion and transcription factor evolution (Wagner and Lynch 2010), or by the mobilization of generic physicochemical behaviors in developmental cell aggregates (Newman 2016). While differing in detail, these approaches have in common that they recognize the origin of novelty as a distinct problem in

evolutionary theory and attribute to development the key to its explanation. Implicitly or explicitly, these views hold that new structural characters, such as skeletal parts, may arise as byproducts of untargeted evolutionary variation of developmental parameters. This view attributes the decisive causal role in the origination of phenotypic characters, body parts, and organismal form to development, not to the concomitant genetic variation that is exploited in reiterated rounds of variation and selection (see chapter ▶ "Developmental Innovation and Phenotypic Novelty").

There are many aspects to the integration of these and other components of evo-devo theory into the evolutionary framework that are vividly debated (Laland et al. 2014; Fábregas-Tejeda and Vergara-Silva 2018). But at a general level, evo-devo illustrates that the causality of evolution is not unidirectional, with endless variation being produced and natural selection weeding out. Instead, evolutionary causation is reciprocal and systemic, with multiple feedback relations between organismal activity and environment on the one hand, and between developmental processes and phenotypic structure on the other hand. As noted in more detailed treatments of the EES (Laland et al. 2015; Müller 2017), a series of specific predictions for a distinct research agenda derive from the integration of evo-devo principles into evolutionary theory: phenotypic variation in populations will be systematically facilitated and biased by development; nongradual variation and nonlinear phenotypic effects are possible; the environment has a direct capacity to alter development prior to selection; and the origin of complex organismal forms and phenotypic novelty is based on emergent and self-organizing capacities that are inherent in the dynamical processes of development. Many of these predicted effects have already found confirmation in the results of evo-devo research. Note that these are expectations that specifically derive from evo-devo, whereas EES theory formulates more predictions pertaining also to other domains of evolution (Laland et al. 2015).

Beyond Evolvability

It has been argued that the theoretical contributions of evo-devo primarily enhance our understanding of evolvability, in the sense of a "capacity of developmental systems to evolve" (Hendrikse et al. 2007). This interpretation seems to point to possible ways of bridging evo-devo with the population theoretical approach, in which evolvability is understood as "the ability of random variations to sometimes produce improvement" (Wagner and Altenberg 1996) or "the ability of a biological system to respond to selection" (see chapter ▶ "Variational Approaches to Evolvability: Short- and Long-Term Perspectives"). However, this joint usage of "evolvability" blurs the fact that its pervasive current understanding is rooted in a genetic primacy tradition of thought. Originally, evolvability referred to an abstract rapport between genes and phenotype, sometimes called the genotype-phenotype

map, which was taken to be itself under genetic control and therefore evolvable in the neo-Darwinian sense, i.e., by random variation and natural selection (Wagner and Altenberg 1996). A possible way to integrate this view with evo-devo is to use pleiotropy as a proxy for development (see chapters ▶ "Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics" and ▶ "A Macroevolutionary Perspective on Developmental Constraints in Animals"). But whereas this approach also harkens back to a causal primacy of genetic evolution, the evo-devo account is rooted in physical development, i.e., it attributes the origins of constructional specificity to the processes that build phenotypic structures through reiterated feedback interactions between genes, cells, and tissue aggregates, which include non-genetic elements such as physics, function, or environmental factors. On this view, the phenotype is not a mere extrapolation of the genotype but gains its distinctive constitution through the constructive processes of development. It is important to pay attention to these different connotations of "evolvability" and their implications (Nuño de la Rosa 2017) in order to fully appreciate the contribution of evo-devo to evolutionary theory. A refined developmental view of evolvability is a significant outcome (see chapters ▶ "Variational Approaches to Evolvability: Short- and Long-Term Perspectives" and ▶ "Evolvability"), but the theoretical consequences of evo-devo extend much farther.

Evo-devo is one of several areas in evolutionary biology that are contributing to a revision of the classical paradigm established by the Modern Synthesis in the early decades of the twentieth century. Whereas inputs from other fields primarily concern new features of genetic and genomic evolution, the structure and modes of transgenerational inheritance, and niche construction theory, evo-devo imports the formative principles underlying the developmental generation of phenotypic variation and complexity. The resulting reorganization, implicit in the extended evolutionary framework briefly outlined in the introduction, relaxes the gene centrism, gradualism, and adaptationism of the MS model. Instead, the inclusion of evo-devo shifts the theoretical emphasis from the sorting of variation in populations to the generative processes that effectuate diverse kinds of evolutionary change, not merely the adaptive kind. This has made it possible to address phenomena of phenotypic evolution that lie outside the evolvability focus, such as character discontinuity, phenotypic organization, and novelty formation discussed in this chapter.

The challenges to the MS framework afforded by evo-devo and other domains of evolutionary biology are often downplayed by arguing that adaptive evolution works well without assuming any additional factors besides the classical neo-Darwinian ones: allele frequency change caused by natural selection is still called "the only credible process underlying the evolution of adaptive organismal traits" (Charlesworth et al. 2017). However, as indicated above, the focus of evo-devo is not on *adaptive* evolution. The explanandum is not selectional maintenance of advantageous variants and gradual optimization of fitness, but the origin of phenotypic discreteness. Whereas MS theory cannot predict preferred phenotypic outcomes of evolution, evo-devo can. Here, the variations that arise are defined by the

inherent material properties and dynamical interactions of developmental processes that can be experimentally explored. This principle of organismal evolution, contrasting evo-devo with the omnipotent genetic variation and selection tenet, is best summarized by the term "inherency" (Newman and Müller 2006). Inherency represents the intrinsic propensities of developmental systems. It characterizes what is always poised to appear (though not necessarily all at once), regardless of whether the impulse for change comes from mutation, selection, environmental induction, or artificial perturbation (see chapter ▶ "Inherency"). Inherency defines the range of forms that can emerge through constructive development, whereas evolvability addresses the developmental capacity for fine-tuning during subsequent cycles of variation and selection.

For these reasons, but also due to other theoretical components such as niche construction and inclusive inheritance, the EES should not be misunderstood as an *extension of* the MS, appending merely peripheral add-ons to an established core framework. "Extended" is used in the sense of "broad," "comprehensive," or "encompassing." Although the EES incorporates standard parts of the MS, its resulting logical structure and the derived predictions are radically different. Instead of a view in which continuous genetic change propels evolution forward and all organismal manifestations are attributed to adaptation, the EES posits that reciprocal interactions between organism-based properties (including genes) and their non-genetic components control phenotypic evolution. Rather than being a smooth extrapolation of an earlier account, this is a proposal for a reorganized and more encompassing framework that constitutes an alternative theoretical structure from which different and new predictions derive. It fulfils Love's criterium (in Walsh and Huneman 2017) that, rather than through additions of more content to an existing framework, theory change proceeds by innovation of theory structure.

Cross-References

▶ A Macroevolutionary Perspective on Developmental Constraints in Animals
▶ Concept of Burden in Evo-Devo
▶ Computational Modeling at the Cell and Tissue Level in Evo-Devo
▶ Developmental Homology
▶ Developmental Innovation and Phenotypic Novelty
▶ Developmental Plasticity and Evolution
▶ Eco-Evo-Devo
▶ Evolvability
▶ Evolution of Complexity
▶ Inherency
▶ Interdisciplinarity in Evo-Devo
▶ Mechanisms of Pattern Formation, Morphogenesis, and Evolution
▶ Morphological Disparity
▶ Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics
▶ Variational Approaches to Evolvability: Short- and Long-Term Perspectives

References

Alberch P (1982) Developmental constraints in evolutionary processes. In: Bonner JT (ed) Evolution and development. Springer, Berlin/Heidelberg, pp 313–332

Badyaev AV (2011) Origin of the fittest: link between emergent variation and evolutionary change as a critical question in evolutionary biology. Proc Biol Sci 278:1921–1929. https://doi.org/10.1098/rspb.2011.0548

Charlesworth D, Barton NH, Charlesworth B (2017) The sources of adaptive variation. Proc R Soc B Biol Sci 284:20162864. https://doi.org/10.1098/rspb.2016.2864

Fábregas-Tejeda A, Vergara-Silva F (2018) The emerging structure of the extended evolutionary synthesis: where does Evo-devo fit in? Theory Biosci 137:1–16. https://doi.org/10.1007/s12064-018-0269-2

Foty RA, Pfleger CM, Forgacs G, Steinberg MS (1996) Surface tensions of embryonic tissues predict their mutual envelopment behavior. Development 122:1611–1620

Hallgrímsson B, Jamniczky H, Young NM, Rolian C, Schmidt-Ott U, Marcucio R (2012) The generation of variation and the developmental basis for evolutionary novelty. J Exp Zool B Mol Dev Evol 318:501–517. https://doi.org/10.1002/jez.b.22448

Hendrikse JL, Parsons TE, Hallgrímsson B (2007) Evolvability as the proper focus of evolutionary developmental biology. Evol Dev 9:393–401. https://doi.org/10.1111/j.1525-142X.2007.00176.x

Jablonka E (2006) Genes as followers in evolution – a post-synthesis synthesis? Biol Philos 21:143–154. https://doi.org/10.1007/s10539-004-0319-7

Jablonka E, Lamb MJ (2005) Evolution in four dimensions. MIT Press, Cambridge

Kondo S, Miura T (2010) Reaction-diffusion model as a framework for understanding biological pattern formation. Science 329:1616–1620. https://doi.org/10.1126/science.1179047

Koonin EV (2011) The logic of chance: the nature and origin of biological evolution. FT Press, Upper Saddle River, New Jersey

Laland K, Uller T, Feldman M, Sterelny K, Müller GB, Moczek A, Jablonka E, Odling-Smee J, Wray G, Hoekstra HE, Futuyma DJ, Lenski RE, Mackay TFC, Schluter D, Strassmann JE (2014) Does evolutionary theory need a rethink? Nature 514:161–164. https://doi.org/10.1038/514161a

Laland KN, Uller T, Feldman MW, Sterelny K, Müller GB, Moczek A, Jablonka E, Odling-Smee J (2015) The extended evolutionary synthesis: its structure, assumptions and predictions. Proc Biol Sci. https://doi.org/10.1098/rspb.2015.1019

Lange A, Nemeschkal HL, Müller GB (2014) Biased polyphenism in polydactylous cats carrying a single point mutation: the Hemingway model for digit novelty. Evol Biol 41:262–275. https://doi.org/10.1007/s11692-013-9267-y

Lange A, Nemeschkal HL, Müller GB (2018) A threshold model for polydactyly. Prog Biophys Mol Biol 137:1–11. https://doi.org/10.1016/j.pbiomolbio.2018.04.007

Mayr E (1960) The emergence of evolutionary novelties. In: Tax S (ed) Evolution after Darwin. University of Chicago Press, Chicago, pp 349–380

Moczek AP (2008) On the origins of novelty in development and evolution. BioEssays 30:432–447. https://doi.org/10.1002/bies.20754

Müller GB (2017) Why an extended evolutionary synthesis is necessary. Interface Focus 7:20170015–20170011. https://doi.org/10.1098/rsfs.2017.0015

Newman SA (2016) "Biogeneric" developmental processes: drivers of major transitions in animal evolution. Philos Trans R Soc B Biol Sci 371:20150443–20150410. https://doi.org/10.1098/rstb.2015.0443

Newman SA, Bhat R (2008) Dynamical patterning modules: physico-genetic determinants of morphological development and evolution. Phys Biol 5:015008. https://doi.org/10.1088/1478-3975/5/1/015008

Newman SA, Müller GB (2006) Genes and form: inherency in the evolution of developmental mechanisms. In: Neumann-Held EM, Rehmann-Sutter C (eds) Genes in Development. Duke University Press Books, Durham, pp 38–73

Niklas KJ (2000) The evolution of plant body plans—a biomechanical perspective. Ann Bot 85:411–438. https://doi.org/10.1006/anbo.1999.1100

Nuño de la Rosa L (2017) Computing the extended synthesis: mapping the dynamics and conceptual structure of the evolvability research front. J Exp Zool B Mol Dev Evol 37:73–17. https://doi.org/10.1002/jez.b.22741

Odling-Smee FJ, Laland KN, Feldman MW (2003) Niche construction. Princeton University Press, Princeton

Peterson T, Müller GB (2016) Phenotypic novelty in EvoDevo: the distinction between continuous and discontinuous variation and its importance in evolutionary theory. Evol Biol 43:314–335. https://doi.org/10.1007/s11692-016-9372-9

Pigliucci M, Müller GB (2010) Evolution – the extended synthesis. MIT Press, Cambridge

Wagner GP, Altenberg L (1996) Complex adaptations and the evolution of evolvability. Evolution 50:967–976

Wagner GP, Lynch VJ (2010) Evolutionary novelties. Curr Biol 20:R48–R52

Walsh D, Huneman P (2017) Challenging the modern synthesis. Oxford University Press, Oxford

West-Eberhard MJ (2003) Developmental plasticity and evolution. Oxford University Press, Oxford

Evo-Devo and Phylogenetics

Alessandro Minelli

Contents

Abstract

The contribution of evolutionary developmental biology (evo-devo) to phylogenetics has two aspects. The first is methodological: how to partition the phenotype into independent characters, in the light of the evolvability and modularity of developing systems. Evolvability, the ability to produce heritable phenotypic variation, has taken central role in explanations of evolutionary change, together with an increasing appreciation of the complex relationships between genotype and phenotype, which are characterised by (1) pleiotropy, (2) the involvement of a large number of genes in controlling single phenotypic traits, (3) the presence of polyphenism due to the influence of external, nongenetic factors, and (4) the

A. Minelli (✉)
University of Padova, Padova, Italy
e-mail: alessandro.minelli@unipd.it

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_40

modular architecture of developing systems. This allows for the occasional manifestation of saltational evolution. The second contribution of evo-devo to phylogenetics relates to specific sources of information that can be used in phylogenetic analysis, as provided by differences in the spatial and temporal patterns of expression of developmental genes or whole gene regulatory networks and by heterochronic patterns, especially in the framework of sequence heterochrony where changes in the temporal sequence of individual developmental events are considered relative to other events in the ontogeny of the same organism. In turn, a sound understanding of phylogenetics can benefit evo-devo in the selection of new model species.

Keywords

Heterochrony · Homology · Model species · Phylogeny · Saltational evolution

Introduction

Evolutionary developmental biology, or evo-devo, emerged as an independent discipline within the life sciences in the last quarter of the twentieth century. However, to some extent its historical antecedents can be traced back to the efforts of Etienne Geoffroy Saint-Hilaire (1772–1844), who systematically looked for equivalent structural elements in the body plans of animals as different as a vertebrate, a crayfish, and a squid, and to the long, although discontinuous, tradition of studies on *heterochrony*. When Ernst Haeckel (1834–1919) first introduced the latter term, this was intended to label a deviation from Haeckel's biogenetic "law" according to which ontogeny (the development of the individual) recapitulates phylogeny (the evolutionary history of the species). Heterochrony thus referred to circumstances where the comparative study of developmental sequences of different animals cannot be straightforwardly used to infer their evolutionary relationships. In the twentieth century, these deviations from the *biogenetic law* were the subject of Gavin de Beer's (1899–1972) ground-breaking work. This author established that an animal's ontogenetic progression towards sexual maturity does not necessarily proceed in strict conjunction with the development of its nonreproductive (somatic) structures. This decoupling allows for the two processes to run at different pace and eventually to evolve via changes in the relative time of onset or offset of somatic versus reproductive development or in their relative speed. This way, heterochrony emerged as a pervasive and variegated developmental basis of evolutionary change (Gould 1977).

However, when evo-devo eventually took form in the 1980s, this discipline's focus was largely divorced from those original ties to phylogenetics. A quarter of century later, Wiens et al. (2005) could still write that up to the time the overall contribution of evo-devo to phylogenetics had been quite small. However, a growing appreciation of the mutual benefits that can derive to both evo-devo and phylogenetics from reciprocal interactions has surfaced at last in recent years.

The contribution of evolutionary developmental biology (evo-devo) to phylogenetics has two aspects. The first is methodological: how to partition the phenotype into independent characters, in the light of the evolvability and modularity of developing systems. Evolvability, the ability to produce heritable phenotypic variation, has taken central role in explanations of evolutionary change, together with an increasing appreciation of the complex relationships between genotype and phenotype. The second contribution of evo-devo to phylogenetics relates to specific sources of information that can be used in phylogenetic analysis. In turn, a sound understanding of phylogenetics can benefit evo-devo in the selection of new model species.

Evo-Devo's Contribution to Phylogenetics

The advent of evolutionary developmental biology offers indeed new opportunities to extract phylogenetic information from a comparison of developmental schedules of different species (Telford and Budd 2003; Minelli et al. 2007). Evo-devo's contribution to phylogenetics has two aspects. The first is methodological: how to partition the phenotype into independent characters; the second relates to specific sources of information to be used in phylogenetic analysis, as suggested by heterochrony or by comparative patterns of expression of developmental genes.

Partitioning the Phenotype into Individual Characters

One of the main steps in a phylogenetic analysis is filling a data matrix: the rows are the taxa (usually, species) to be compared, the columns are the characters for which the taxa are compared. A basic requirement is, to include only mutually independent characters, to avoid giving more weight to those that are instead interdependent. In practice, however, it is often difficult to determine if two characters are actually independent or to which extent.

Independence between two characters means that changes in one of them are not necessarily accompanied by changes in the other. On one side, this lack of correlation can be due to a lack of functional coupling, on the other side it reveals the autonomy of the developmental processes on which each character depends, a circumstance that in principle corresponds to the expression of different genes, or at least to spatial, temporal, or quantitative differences in the expression of the same genes. Here is an area where evo-devo can positively contribute to a phylogenetic analysis. To see how, we must refer to two key concepts of evolutionary developmental biology: evolvability and modularity.

With the advent of evolutionary developmental biology, *evolvability*, i.e., the ability to produce heritable phenotypic variation (Hendrikse et al. 2007) has taken central role in explanations of evolutionary change, together with an increasing appreciation of the complex relationships between genotype and phenotype (the so-called genotype → phenotype map; Pigliucci 2010; Wagner and Zhang 2011).

This complexity has many causes, among which (1) *pleiotropy*, i.e., the fact that the expression of one gene has commonly an effect on many phenotypic traits, (2) the involvement of a large number of genes in controlling the developmental processes culminating in the production of a single phenotypic trait, (3) the influence of external, nongenetic factors (discussed in a later section of this entry), and (4) the *modular architecture* of developing systems.

To some extent at least, a developing organism can be described indeed as a system of local units, or *modules*, dominated by specific developmental dynamics, such as those generating a leaf primordium in a plant or those responsible for the production of segments in an insect.

Evolutionary changes are also often modular, affecting individual characters that emerge as hot points of morphological evolution. In many rapid radiations, the explosion of phenotypes is essentially restricted to large variation in a well-circumscribed module. This is the case with the copulatory structures of a great number of insect groups, with the chewing structures (mastax) of rotifers, and with the stamens or the petals in flowering plants.

Even within a series of homologous parts do individual elements, or group of elements, often behave as partly independent modules. Examples in the animal kingdom are found, e.g., among the teeth of mammals (incisors, canines, premolars, molars) and the segments of arthropods (e.g., in insects, thoracic segments with legs versus abdominal segments without legs). In plants, examples of developmentally independent modules are the nectariferous petals of *Delphinium* (Ranunculaceae) and the individual petals and stamens of *Bauhinia* (Fabaceae): in this genus there are species like *B. blakeana*, with five petals and three fertile stamens, alongside species like *B. divaricata*, with two petals and one functional stamen only.

In phylogenetic analyses, understanding or at least estimating the modularity of the developmental processes underlying the morphological traits of species or lineages to be compared is important also when the evolutionary changes in developmental processes have been *systemic*, affecting many dimensions and body parts in integrated way. Systemic, that is, nonmodular change may conceal the actual relationships between phylogenetically related taxa. This is one of the contexts in which morphological evidence must be used most cautiously, and we must definitely acknowledge its subordinate importance in respect to comparative molecular data. A fitting example is provided by the duckweeds, long considered to form an easily diagnosable plant family, but eventually reduced to a subfamily of the Araceae (Henriquez et al. 2014). The systemic evolution the duckweed lineage has undergone has completely cancelled the modular architecture of the other Araceae and, indeed, of the overwhelming majority of plants, only leaving behind a thallus-like blob of green matter, sometimes (but not always) accompanied by simple roots and occasionally producing a rudimentary stamen or carpel, all that remains of a typical flower.

Genes Versus Environment and the Genotype → Phenotype Map

One of the reasons why an organism's phenotype cannot be fully predicted from the genotype is the frequent occurrence of alternative phenotypes in the absence

of genetic differences: this occurs when specific environmental cues are "interpreted" by the developing system as a switch between alternative pathways. This phenomenon is known as *phenotypic plasticity* and the multiplicity of resulting phenotypes is described as a case of *polyphenism* (reviewed in Fusco and Minelli 2010). Environmental influences are often due to differences in the relative length of day and night, a result of which is the seasonal polyphenism of some butterflies, e.g., the European *Araschnia levana*, with two generations per year (a spring and a summer one), dramatically different in their wing color patterns to the extent that they were originally described as different species. The temperature at which the embryo is exposed during incubation is involved instead in the environmental determination of sex in the American alligator, many turtles, and other reptiles, while in *Schistocerca gregaria* and other grasshoppers mechanical stress due to exceedingly frequent contacts of juveniles with theirs conspecifics results in the production of gregarious and migratory adults rather than solitary and sedentary ones.

The divide between environmentally controlled polyphenism and genetically determined polymorphism, however, is not necessarily strong. How easily this divide can be crossed is shown by the pea aphid (*Acyrthosiphon pisum*), a species where males as well as females occur in two different morphs, winged and wingless, respectively. The mechanisms responsible for the presence versus absence of wings are different in the two sexes: the male morphs represent a genetic polymorphism, whereas the female morphs depend on the photoperiod. However, the developmental pathways leading to these alternative phenotypes are nearly the same in both sexes: the product of the gene locus (*aphicarus*) controlling wing development in the male is also involved in the polyphenic response of the female.

There is growing evidence that populations harbor variable amounts of *cryptic variation*, that is, of variation that is not expressed under the environmental conditions under which the population currently lives; a change of external conditions, however, may uncover this variation and cause the expression of novel phenotypes. This can be of consequence in phylogenetic analyses. On the one hand, the previously unobserved phenotypes may wrongly suggest a phylogenetic distance quite higher than eventually demonstrated by molecular studies; on the other, the newly expressed phenotypes can offer new targets to selection and thus accelerate evolutionary divergence and perhaps the emergence of evolutionary innovations (Moczek et al. 2011).

Saltational Evolution

Before the advent of evo-devo, a serious obstacle to reconstructing phylogeny was the nearly universally (although mostly tacitly) accepted principle, that evolution necessarily proceeds by progressive accumulation of small changes; as a consequence, species differing in very conspicuous aspects could hardly be acknowledged to be phylogenetically close relatives. The strength of this preconception has been strongly reduced by evo-devo. Several lines of evidence concur indeed in demonstrating that the changes in developmental processes necessary to obtain a new,

strongly divergent phenotype are not necessarily proportional to the morphological distance between the old and the new phenotypes. On the other hand, in many evolutionary lineages, some hypothetical phenotypes differing from those occurring in nature only in minor detail do never occur because of internal constraints in the developmental processes by which the phenotype is produced. As a consequence, the actual distribution of phenotypes within a clade may reflect developmental constraints (inequalities of evolvability in different directions) rather than phylogenetic affinities, thus inviting caution in the course of phylogeny reconstructions. Segment number in centipedes offers a case in point.

All adult specimens of all centipede species have an odd number of leg-bearing segments. This number is fixed and identical in all species of some subgroups (e.g., it is always 15 in all of the ca. 1000 species of lithobiomorph centipedes described to date) but variable in others, often even within a brood issued from the same parents, for example, between 51 and 59, but limited to the odd values: specimens with 52, 54, 56, or 58 pairs of legs simply do not occur. Thus, moving from a "permitted" number, e.g., 57, to one of the arithmetically closest values (56 or 58) is not possible. Nevertheless, a much larger change, i.e., sudden and likely very recent duplication of the number of leg pairs has been observed in a lineage of scolopendromorph centipedes. Most of the *ca*. 700 species belonging to this clade have a fixed number of 21 pairs of legs, although several have 23, with a single species (*Scolopendropsis bahiensis*) including specimens with 21 leg-bearing segments along with others with 23. A duplication of the whole set of trunk segments has been suggested (Minelli et al. 2009) to account for the origination of the closely related *Scolopendropsis duplicata*, a newly discovered species where leg-bearing segments are either 39 or 43. This species has likely diverged from *S. bahiensis* quite recently, as the dramatic increase in segment number is the only obvious difference between the two *Scolopendropsis* species. It has been hypothesized that the duplication of trunk segment number, a phenotypically major leap, was very likely the effect of a minor genetic and developmental change.

Developmental Genes and Phylogenetic Inference

The most conspicuous body of information generated thus far within evolutionary developmental biology is about the so-called "developmental genes," i.e., genes demonstrably involved in the control of specific ontogenetic events or in the shaping of specific traits of body architecture. Data spans from the mere identification of these genes and of their nucleotide sequence, to the temporal and spatial patterns of expression, and the mechanisms by which the expression of these genes is modulated and the way by which, in turn, their products modulate the spatial or temporal expression of other genes.

These genes can be studied at different levels for their potential phylogenetic signal.

A first step is to use the gene sequences to reconstruct the phylogeny of the organisms from which the genes have been isolated. In the case of animals, several authors have looked at the *Hox genes* as to privileged genes supposed to carry

important phylogenetic signal because of their roles in controlling key aspects of the animal's body architecture such as the orderly sequence of organs along the animal's antero-posterior body axis. There are studies, for example, where Hox gene sequences are used in reconstructing the relationships among the bilaterian phyla or the major clades within the Arthropoda.

The next step is to search for homologies at the level of gene expression patterns. This way, largely accepted homologies between body regions of distantly related arthropod taxa, such as insects and arachnids, have been traced by comparing the expression patterns of *Hox* genes.

These patterns are nevertheless subject to evolution, because of *gene duplication* followed by functional divergence of the *paralogous* copies thus obtained, or by *changes in the gene's regulatory sequences*, not to mention *gene loss*. As a consequence, we observe changes in the spatial extent or in the timing or level of gene expression.

Examples of the different way in which a morphological trait can be modified by changes in the regulation of the expression of the same genes are provided by the pattern of dorsal bristles on the thorax of Diptera, which is controlled by the expression of the gene *scute*. Changes in the spatial expression of *scute* account for the differences between more distantly related taxa, such as *Ceratitis capitata*, *Drosophila melanogaster*, and *Calliphora vicina*, whereas differences in the timing of this gene's expression underlie the differences in the bristle pattern of *Calliphora vicina* compared to another calliphorid, *Protophormia terranovae.* Changes in the expression level of just two genes (*bone morphogenetic protein 4* and *calmodulin*) are instead responsible for the conspicuous differences in beak shape among Darwin's finches (*Geospiza*).

With the rapidly increasing knowledge on gene control cascades, attention has shifted from individual genes to whole gene regulatory networks. An exceptional example of the evolvability of developmental gene networks has been revealed in a comparison of notochord development in the pelagic urochordate *Oikopleura* and the ascidian *Ciona intestinalis*. In the latter, some 50 genes are known to be activated downstream of *Brachyury*, but 24 of them do not have a homologue in the small, very compact genome of *Oikopleura*. Some of the latter have undergone a lineage-specific duplication, but less than a half of them are apparently expressed in the context of notochord formation.

However, the different components of a gene regulatory network do not necessarily evolve at the same pace. For example, within the gene regulatory network controlling the specification of the endomesoderm in nematodes, evolution is most rapid for some genes involved in the specification of blastomere identity, as suggested by a comparison between the genomes of *Haemonchus contortus* and *Brugia malayi* (Maduro 2006).

Evolving Gene Functions

In animals as distantly related as are squids, insects, and vertebrates, the morphogenesis of the eye is controlled in part by the lineage-specific homologues of the

same genes. The best known and arguably the most important of these genes is *Pax6* (also known as *eyeless* in *Drosophila*). The widespread involvement of *Pax6*/*ey* homologues in eye morphogenesis has suggested a common (monophyletic) origin of all animal eyes (Gehring and Ikeo 1999), despite the gross morphological differences between ciliary-type eyes, as are those of vertebrates, and rhabdomeric-type eyes, as are those of insects, a large structural difference that would instead suggest that eyes originated at least twice independently.

However, its involvement in building eyes is not necessarily the original developmental role of *Pax6*. First, the expression of *Pax-6* is not restricted to the eyes. For example, in the squid, *Pax6* expression extends to the brain and the arms; in vertebrates, to a large part of the nervous and sensory systems, including nasal placodes, diencephalon, latero-ventral hindbrain, and the spinal cord; in *Drosophila*, its homolog *ey* is expressed, other than in the eye, also in the brain and the ventral nerve cord. Second, *Pax6* homologues are also present in eyeless animals, for example, in the roundworms (nematodes) and in the sea urchins. In the latter, a *Pax6* homolog is expressed in the tube feet. A likely conclusion is that *Pax6* was a patterning gene, originally expressed in the head, which has been co-opted several times in the regulation of eye development.

Dramatic functional changes have been recorded in the evolution of two members of the Hox gene family in some arthropod lineages. Hox genes, as mentioned above, are best known to specify positions along the main body axis of bilaterian animals. In the arthropods, however, one of these genes (re-named here *fushi tarazu*) is involved instead in the segmentation of the trunk and also (limited to the insects) in neurogenesis. Another Hox gene (*zerknüllt*, shortly *zen*) is involved in dorso-ventral patterning. In the flies (Diptera), a duplication of *zen* has given rise to a new functionally divergent gene, *bicoid*. In *Drosophila*, *bicoid* is required for the normal development of head and thorax, and in another dipteran, the scuttle-fly *Megaselia abdita*, it is also required for the development of four abdominal segments.

These examples show that major changes in gene functions do not necessarily determine an acceleration of morphological evolution. In other terms, homology at the level of genes, and genes expression patterns, does not necessarily suggest homology of morphological features and vice versa.

The Phylotypic Stage

The independence of developmental modules is limited by constraints, more evident at specific times along the ontogeny: specifically, largely invariant stages shared by (most of) the members of a large group such as vertebrates, or insects, can be often recognized. These stages do not coincide with the earliest embryonic stages, which are dramatically affected by conditions such as the amount and spatial distribution of the yolk in the egg, which is sometimes very different between closely related species. For example, the sea urchin *Heliocidaris tuberculata* produces small eggs, with a modest amount of yolk, which develop into a typical pluteus larva, but the very closely related species *H. erythrogramma*, barely distinguishable from

H. tuberculata in the adult stage, produces instead much larger eggs, full of yolk, from which a juvenile develops directly, bypassing the conventional larval stage. The initially divergent developmental trajectories converge however towards a later, much more conserved stage (Richardson 2012). This stage, which is called the *phylotypic stage*, is sometimes recognizable as characteristic for a whole phylum, although it must be acknowledged that often, rather than a point in development, what is conserved is instead a *phylotypic period*. This term refers to a more or less extended segment of the developmental trajectory, within which the traits shared by the members of a phylum are more or less faithfully conserved among a smaller or larger number of species. As expected, gene expression is maximally conserved around the phylotypic stage or period.

Phylogenetic Signal from Heterochronic Patterns

From the old-fashioned perspective of Haeckel's recapitulation principle (ontogeny recapitulates phylogeny), heterochrony was nothing but noise obscuring the potential contribution of a detailed knowledge of ontogeny to the reconstruction of phylogeny. Subsequent studies, however, have shown that heterochrony per se can be informative about affinities, that is, in technical language, that heterochrony may contain phylogenetic signal.

The traditional approach to heterochrony focused on developmental changes in size and shape relationships: in terms of *growth heterochrony*, two major patterns were distinguished, *paedomorphosis* and *peramorphosis*, according to whether maturation is anticipated or delayed and/or the growth period is shortened or extended, respectively.

Recognizing these evolutionary patterns of change in the developmental sequences of the species to be compared can be dramatically important to avoid serious pitfalls in the reconstruction of phylogeny. A good example is provided by salamanders. Several lineages of salamanders have evolved via paedomorphosis, that is, they retain throughout their life larval traits such as the presence of external gills. This has serious consequences on a phylogenetic analysis based on morphology. Lineages that have independently evolved by paedomorphosis will likely cluster together, irrespective of their actual affinities. In some phylogenetic analyses, most paedomorphic families (Amphiumidae, Dicamptodontidae, Sirenidae, Proteidae) cluster indeed in a single clade, including also individual paedomorphic representatives of the Plethodontidae and Ambystomatidae. This obscures the actual affinities of these lineages. To uncover the latter, it is not necessary to rely on molecular rather than morphological evidence. The same result is obtained by excluding from the data matrix of morphological data those traits that are affected by paedomorphosis, which have been acquired independently by these different lineages, accompanied by the loss of lineage-specific traits retained instead by their nonpaedomorphic relatives (Wiens et al. 2005).

An increasing appreciation of the modularity of developmental processes has fostered a new approach to heterochrony, termed *sequence heterochrony* (Smith

2001), focused on the changes in the temporal sequence of individual developmental events relative to other events in the ontogeny of the same organism. Given two events A and B in a developmental sequence, these can occur in one of the following orders: (i) A occurs before B, (ii) A and B are simultaneous, or (iii) A occurs after B. Translated into numerical codes, these timing relationships are assembled in a matrix which is subsequently subjected to phylogenetic analysis according to the current methods.

In the flowering plants, heterochronies in the production of individual floral parts can be responsible for conspicuous and phylogenetically informative differences. For example, a number of clades in the legume family (Fabaceae) are characterized by heterochronies such as the anticipation or retardation in the production of a whole whorl with respect to another (e.g., stamens vs. petals) or of a single organ (e.g., a sepal or a petal) with respect to the other elements of its whorl.

In vertebrates, the relative times at which the fore and hind limbs differentiate are characteristic of different clades (Bininda-Emonds et al. 2007). In the primitive condition, as seen in cartilaginous and bony fishes, forelimbs develop earlier than the hind limbs. In the frogs (anurans), hind limb development precedes instead the differentiation of the anterior pair of appendages. In most of the remaining vertebrates, the development of the two limb pairs is nearly synchronous.

A study of sequence heterochrony involving numerous characters has proved effective in fixing the phylogenetic position of the turtles in the phylogeny of amniotes (Werneburg and Sanchez-Villagra 2009).

Phylogenetics to the Benefit of Evo-Devo

Selecting New Model Species

Despite the pervasive comparative attitude that differentiates evo-devo from many other disciplines in the life sciences, most of the experimental results thus far contributed by evolutionary developmental biology have been obtained on a small number of model species. The list of evo-devo's choice model organisms includes animals such as mice (*Mus musculus*), chicken (*Gallus gallus*), zebrafish (*Danio rerio*), the sea squirt (ascidian) *Ciona intestinalis*, a few sea urchin species such as *Heliocidaris tuberculata* and *H. erythrogramma*, the fruitfly *Drosophila melanogaster*, and a tiny nematode worm (*Caenorhabditis elegans*), plants as the thale cress (*Arabidopsis thaliana*), tomato (*Lycopersicon esculentum*), snapdragon (*Antirrhinum majus*), and rice (*Oryza sativa*) plus the moss *Physcomitrella patens*. Except perhaps for *Ph. patens*, all these organisms were selected only because of practical advantages, such as short generation time and easy adaptation to artificial environment. Of recent, however, most suggestions for new entries to be added to the list of model organisms advocate the phylogenetic position of a species as anticipating its value in future comparisons (Milinkovitch and Tzika 2007). However, there are no scientific reasons to expect that the topology of the phylogenetic tree will inform us unambiguously about historical changes affecting characters

other than those that have been used to build the tree. Unfortunately, there are no macroevolutionary laws suggesting strong consistent trends in character variation across the different branches of the tree.

Attention has been primarily targeted towards "basal" representatives of a smaller or larger branch of the tree of life (i.e., organisms belonging to lineages that are considered to have branched off early from the common ancestor): the expectation is that a "basal" branch will be a good proxy for the unknowable common ancestor of a major clade. However, time (thus, opportunity to change) has run to the same length for all branches stemming from the same common ancestor, irrespective of their branching order; in other terms, a "basal" species is not necessarily a more conserved model of an ancestor, that is, a repository of primitive character states (Jenner 2006; Minelli and Baedke 2014).

The inadequate concern hitherto demonstrated by evo-devo researchers for comparisons within a suitable phylogenetic context is also shown by the fact that many of the popular model species belong to taxonomically small or very small genera, e.g., *Arabidopsis* (12 species), *Ciona* (9), *Heliocidaris* (6), *Caenorhabditis* (4), *Gallus* (4), and *Physcomitrella* (2). From this perspective, a welcome new entry is the scarab beetle genus *Onthophagus*, with about 2000 species, some of which are emerging as evo-devo models for the study of the developmental genetic basis of evolutionary novelties, their head and thoracic horns.

Cross-References

- ▶ Developmental Homology
- ▶ Developmental Plasticity and Evolution
- ▶ Evolvability
- ▶ Heterochrony
- ▶ Methods and Practices in Paleo-Evo-Devo
- ▶ The Developmental Hourglass in the Evolution of Embryogenesis

References

Bininda-Emonds ORP, Jeffery JE, Sánchez-Villagra MR, Hanken J, Colbert M, Pieau C, Selwood L, Ten Cate C, Raynaud A, Osabutey C, Richardson MK (2007) Forelimb-hindlimb developmental timing across tetrapods. BMC Evol Biol 7:182

Fusco G, Minelli A (2010) Phenotypic plasticity in development and evolution: facts and concepts. Introduction. Philos Trans R Soc B Biol Sci 365:547–556

Gehring WJ, Ikeo K (1999) Pax 6: mastering eye morphogenesis and eye evolution. Trends Genet 15:371–377

Gould SJ (1977) Ontogeny and phylogeny. Belknap Press of Harvard University Press, Cambridge, MA

Hendrikse JL, Parsons TE, Hallgrímsson B (2007) Evolvability as the proper focus of evolutionary developmental biology. Evol Dev 9:393–401

Henriquez CL, Arias T, Pires JC, Croat TB, Schaal BA (2014) Phylogenomics of the plant family Araceae. Mol Phylogenet Evol 75:91–102

Jenner RA (2006) Unburdening evo-devo: ancestral attractions, model organisms, and basal baloney. Dev Genes Evol 216:385–394
Maduro MF (2006) Endomesoderm specification in Caenorhabditis elegans and other nematodes. Bioessays 28:1010–1022
Milinkovitch MC, Tzika A (2007) Escaping the mouse trap: the selection of new Evo-Devo model species. J Exp Zool B Mol Dev Evol 308:337–346
Minelli A, Baedke J (2014) Model organisms in evo-devo: promises and pitfalls of the comparative approach. Hist Philos Life Sci 36:42–59
Minelli A, Negrisolo E, Fusco G (2007) Reconstructing animal phylogeny in the light of evolutionary developmental biology. In: Hodkinson TR, Parnell JAN (eds) Reconstructing the tree of life: taxonomy and systematics of species rich taxa. Taylor and Francis – CRC Press, Boca Raton, pp 177–190
Minelli A, Chagas AJ, Edgecombe GD (2009) Saltational evolution of trunk segment number in centipedes. Evol Dev 11:318–322
Moczek AP, Sultan S, Foster S, Ledon-Rettig C, Dworkin I, Nijhout HF, Abouheif E, Pfennig DW (2011) The role of developmental plasticity in evolutionary innovation. Proc R Soc B 278:2705–2713
Pigliucci M (2010) Genotype-phenotype mapping and the end of the 'genes as blueprint' metaphor. Philos Trans R Soc B 365:557–566
Richardson MK (2012) A phylotypic stage for all animals? Dev Cell 22:903–904
Smith KK (2001) Heterochrony revisited: the evolution of developmental sequences. Biol J Linn Soc 73:169–186
Telford MJ, Budd GE (2003) The place of phylogeny and cladistics in Evo-Devo research. Int J Dev Biol 47:479–490
Wagner GP, Zhang J (2011) The pleiotropic structure of the genotype-phenotype map: the evolvability of complex organisms. Nat Rev Genet 12:204–213
Werneburg I, Sanchez-Villagra MR (2009) Timing of organogenesis support basal position of turtles in the amniote tree of life. BMC Evol Biol 9:82
Wiens JJ, Bonett RM, Chippindale PT (2005) Ontogeny discombobulates phylogeny: paedomorphosis and higher-level salamander relationships. Syst Biol 54:91–110

Methods and Practices in Paleo-Evo-Devo

Carolin Haug and Joachim T. Haug

Contents

Abstract

Paleo-evo-devo is the discipline studying the developmental biology of fossil organisms and its evolutionary implications. In adopting a paleo-evo-devo approach, fossils have to be understood as once-living organisms, and the developmental patterns of extant organisms have to be comparatively investigated. For some types of fossils, it is comparably easy to investigate ontogeny, as they preserve earlier portions of the process throughout their entire life, for example as growth lines, or as they have been fossilized while bearing offspring inside their bodies. Yet, in most cases the ontogeny of fossil organisms (and also of some extant ones) has to be reconstructed based on plausibility. Major aspects for this approach are increasing differentiation or number of structures as well as continuity in development. Despite the difficulties in reconstructing the ontogenies of fossil organisms, studying fossilized development can provide important insights into the evolution of developmental patterns not available only from the study of extant organisms. Also the workflow in the practical work in paleo-evo-devo is shortly outlined.

C. Haug (✉) · J. T. Haug
Department Biology II, Functional Morphology Group, LMU Munich, Planegg-Martinsried, Germany
e-mail: carolin.haug@palaeo-evo-devo.info; jhaug@bio.lmu.de

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_41

Keywords

Fossilized development · Deep time · Evolutionary reconstruction · Heterochrony · Character polarization

Introduction

Paleo-evo-devo or evolutionary developmental paleobiology is a biological discipline combining approaches from developmental biology and paleobiology into an evolutionary framework. The discipline is still developing; the term summarizing the principal approach – paleo-evo-devo – has to our knowledge first appeared in the foreword to Minelli and Fusco in 2008. While some of the principal approaches are definitely significantly older, the comparably recent appearance of a distinct term for such approaches underlines that the discipline is still maturing. In the following, a short outline of paleo-evo-devo is provided (see also Sánchez-Villagra 2012; Urdy et al. 2013).

Paleo-evo-devo is a research field in which data and knowledge from developmental biology and paleontology are combined to draw conclusions about the evolution of a group of organisms. The inclusion of these data can help to explain how the stepwise transformation of morphological characters evolved, especially in lineages where the extant adults possess very distinctly differing morphologies. With such an approach, a more complete view on the evolutionary history of a group of organisms can be achieved (see also Haug and Haug 2016a).

This chapter is focused on the methodological aspects of paleo-evo-devo to provide a guideline for practical research. The methods which are applied in paleo-evo-devo are significantly different from those used in most other fields of evo-devo, especially due to special challenges of the data acquisition, which makes the explanation of the practical aspects necessary. The theoretical aspects of paleo-evo-devo can be retrieved from other publications (e.g., Hall 2002; Wilson 2011; Urdy et al. 2013).

Fossils as Biological Entities

Fossils are remains of once-living organisms. As such, they offer a view into the past. Reconstructing the extinct organism based on its fossil remains is a truly biological task. Although paleontology, the scientific discipline focusing on fossils, is in many modern university curricula deeply nested in the geological sciences, understanding the biology of an extinct organism obviously demands for *biological approaches*. This is also true for developmental aspects of the extinct organism. Although it might sound trivial, one needs to be aware that also extinct organisms, like living ones, developed from a single cell, then through embryogenesis, were born or hatched, grew up, and ideally reached adulthood. This statement is in fact far from trivial given its consequences: one needs to recognize that a fossil specimen

might not necessarily represent an adult. This also means in further consequence that two morphologically differing specimens do not necessarily represent different species, but could potentially represent *different developmental stages of the same species*. Making this distinction in a specific case is far from simple and has given rise to significant debates in recent years concerning various groups of organisms (e.g., dinosaurs: Horner and Goodwin 2009; arthropods: Haug et al. 2012; cycloneuralian worms: Haug and Haug 2015a; for a detailed discussion see Haug and Haug 2016a). Hence, careful considerations are required within a biological framework, developmental biology included, when trying to identify and interpret a fossil. Such considerations are therefore some of the basic tasks for paleo-evo-devo.

Reconstructing the Ontogeny of a Fossil Organism

In general, one could assume that paleo-evo-devo faces three major methodological problems: (1) the problem of reconstructing ontogenies; (2) the problem of recognizing conspecifity; and (3) how problems 1 and 2 are to be confronted in the case of fossils. Problems 1 and 2 are highly interlinked and are often part of an iterative process where first an ontogenetic series is reconstructed and only after certain inconsistencies in this series may lead the researcher to conclude that there are in fact two species in the sequence. The specimens included into the ontogenetic sequence are sorted again, and two ontogenetic sequences are reconstructed, which are again checked for consistency (see the example in Haug et al. 2010a, where Cambrian specimens originally described as one crustacean species turned out to be two species). Hence, in the following no distinctions will be made between these problems.

When dealing with fossils, the important question is: *how can we infer how an extinct organism developed throughout ontogeny?* Obviously, a direct observation of the developing organism is excluded in fossils. Therefore, one needs to have a look into modern developmental biology and assess which of the approaches used there for observing the development of living organisms can also be applied to fossil organisms.

1. Preserved ontogeny
   Some organisms are very "cooperative" to the researcher interested in development, preserving aspects of their ontogeny in their morphology, or at least in certain parts of their morphology. As a result, a single specimen may be informative for its entire ontogeny or at least parts of it. Well-known examples are shells and other hard parts of various metazoans. These often show distinct *growth lines*, which indicate the shape of the shell at an earlier time slice of the ontogeny (Fig. 1). Molluscan shells, for example, often even preserve the embryonic shell (protoconch) and hence provide a comparably complete developmental history of their shell. Such information can be and has been successfully exploited also in fossil organisms (e.g., gastropods: Nützel 2014; ammonites: De Baets et al. 2012; echinoderms: Sumrall 2008).

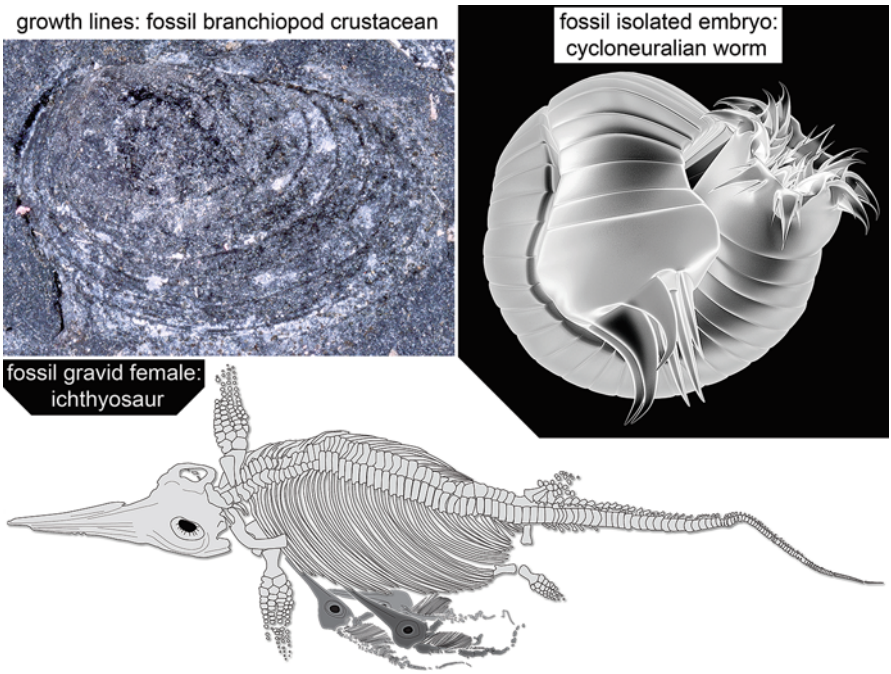


Fig. 1 Examples of fossilized development. *Upper left*: A 300-million-year-old fossil branchiopod crustacean (FMNH, Mazon Creek Formation, USA); the growth lines on the shield make it possible to partly reconstruct the ontogeny. *Upper right*: 3D model of a representative of *Markuelia*, the embryo of a cycloneuralian worm from ca. 500-million-year-old Orsten deposits from Australia. *Bottom*: Drawing of a gravid female ichthyosaur, ca. 180 million years old (SMNS, Holzmaden lagerstätte, Germany); there are several embryos inside the womb, three partly expelled ones have been drawn here

2. Serially organized organisms
   A related case to preserved ontogeny is provided by still developing, serially organized organisms. In animals that add body segments during their ontogenetic sequence, such as annelid worms or many arthropods, the anterior segments are usually further developed than the posterior ones (e.g., Zhang et al. 2007). With such an *anterior-posterior gradient,* a single specimen can be (at least roughly) informative of the ontogenetic changes of certain structures. In arthropods, it is for example possible to compare the morphology of trunk appendages along the series and reconstruct the ontogenetic changes based on these segments (see also below, 4d) Increase in differentiation).
3. Breeding
   If an organism can be bred in captivity, it is indeed possible to directly observe its development. Yet, even in this apparently ideal case it is often necessary to deviate from studying the entire ontogeny of a single individual continuously as certain ontogenetic stages might be missed and most methods for

microscopical inspection require preparation of the specimen and thus to sacrifice it. Hence, the ontogenetic sequence of an organism is *reconstructed* based on a series of individuals of different ages, which is a well-established approach in neontology. Such a strategy can, in principle, be transferred also to fossil organisms, although identifying the conspecifity of all individuals used to reconstruct the sequence may be tricky; relatively complete ontogenetic sequences are needed here.

There are certain exceptions among fossils that allow the identification of conspecifity through aspects of their *breeding biology*. One example includes gravid animals, where the mother is still carrying the developing embryo. Very well known are ichthyosaur females with embryos in their womb (Fig. 1). Further examples of clear conspecifity in fossil organisms are those in which already hatched immatures are retained in a special cavity of the mother. Such brood pouches occur for example in ostracod crustaceans (Siveter et al. 2007).

4. Plausibility

In cases in which neither breeding nor barcoding (i.e., molecular methods to identify which species a specimen belongs to) is successful (or practical), alternative criteria have to be used for identifying conspecifity. In modern forms (e.g., from plankton or meiofauna samples), one is indeed also sometimes forced to apply a chain of reasoning to construct plausible cases of conspecifity; a similar concept can be well applied to fossils (the exact criteria are listed below, a–e). Criteria applicable here are related to the fact that there are certain expectations for ontogenetically differing conspecific forms. Accordingly, it can be partly tested whether several individuals are likely to represent separate stages of a single ontogenetic sequence or are separate species:

(a) *Co-occurrence*: Co-occurrence is an important datum, as two specimens from the same sample have a higher probability to be conspecific than specimens belonging to samples from different locations. This also applies to fossils: two fossil specimens from the same horizon (namely, a layer of rock with a specific composition) in the same locality are more likely to be conspecific than two fossils from different horizons and/or different localities. As a rule of the thumb, the longer the distance in space and time, the less likely the specimens are to be conspecific. But also here one can find exceptions. Like today, plausibly also in former times there were globally distributed species. For such species, it might easily be possible to find two conspecific specimens on different continents. The same is true for time. A properly formulated and applicable concept for species in time is still lacking, hence even a distance of 20 million years between two specimens may not be enough to discard conspecifity. Therefore, absence of co-occurrence in the fossil record is not sufficient to exclude conspecifity of two specimens, while direct co-occurrence may point to it.

(b) *Morphometric aspects:* One can assume in general an increase in size throughout ontogeny, but this is not universal. It does, for example, not necessarily account for embryos developing within an egg. Additionally, there are cases of nonfeeding larvae that slightly decrease in size during

ontogeny. Furthermore, one needs to accept a certain variance of size also in corresponding developmental stages, which might lead to cases in which supposedly further developed individuals are smaller than less developed ones. Still, with a larger sample size of specimens, it should be possible to test whether all presumed conspecific forms cluster around a single trend line in a scatter plot or not. If they do not, conspecifity cannot be excluded but is more difficult to explain. Even though clustering around the same trend line is not a fully conclusive argument for conspecifity, it makes the case more plausible.

(c) *Increase in number of structures:* At least for certain organisms, one can expect an increment of certain structures throughout ontogeny. This includes, for example, an increase in number of plates in echinoderms, ribs and whorls in ammonoid molluscs, body segments in annelid worms and many arthropods, or for the latter also number of appendages (e.g., Sumrall and Wray 2007; De Baets et al. 2012; Haug and Haug 2016a; Fig. 2). Such an increase should be (at least roughly) coupled with a gain in size. If there is no (or only a weak) correlation between difference in number of structures and growth, it is unlikely that the individuals represent the ontogenetic sequence of a single species. Rather, it is more likely that they represent different species or different morphs of a species. Yet, also here certain variation in size is possible, leading to slightly smaller individuals, but with a moderately higher number of structures. If there is a positive correlation between increase in size and increase in number of structures, conspecifity is more likely.

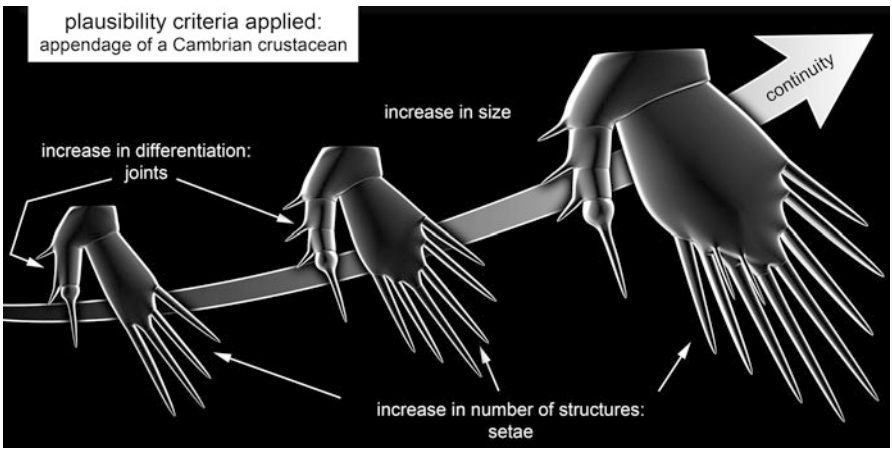


Fig. 2 The plausibility criteria applied on an appendage of a Cambrian crustacean (ca. 500 million years old). During development the appendage increases in size, further differentiates by adding joints, and increases in the number of structures, in this case setae; the entire development proceeds relatively continuously

(d) *Increase in differentiation:* One can also assume an increasing differentiation, i.e., the individual structures become better developed throughout ontogeny. This can be recognized partly by a relative size (e.g., when limb buds become functional limbs) or by an increment in number of substructures (e.g., joints on an arthropod appendage; Fig. 2). Such an increase in differentiation should also show a distinct correspondence to growth. Also here a certain degree of variation is to be expected. There are indeed cases where it is also possible that certain structures degenerate during ontogeny (e.g., the horns in pachycephalosaurid dinosaurs, Horner and Goodwin 2009). Yet, also such a decrease should then show a more or less strict correlation to size increase, though a negative one.

(e) *Continuity:* Considering all correlations discussed above, a certain continuity in development should also be expected, i.e., no repeated change from increase of a structure to decrease of this structure and back to an increase of it. It is thus clear that it is easier to recognize gradual developmental patterns as these provide such continuity. In these cases, the different stages of the studied species will also show more morphological similarities with each other. In forms that develop in a less gradual way, possibly even involving metamorphosis, it will be difficult to link premetamorphic and postmetamorphic forms.

With such considerations in mind, plausible cases can be constructed that indicate possible conspecifity of morphologically differing specimens, identifying those as different life stages. Such approaches can be applied to fossils as well, but remain plausible assumptions. Therefore, a *close comparison to modern forms* for which ontogenetic sequences are already (at least partly) available is necessary (but see below, Practical Work, (1) Primary data acquisition).

One also has to accept certain limitations. In a hypothetical case of a progenetic (small-sized larva-like) species and its "normal" sister species, it would most likely not be easy to distinguish reliably between a juvenile of the "normal" species and the adult of the progenetic one. Most likely both would be recognized as a single species. Notwithstanding, such a case mainly demonstrates the possible resolution (or its limitations) of paleo-evo-devo approaches instead of disproving their usefulness.

It is also important to note that in many cases it will be easier to reconstruct certain phases of the ontogeny than others. First of all, the embryonic phase is rarely accessible, but also here notable exceptions have been found, such as ichthyosaur embryos inside the mother or isolated embryos of cycloneuralian worms (Fig. 1; e.g., Donoghue et al. 2006; Haug and Haug 2015a). Larval phases of different animals can be reconstructed comparably reliably, but it is less easy to link them to possible postmetamorphic/non-larval juvenile or adult forms. Non-larval juvenile development can again be comparably easily reconstructed although also here pronounced morphological changes may occur complicating the process. Also in many groups of extant organisms, juvenile development is less well documented than the embryonic or larval phase (and the adult morphology), and therefore often less comparative data are available.

The latter discussed cases for reconstructing the ontogeny of extinct organisms all demand for a large sample size. Yet, in many cases only very few specimens might be available. As pointed out above, in some groups of organisms a single specimen can be informative for its developmental history. In cases in which only a single immature fossil is available, the reliability of any interpretation must be seen as even less strong, but also in such cases some inferences might be possible. When restricting attention to a distinct well-delineated systematic group also information of parts of the ontogeny of clearly nonconspecific specimens might be important. In the right framework even the finding of a single specialized immature form may be telling.

Fossils in Evolutionary Reconstructions

Even though a reconstructed ontogenetic sequence of a fossil organism remains less reliable than that of (many) modern forms, there is still a benefit in reconstructing it. In former times, fossils have played the sole role for reconstructing the evolutionary history of a systematic group. The advent of Hennigian phylogenetic systematics has provided a framework in which it is possible to reconstruct evolutionary history on the basis of the comparison of extant forms, partly pushing fossils away from the prime position. Still, fossils indeed provide crucial insights that cannot be simply inferred from extant forms:

1. Fossils provide minimum ages
   The advantage that fossils can deliver information which cannot be inferred from extant forms has even been recognized in a modern molecular-dominated biology: fossils act as anchor points in time. This is also true for developmental aspects. Fossils may give minimum ages for the occurrence of specific developmental patterns or specific larval forms (Fig. 3). In many instances, such occurrences seem to be inferred based on the presence of specific adult morphologies. Yet, larval morphology or more general developmental patterns are not necessarily strictly coupled with a specific adult morphology (e.g., Scholtz 2004, 2005). In other words, there might be modern looking adult forms in a certain horizon, not necessarily meaning that they did already have the same developmental pattern as their modern relatives. And vice versa, specific developmental patterns or larvae might have evolved before the modern-type adult morphologies, for example, in brachyuran crabs (Haug et al. 2015a). Only direct fossil evidence will therefore allow a reliable reconstruction of the time when a specific novelty in a developmental pattern first evolved. For such cases also single (isolated) larval forms may be highly informative as recent descriptions of holometabolous insect larvae have shown (e.g., Nel et al. 2013; Haug et al. 2015b).
2. Fossils provide important character polarizations
   When reconstructing evolutionary changes along a phylogenetic tree, the direction of character change is crucial. If two different character states are known in

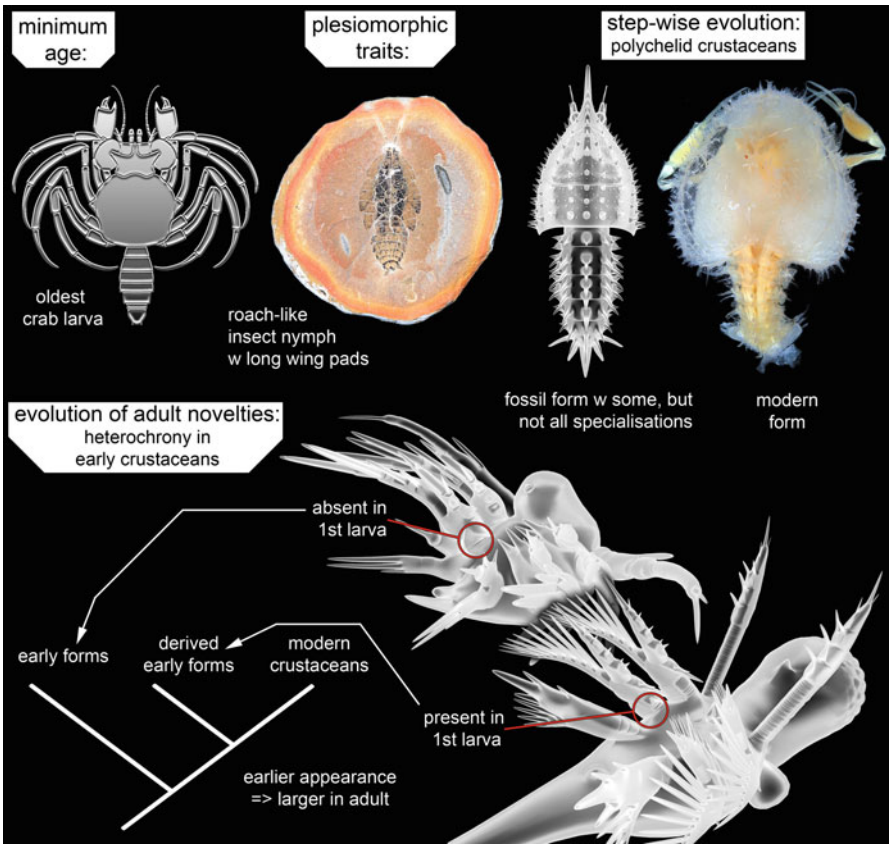


Fig. 3 Fossils in evolutionary reconstructions. *Upper left*: The oldest crab larva from the Solnhofen Lithographic Limestones, Germany (SMNS, ca. 150 million years old), with a relative modern morphology, although adults during that time have a rather ancestral appearance (Haug et al. 2015a). *Upper middle*: 300-million-year-old roach-like insect nymph (ROM, Mazon Creek Formation, USA) with plesiomorphic long wing pads; modern representatives of the group have short wing pads. *Upper right*: Comparison of 90-million-year-old (MNHN, Hadjoula, Lebanon) and extant larva (MNHN) of polychelid crustaceans; the fossil larva shows already some, but not all characters of the modern larva. *Bottom*: Heterochrony in early crustaceans from the Orsten deposits (ca. 500 million years old), resulting in adult novelties; in the first larva in early crustaceans a feeding structure is not yet present on the third appendage (mandible), but it is already present in the first larva of derived early forms; due to the earlier ontogenetic appearance in the derived early forms, the feeding structure is larger in the adult

two sister groups, a comparison to the closest related group of the two, a so-called outgroup comparison, helps to evaluate which of the two states was ancestral (= part of the ground pattern) and which state is derived. Such an evaluation is called character polarization. Often evo-devo scenarios are heavily influenced by model organisms. Yet, model organisms are not necessarily chosen because of their plesiomorphic appearing developmental patterns, but mostly according to

their availability. Hence, especially for polarizing detailed character reconstructions along phylogenetic trees, fossils (but also some modern representatives of the "forgotten branches") may act as important polarization points, providing a clear direction for ordering step-wise character evolution, for example, in early arthropods (e.g., Maas et al. 2006; see also review in Edgecombe 2010).

3. Fossils may display more plesiomorphic traits
   Many fossils indeed show ontogenetic patterns that are also known from modern forms. Such patterns are comparably easy to recognize. Yet, fossils have been heralded for cases in which they possess character combinations that are no longer represented in the modern fauna (e.g., Donoghue et al. 1989). This holds also true for developmental patterns. Fossil species may have developed in a different manner than their modern counterparts (Fig. 3). They might have differed in pattern, i.e., characters appearing in a specific order might have appeared in a different order in the past, or might have developed through quite different larval stages. Such cases are much more difficult to infer, yet they are especially interesting. They again demonstrate that also ontogeny evolves. Identifying such cases is one of the main strengths of the paleo-evo-devo approach.

   In some cases, it could be shown that all modern representatives of a group exhibit derived ontogenetic patterns, but that this pattern is not ancestral for the modern group, but that several lineages have evolved it convergently from a now extinct pattern. Such cases are known, for example, from spiny and slipper lobsters (Haug and Haug 2015b, 2016b) and various lineages of insects (e.g., Shear and Kukalová-Peck 1990; Haug et al. 2016a). Such findings are therefore only possible with the inclusion of fossils.
4. Fossils can break a single "evolutionary jump" into substeps
   Often fossils have been termed "missing links" or "connecting links." Both terms show that there is a general misunderstanding of evolutionary theory and should thus not be used. The fossils often referred to with these inappropriate expressions usually have already evolved some, but not yet all, characteristics of a specific modern group (often these are therefore referred to with the likewise infelicitous term "stem-representative"). As a result, such fossils break down an apparent evolutionary jump, the acquisition of several characters in one step, into at least two substeps. In this way, they provide a clear order in which certain characters evolved (A before B). Such cases can also be found for developmental patterns. Most simple, larval forms that possess only part of the characteristics of their modern counterparts provide an important marker point for determining in which order such larval specializations evolved (Fig. 3).
5. Heterochrony and the emergence of adult "novelties"
   While developmental data are mostly informative about the evolution of developmental patterns on first sight, paleo-evo-devo may also be informative for the evolution of modern morphologies or "novelties" in adults. Heterochrony, the evolutionary shift of developmental timing, plays an important role in this respect (Fig. 3). Numerous examples of fossil animal species whose ontogeny has been reconstructed show a rather gradual developmental pattern compared to their modern counterparts; especially well documented examples come from early

crustaceans and insects (e.g., Shear and Kukalová-Peck 1990; Walossek 1993; Haug et al. 2016a). This thus often demonstrates a step-wise formation of certain structures that appear in modern forms in a single step. Alternatively, some adult characters in derived forms might be identified as former larval characters that become shifted into the adult phase. For instance, species of the Cambrian arthropod group *Naraoia* lack dorsal joints of the trunk segments, a feature originally present in larval stages of the closely related trilobites (Fortey and Theron 1994; Haug et al. 2010b). With such examples, also quite aberrant appearing morphologies can be explained by small steps, for example, the evolution of the shovel-shaped antennae in slipper lobsters from elongate feeler-type antennae (Haug et al. 2016b). This again emphasizes the strength and necessity for a paleo-evo-devo approach.

Practical Work

In the following, practical aspects of performing paleo-evo-devo studies are presented. In the four steps of the workflow, the primary data are acquired, developmental sequences are reconstructed, followed by a proximate and an ultimate interpretation.

1. Primary data acquisition
   Actual work in paleo-evo-devo involves direct work on fossils. As indicated above in the section "Morphometric Aspects," larger sample sizes, larger sample sizes are of advantage. Yet, when exceptionally preserved fossils are studied, only some few or even a single specimen may be quite informative. For extracting the maximum morphological information from each fossil specimen different up-to-date methods can be applied, for example, computed tomography, virtual surface reconstruction, or contrast enhancing methods in macro- and microphotography (e.g., Haug et al. 2012; Sutton et al. 2014). Maximum information should include also details, which are not necessarily studied for pure taxonomic or stratigraphic reasons.

   For comparison, the study of modern forms is necessary. Generally speaking, it is more or less impossible to exhaustively investigate the morphology of modern organisms. Therefore, studies are often restricted to specific aspects of their morphology, usually those that are considered taxonomically important. This is also true for the study of developmental sequences. Unfortunately, these characters are not necessarily the characters that are available in the fossil specimens. This makes in many cases necessary to study the modern species, specifically for the characters available in the fossils. Extensive data acquisition is therefore a crucial step for a paleo-evo-devo approach.
2. Reconstruction of developmental sequences
   Based on the acquired primary data, ontogenetic sequences of the extinct species can be reconstructed. For each of the ontogenetic stages, the morphology of all structures needs to be reconstructed, which makes changes of these structures during ontogeny visible. In general, one expects an increase in size as well as in

the number of substructures, such as joints, setae, or spines. However, not all structures usually grow at the same speed in size or number, which results in a mosaic-like development.

3. Proximate interpretation
   Based on the reconstructed morphologies, "local" or proximate interpretations are possible. Among them rough systematic identifications can be provided. Comparison to such forms may then alter some of the morphological or developmental interpretations. Hence, such steps should be followed in a reciprocal manner. Also functional and, based on these, ecological interpretations should be attempted to better understand the entire biology of the studied organism.
4. Ultimate interpretation
   Within a phylogenetic framework, it is then possible to make ultimate (evolutionary) interpretations. These may include aspects of time of appearance and/or detailed reconstruction of character transformation. Also based on ecological interpretations, findings may contribute to further-reaching interpretations of faunal and ecology evolution.

Cross-References

- ▶ Evo-Devo and Phylogenetics
- ▶ Form and Function in Evo-Devo
- ▶ Gavin de Beer (1899–1972)
- ▶ Heterochrony
- ▶ Interdisciplinarity in Evo-Devo
- ▶ Stephen Jay Gould (1941–2002)

Acknowledgments We would like to thank curators and collection managers from different museums providing specimens (see figure captions). Furthermore, we are grateful to Roger Frattigiani, Laichingen, for providing the crab megalopa from Solnhofen limestones. JTH was supported by the German Research Foundation (DFG HA 6300/3-1); CH was supported by the LMU through a Bavarian Equal Opportunities Sponsorship (BGF). Both authors would like to thank J.M. Starck, Munich, for his support.

References

De Baets K, Klug C, Korn D, Landman NH (2012) Early evolutionary trends in ammonoid embryonic development. Evolution 66:1788–1806

Donoghue MJ, Doyle JA, Gauthier J, Kluge AG, Rowe T (1989) The importance of fossils in phylogeny reconstruction. Ann Rev Ecology Syst 20:431–460

Donoghue PCJ, Bengston S, Dong XP, Gostling NJ, Huldtgren T, Cunningham JA, Yin C, Yue Z, Oeng F, Stampanoni M (2006) Synchroton X-ray tomographic microscopy of fossil embryos. Nature 442:680–683

Edgecombe GD (2010) Palaeomorphology: fossils and the inference of cladistic relationships. Acta Zool 91:72–80

Fortey RA, Theron JN (1994) A new Ordovician arthropod, *Soomaspis*, and the agnostid problem. Palaeontology 37:841–861
Hall BK (2002) Palaeontology and evolutionary developmental biology: a science of the nineteenth and twenty-first centuries. Palaeontology 45:647–669
Haug JT, Haug C (2015a) Worm paleo-evo-devo – the ontogeny of *Ottoia prolifica* from the Burgess Shale. Res Rev J Zool Sci 3(1):3–14
Haug JT, Haug C (2015b) "Crustacea": comparative aspects of larval development. In: Wanninger A (ed) Evolutionary developmental biology of invertebrates 4: Ecdysozoa II: Crustacea. Springer, Wien, pp 1–37
Haug C, Haug JT (2016a) Developmental paleontology and paleo-evo-devo. In: Kliman RM (ed) Encyclopedia of evolutionary biology, vol 1. Academic, Oxford, pp 420–429
Haug JT, Haug C (2016b) "Intermetamorphic" developmental stages in 150 million-year-old achelatan lobsters – the case of the species *tenera* Oppel, 1862. Arthropod Struct Dev 45:108–121
Haug JT, Waloszek D, Haug C, Maas A (2010a) High-level phylogenetic analysis using developmental sequences: the Cambrian †*Martinssonia elongata*, †*Musacaris gerdgeyeri* gen. et sp. nov. and their position in early crustacean evolution. Arthropod Struct Dev 39:154–173
Haug JT, Maas A, Waloszek D (2010b) †*Henningsmoenicaris scutula*, †*Sandtorpia vestrogothiensis* gen. et sp. nov. and heterochronic events in early crustacean evolution. Earth Environ Sci Trans R Soc Edinb 100:311–350
Haug C, Van Roy P, Leipner A, Funch P, Rudkin DM, Schöllmann L, Haug JT (2012) A holomorph approach to xiphosuran evolution: a case study on the ontogeny of *Euproops*. Dev Genes Evol 222:253–268
Haug JT, Martin JW, Haug C (2015a) A 150-million-year-old crab larva and its implications for the early rise of brachyuran crabs. Nature Comm 6:art.6417
Haug JT, Labandeira CC, Santiago-Blay JA, Haug C, Brown S (2015b) Life habits, hox genes, and affinities of a 311 million-year-old holometabolan larva. BMC Evol Biol 15:art.208
Haug JT, Haug C, Garwood R (2016a) Evolution of insect wings and development – new details from Palaeozoic nymphs. Biol Rev 91:53–69
Haug JT, Audo D, Charbonnier S, Palero F, Petit G, Abi Saad P, Haug C (2016b) The evolution of a key character, or how to evolve a slipper lobster. Arthropod Struct Dev 45:97–107
Horner JR, Goodwin MB (2009) Extreme cranial ontogeny in the Upper Cretaceous dinosaur *Pachycephalosaurus*. PLoS One 4(10):e7626
Maas A, Braun A, Dong X, Donoghue PCJ, Müller KJ, Olempska E, Repetski JE, Siveter DJ, Stein M, Waloszek D (2006) The "Orsten" – more than a Cambrian Konservat-Lagerstätte yielding exceptional preservation. Palaeoworld 15:266–282
Minelli A, Fusco G (2008) Evolving pathways. Key themes in evolutionary developmental biology. Cambridge University Press, Cambridge
Nel A, Roques P, Nel P, Prokin AA, Bourgoin T, Prokop J, Szwedo J, Azar D, Desutter-Granscolas-L, Wappler T, Garrouste R, Coty D, Huang D, Engel MS, Kirejtshuk AG (2013) The earliest known holometabolous insects. Nature 503:257–261
Nützel A (2014) Larval ecology and morphology in fossil gastropods. Palaeontology 57:479–503
Sánchez-Villagra MR (2012) Embryos in deep time. University of California Press, Berkeley
Scholtz G (2004) Baupläne versus ground patterns, phyla versus monophyla: aspects of patterns and processes in evolutionary developmental biology. In: Scholtz G (ed) Evolutionary developmental biology of Crustacea. AA Balkema, Lisse, pp 3–16
Scholtz G (2005) Homology and ontogeny: pattern and process in comparative developmental biology. Theory Biosc 124:121–143
Shear WA, Kukalová-Peck J (1990) The ecology of Paleozoic terrestrial arthropods: the fossil evidence. Can J Zool 68:1807–1834
Siveter DJ, Siveter DJ, Sutton MD, DEG B (2007) Brood care in a Silurian ostracod. Proc R Soc B Biol Sci 274(1609):465–469

Sumrall CD (2008) The origin of Lovén's law in glyptocystitoid rhombiferans and its bearing on the plate homology and the heterochronic evolution of the hemicosmitid peristomal border. In: Ausich WI, Webster GD (eds) Echinoderm paleobiology. University of Indiana Press, Bloomington, pp 228–241

Sumrall CD, Wray GA (2007) Ontogeny in the fossil record: diversification of body plans and the evolution of "aberrant" symmetry in Paleozoic echinoderms. Paleobiology 33:149–163

Sutton M, Rahman I, Garwood R (2014) Techniques for virtual palaeontology. Wiley-Blackwell, Chichester

Urdy S, Wilson LAB, Haug JT, Sánchez-Villagra MR (2013) On the unique perspective of paleontology in the study of developmental evolution and biases. Biol Theory 8:293–311

Walossek D (1993) The Upper Cambrian *Rehbachiella* and the phylogeny of Branchiopoda and Crustacea. Lethaia 26:1–318

Wilson LAB (2011) The contribution of developmental palaeontology to extensions of evolutionary theory. Acta Zool 94:254–260

Zhang X, Siveter DJ, Waloszek D, Maas A (2007) An epipodite-bearing crown-group crustacean from the lower Cambrian. Nature 449:595–598

Eco-Evo-Devo

Sonia E. Sultan

Contents

Abstract

Because environmental conditions influence gene expression as well as metabolic processes, an organism's development is not scripted in its DNA but takes shape through the interaction of genotype and environment. This expanded understanding calls for several significant lines of investigation. *Ecological developmental biology* ("eco-devo") extends the study of developmental mechanisms and their

S. E. Sultan (✉)
Biology Department, Wesleyan University, Middletown, CT, USA
e-mail: sesultan@wesleyan.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_42

phenotypic outcomes to include environmental context dependency. The starting point for an ecological development approach is to characterize the environmental response patterns or *norms of reaction* of genotypes in taxa of interest to ecological factors relevant to their natural settings. Another major line of eco-devo research is to determine precisely how developmental pathways incorporate environmental inputs so as to generate these context-dependent phenotypes. Answers to this question of mechanism include a fascinating array of regulatory systems, from well-studied hormonal transduction pathways to environmentally induced molecular epigenetic changes. More fundamentally, an eco-devo approach points to two further research questions of broad resonance for evolutionary biology: How do such environmentally responsive developmental systems evolve? And how does this developmental flexibility itself affect the processes of adaptive evolution and diversification? This chapter provides a brief overview of the central issues that comprise this "eco-evo-devo" territory, ending with a section on practical considerations for the design of empirical studies.

Keywords

Phenotypic Plasticity · Norm of Reaction · Epigenetics · Transgenerational Plasticity · Niche Construction

Introduction

Because environmental conditions influence gene expression as well as metabolic processes, an organism's development is not scripted in its DNA but takes shape through the interaction of genotype and environment. This expanded understanding calls for several significant lines of investigation. *Ecological developmental biology* ("eco-devo") extends the study of developmental mechanisms and their phenotypic outcomes to include environmental context dependency (Gilbert 2001; Sultan 2007). The starting point for an ecological development approach is to characterize the environmental response patterns or *norms of reaction* of genotypes in taxa of interest to ecological factors relevant to their natural settings. Another major line of eco-devo research is to determine precisely how developmental pathways incorporate environmental inputs so as to generate these context-dependent phenotypes. Answers to this question of mechanism include a fascinating array of regulatory systems, from well-studied hormonal transduction pathways to environmentally induced molecular epigenetic changes.

More fundamentally, an eco-devo approach points to two further research questions of broad resonance for evolutionary biology: How do such environmentally responsive developmental systems evolve? And how does this developmental flexibility itself affect the processes of adaptive evolution and diversification? This chapter provides a brief overview of the central issues that comprise this "eco-evo-devo" territory, ending with a section on practical considerations for the design of empirical studies. For further discussion and references, see Stearns 1989; Lewontin 2000; Abouheif et al. 2014; Gilbert and Epel 2015; Laland et al. 2015; Sultan 2015.

Ecological Development as a Source of Phenotypic Variation

The environmental context dependence of development plays a critical evolutionary role by shaping the phenotypic traits and resulting fitness differences that lead to natural selection. Both inevitable and adaptive effects of environmental variation on phenotypic expression can lead to environmentally variable or *plastic* norms of reaction.

Inevitable Effects of Environmental Conditions

The elegant mathematical apparatus of twentieth-century population genetics assigned particular fitness levels to alternative genotypes to predict selective trajectories. In real populations, however, the absolute and relative fitness of genetic individuals are strongly influenced by levels of environmental factors that directly affect growth and function, and hence reproductive output (Kingsolver et al. 2012). Individual biomass and offspring number may be highly correlated with the availability of patchily distributed resources such as food or light, or with temperature, pH, and other abiotic variables. These direct effects of external conditions on individual phenotypic outcomes, whether positive or negative, may be understood as inevitable consequences of variation in environmental quality.

Adaptive Plasticity

Phenotypic differences expressed in alternative environmental conditions may also include beneficial developmental adjustments or *adaptive plasticity* expressed by individual organisms in response to specific environmental cues (Stearns 1989; Dewitt and Scheiner 2004). In certain cases, adaptive plasticity can lead to the production of distinct alternative phenotypes rather than continuous variation; cases of such threshold-inflected norms of reaction are often termed *polyphenisms.* The specificity of these characteristic developmental cue-and-response systems, together with their positive contributions to function and fitness, indicate that they have been shaped by natural selection (next section). Adaptive plastic adjustments include a fascinating array of animal and plant systems. Examples include changes in the feeding structures of fish, reptiles, and insects in response to differences in food type and availability, faster metamorphic transitions in toads that sense as juveniles that their ponds are drying up, enlarged alveolar surface area in lungs of oxygen-limited mammals, flexibility in the size and structure of plant leaves in response to light intensity, the production of customized deterrents by plants attacked by particular insect herbivores, and defensive shell morphologies produced by aquatic invertebrates threatened by predators (references in Gilbert and Epel 2015; Sultan 2015).

In adaptive plasticity, a particular (abiotic or biotic) aspect of the environment acts as a cue that is transduced via a variety of internal chemical and physical signals to produce a specific phenotypic response (Knight and Knight 2001; Lema and

Kitano 2013). In both animal and plant systems, signaling factors can include hormones, mineral ions such as calcium, sugars and other metabolites, peptides and nucleic acids, pigments and other receptor molecules, and osmotic, electrical, and mechanical effects. The organism's physiological state can also create internal feedbacks that act as indirect environmental cues. These complex regulatory pathways shape the expression of gene products such as transcription factors and microRNAs, which in turn mediate downstream gene expression patterns and interactions. Specific environmental states can also induce molecular epigenetic changes that directly alter gene expression, such as the addition of chemical methyl groups to specific DNA loci or chemical modifications to histone proteins (Bateson and Gluckman 2011).

Transgenerational Environmental Effects

When environmentally induced epigenetic factors persist through the process of meiosis, they can be transmitted via maternal and/or paternal gametes to shape the development of one to many progeny generations (Jablonka and Raz 2009; Soubry et al. 2014; Blake and Watson 2016). Effects of maternal environment on the subsequent generation may also include induced changes in the amount and composition of the carbohydrate, mineral, lipid, and protein "provisions" packed into eggs or seeds, and of hormones and other regulatory molecules transferred into the embryo via maternal cytoplasm (West-Eberhard 2003). Through these (often interacting) mechanisms, eco-devo effects can be inherited as well as immediate, resulting in a complex interplay of genotype with both present and previous environments. Again, such effects can be interpreted as either inevitable or adaptive (for examples of adaptive transgenerational plasticity, see Gilbert and Epel 2015; Sultan 2015).

The Internal Environment: Microbial Symbionts

Plant and animal tissues and body cavities house characteristic communities of microorganisms (such as bacteria, archaea, fungi, and protozoa) that are either transmitted to individuals by their mothers (in the egg or via skin contact) or taken up directly from their surroundings. These symbiotic microbial assemblages or *microbiomes* comprise internal biotic environments that, like external conditions, play critical roles in mediating development, function, and behavior (Gilbert and Epel 2015). In mammals, for example, the normal development of nutrient-absorbing blood vessels in the gastrointestinal tract is completed only after specific chemical signals from bacteria inhabiting the animal's gut are received by the epithelial cells that line the small intestine (references in Sultan 2015). Microbial eco-devo signaling has also been implicated in the regulation of mammalian brain function.

Ecologically important microbial symbioses include coral polyps and the photosynthetic dinoflagellates that live in their endodermal cells, nitrogen-fixing bacteria that colonize and morphogenetically shape the roots of legume plants (resulting in

the high protein content of beans), and the metabolic partnership between grazing animals such as cows and the gut bacteria needed to digest grass and other cellulose-rich plant foods. These biological systems exemplify some of the diverse ways that internal environmental elements participate in individual development and function. The term *holobiont* is sometimes used to refer to the functional or developmental unit consisting of an individual organism together with its microbial symbionts (see discussion and references in Gilbert and Epel 2015). Alternatively, the host-microbiome relationship can be viewed as a mode of environmental mediation by the organisms involved (see below, "Eco-Devo Responses as Niche Construction").

Evolution of Eco-Devo Responses

As products of evolution, norms of reaction necessarily reflect phylogenetic, genetic, and biochemical constraints along with past selection, resulting in differences at the level of genotypes, populations, and taxa. These systems of environmentally modulated phenotypic response are thus products of the evolutionary process, as well as causal influences on that process.

Genetic Diversity for Environmental Responses

Norms of reaction vary among genotypes, due to genetic differences in the sensory, regulatory, and developmental elements that interact with environmental factors. These underlying genetic differences result in generally non-parallel patterns of response for any given trait across a set of specified environments (Barton and Turelli 1989). Because norms are neither identical nor parallel, the magnitude and/or rank order of phenotypic differences among a given set of genotypes varies from one environment to another, a pattern of variation known in statistical terms as *genotype by environment* or *GxE variance*. Eco-devo studies consistently show that such genetic diversity for environmental response patterns is both substantial and ubiquitous in naturally evolved systems (Barton and Turelli 1989; Scheiner 1993; Des Marais et al. 2013), with important consequences for adaptive evolution (see "Evolutionary Impact of Eco-Devo Responses" section below).

In contrast to a simplified model that posits the selective accumulation of alleles with favorable deterministic effects, the evolution of eco-devo systems has produced extraordinarily complex and flexible – yet robust – regulatory pathways (Bateson and Gluckman 2011). These response pathways offer numerous potential targets for evolutionary change, including the various genetic elements that affect cue reception and expression of responses by influencing the sensitivity threshold of receptors, the concentration and composition of hormones and other signaling molecules, and the downstream effects of these signals (Moczek et al. 2011; Abouheif et al. 2014). Depending on the trait and environmental states in question (and allowing for inevitable effects of environmental stresses or resource limits as discussed above), these pathways can result in norms of reaction characterized by similar phenotypes

across environments (*canalized development*) or by phenotypes that differ in specific functional traits either continuously (*adaptive plasticity*) or discretely (*polyphenism*). These alternative norms of reaction reflect the selective accumulation of genomic elements that modulate ancestral responses to environmental variation in one of two ways: to minimize disruptive effects of particular environmental states on phenotypes (leading to canalization) or to shape those effects to produce differently adaptive outcomes (Nijhout 2003). The selective refinement of environmental effects on development is termed *genetic accommodation* (discussion and references in West-Eberhard 2003; Gilbert and Epel 2015).

Evolution of Adaptive Plasticity

A substantial theoretical literature has examined the adaptive evolution of relatively plastic versus constant norms of reaction for traits that affect fitness. A consensus has emerged that adaptive plasticity is favored by fine-scale spatial and temporal heterogeneity in the environment, provided that the organism is able to accurately perceive and transduce environmental cues so as to produce phenotypes that match differing conditions (e.g., Sultan and Spencer 2002; Baythavong 2011; Scheiner and Holt 2012). Although the evolution of adaptively plastic developmental systems was initially hypothesized to be constrained by unique "costs" due to presumed added regulatory complexity, despite considerable research effort such costs have not been found (Scheiner and Holt 2012). In view of current insights to the complexity of genomic regulatory systems in general, the absence of inherent "costs" underscores the fact that plasticity is not a special case of development. Rather, whether outcomes are relatively constant across environments or highly plastic, development as a rule is regulated via complex interactions of environmental and genomic factors (Gilbert 2001; Sultan 2015; and references therein).

The accurate phenotypic responses that are necessary for plasticity to be selectively favored depend not only on the organism's developmental cue-and-response system but also on the availability of reliable and timely environmental cues. Such cues range from chemical and physical properties of air, soil, and water (e.g., temperature, photon flux, pH, relative humidity, and concentration of carbon dioxide, oxygen, mineral ions, and other chemical resources) to biotic elements such as predators and herbivores, pathogens, parasites, competitors, mutualist partners, and symbionts. These cues can be surprisingly subtle. For instance, in a number of insect and vertebrate systems, the individual's social environment provides critical developmental signals. Because eco-devo effects can extend across generations via maternal and epigenetic inheritance, the reliability of both immediate and parental cues in predicting selective conditions will influence the selective evolution as well as the expression of adaptive phenotypic responses within a given generation (Leimar and McNamara 2015). In view of the potential negative impact on the ability of organisms to express adaptive phenotypes in future environments, disruption of evolved cues is a particular concern as human activities radically alter myriad aspects of natural habitats and communities (Sultan 2007).

Evolutionary Impact of Eco-Devo Responses

A Norm of Reaction View of Fitness Variation

A central eco-devo insight is that the values of individual fitness traits, such as life-history timing and reproductive output, are strongly influenced by environmental conditions, rather than inherent to the genotype as simplified population genetics models imply. These influences on fitness include both inevitable effects of environmental stresses and adaptive plastic responses that can partially offset those stresses. As noted above, both types of environmental influence cause characteristic, genotype-specific effects on phenotypes. Consequently, since abiotic and biotic factors are highly variable in natural habitats, patterns of fitness variation in real populations reflect not only the set of genotypes present but "complex associations among genotypes, environments and phenotypes" (Kingsolver et al. 2012, p. 1116).

A fundamental departure from conventional views of fitness variation is that the variation on which selection acts is generated by eco-devo systems and not by random genetic mutations (West-Eberhard 2003; Laland et al. 2015). Because phenotypes with different levels of fitness are consistently associated with certain environmental states (rather than randomly distributed across them, as is assumed in gene-based models that treat environmental effects as noise), selective outcomes will depend on the distribution of environments, even for fitness-related traits that show heritable genetic variation (Kingsolver et al. 2012). As a result of transgenerational and longer-term epigenetic effects of environments on phenotypes, fitness variation may also reflect the distribution of previous environmental states, leading to higher-order interactions of genotype and environment on realized fitnesses and hence selective outcomes. Given that environmental heterogeneity and the expression of genetic variation are non-independent, a norm of reaction approach allows for a more nuanced exploration of selective outcomes than one that considers genotypes as fixed fitness variants.

Selective Consequences of GxE Variation within Populations

Decades of eco-devo studies (including many of crop plants and domesticated animals) show that genotypes generally express non-parallel norms of reaction for the developmental, functional, and life-history traits that influence fitness, resulting in changes in the size and/or rank order of genotypic differences among environmental states (see Genetic Diversity for Environmental Responses). The selective impact of this *GxE* variation in populations will depend on (a) the precise pattern of variation among norms of reaction, (b) the distribution and frequency of alternative environmental states, and (c) the intensity of selection in the alternative environments.

In cases where norms of reaction cross for fitness-related traits, the relative fitnesses of the genotypes shift in rank order from one environmental microsite or temporal state to another – in other words, a given genotype will have higher fitness than another genotype in one environment but lower fitness than the other in a different environment. If both environments occur within a population (i.e., at a

spatially or temporally fine scale of variability), then neither genotype will be consistently favored by selection and both genotypes will be maintained. Crossing patterns of *GxE* variation combined with environmental heterogeneity can thus act to buffer selection and maintain genetic variation in natural systems (references in Scheiner 1993; Sultan 2015).

Even without crossing, non-parallel norms of reaction for fitness traits can constrain selective response, since fitness differences may be expressed only in certain environments. When those environments are relatively rare or infrequent, such conditionally expressed genetic variation will undergo "relaxed" selection compared to variants that are exposed to selection in every individual in a population (Van Dyken and Wade 2010). Genetic variation that is thus largely hidden from selection or "cryptic" can accumulate in populations, to be released as a potential fuel for selective change if a formerly rare environment becomes common or if the population colonizes a novel site (Paaby and Rockman 2014). If this rare or novel environment is one in which expressed trait differences cause a particularly large selective advantage, the effect will be to accelerate selective change (Kingsolver et al. 2012). Conversely, genotypic norms of reaction may instead converge in a newly stressful environment, reducing the potential for selective response even if selection would otherwise be strong in this environment (Sultan 2007, 2015).

Effects of Adaptive Eco-Devo Responses on Evolutionary Diversification

The functionally appropriate plastic responses expressed by individual organisms can affect the process of adaptive diversification in several key ways. First, unlike the random occurrence of mutations, environmentally induced phenotypes (including inherited epigenetic effects of parental conditions) can at once provide adaptive adjustment in many or even all individuals of a population to meet environmental demands. Such adaptive eco-devo responses may allow a population to track changing conditions rapidly, without either the lag time or the loss of genetic variation imposed by selection (with resulting limits to subsequent evolutionary potential). Note further that adaptive phenotypes that result from favorable new (and hence rare) mutations are frequently lost due to random drift before they can contribute to such selective change. If the plasticity expressed by existing genotypes in a population provides sufficient adaptive diversity, this plasticity may slow or prevent selective change both among spatially distinct microsites and in response to changes in environmental conditions over time.

Because it decouples adaptation from the time-limited process of selective change, individual plasticity is of particular interest as a possible source of adaptive "rescue" in the face of global climate change and other rapidly emerging environmental challenges that require organisms to adjust development, behavior, and life-history timing (Gienapp et al. 2008). Plasticity may be particularly critical for organisms with limited rates of selective allele-frequency change, due to multi-year life cycles or low genetic variance in novel conditions. Whether plasticity will allow organisms to persist in these often stressful new environments will depend on whether existing

cue-and-response systems can produce adaptive phenotypes in future habitats, with their disrupted environmental cues and altered developmental conditions.

Paradoxically, adaptive plasticity may either oppose or promote evolutionary diversification at larger spatial scales. If plastic norms of reaction provide for sufficiently diverse phenotypes, populations may be able to establish in different habitats without undergoing selective divergence into local ecotypes or, eventually, adaptively distinct taxa. In such cases, the existence of adaptive plasticity may both maintain genetic diversity locally and inhibit among-population adaptive diversification, as seen in certain geographically widespread colonizing species. In highly plastic systems, genetic divergence among populations and species may result largely from population structure rather than adaptive differentiation.

In other cases, plasticity may play the very different role of facilitating adaptive diversification (West-Eberhard 2003; Moczek et al. 2011). When individuals in a population encounter a novel environment, their existing developmental systems may produce an altered, previously unknown phenotype in response. Phenotypic innovations that originate as eco-devo responses may initiate new adaptive possibilities that are then further refined selectively via genetic accommodation of cue-and-response pathways (Nijhout 2003; West-Eberhard 2003). A well-studied example is head and jaw plasticity in stickleback fish in response to different types of diet. This type of functional plasticity appears to have provided the initial basis for repeated, parallel evolutionary divergence between limnetic and benthic stickleback ecotypes (M. Wund and colleagues, discussed in Abouheif et al. 2014). Starting with Conrad Waddington's work in the mid-twentieth century, evolutionists have theorized that initially plastic responses that are favorable may be modified by selection to become constitutive (a subset of genetic accommodation known as *genetic assimilation*). However, few empirical examples exist of this evolutionary "assimilation" process, and it remains controversial (reviewed by Ehrenreich and Pfennig 2016). With regard to the role of eco-devo responses as sources of evolutionary novelty, the question of genetic assimilation may have received undue emphasis: the key point is not whether or not these novel phenotypes become constitutive but the fact that they result from an environmental change rather than a genetic mutation. Recognizing this source of new traits for adaptive diversification shifts the evolutionary focus from a simplified gene-based model of variation to the organism's environmentally responsive developmental system.

Eco-Devo Responses as Niche Construction

An exciting new focus in evolutionary biology is *niche construction*: the active role that organisms play in "constructing" both the environmental conditions of their existence and their realized experience of those conditions (Odling-Smee et al. 2003; Laland et al. 2015; Sultan 2015; and references therein). Because these niche-constructing roles result from the realized phenotypes that organisms express (including individual eco-devo responses to specific conditions), an eco-evo-devo perspective encompasses this aspect of selective causation.

Organisms themselves shape their external environments in two major ways. First, by means of relocation, dispersal, and directional growth and proliferation, individual animals, plants, and other organisms "choose" the spatial and temporal conditions in which their life cycles take place. Second, the resource-collecting and social behaviors of organisms in their natural habitats, as well as their presence and metabolic processes, directly and inadvertently alter the abiotic and biotic components of those habitats in a multitude of ways. Through these niche-constructing activities, organisms themselves help to shape the environmental circumstances that in turn feed back as selective pressures to alter the organisms' subsequent evolution and that of cohabiting species. For instance, the retention of dead lower branches by certain species of pine tree, combined with the characteristic architecture and volatile chemical constituents of pines more generally, comprises a suite of flammability traits that lead to more frequent and intense wildfires in habitats where these species predominate. This niche-constructing effect on fire regimen appears to have caused a selective feedback on these species in the *Pinus* clade, as dead branch retention is phylogenetically associated with fire-adaptive traits such as fire-dependent release of seeds from cones (Schwilk and colleagues, discussed in Sultan 2015, Chap. 2).

Apart from niche-constructing effects on environmental conditions such as wildfire regimes, the phenotype expressed by an individual organism in a given environment will shape the individual's encounter with that environment. For instance, when plants in light-limited conditions produce enlarged leaf surfaces, this increases the number of photons the plants can intercept for photosynthesis although it does not change the light environment per se. Even without altering the environment as such, then, eco-devo responses can affect the degree to which an environment is *experienced* by individuals as favorable or stressful, and hence its selective impact on the population. Both inevitable and adaptive eco-devo responses of individual organisms to their environments can thus be considered as an experiential aspect of niche construction, with the potential to create an evolutionary feedback (discussion and examples in Sultan 2015, Chap. 4).

Niche construction as an ecological and eco-devo phenomenon is indisputable: organismic effects on external (and internal) environments are ubiquitous, and realized phenotypes will necessarily influence how environments are experienced. The critical question is whether these effects alter selective pressures strongly enough to play an important role in the evolutionary process. Although there has been a good deal of controversy about this question, a definitive answer awaits further investigation. As yet, relatively few empirical studies have directly tested the evolutionary impact of niche construction via either external effects or eco-devo-mediated experiential effects.

Eco-Evo-Devo Research Challenges

Evolutionary biologists are expanding their investigations to fully encompass the fact that phenotypes are influenced by environmental conditions, and often dramatically so. This environmental context-dependency includes both inevitable and adaptive

dimensions, raising several key "eco-evo-devo" questions. How much genetic diversity is revealed to selection versus cryptic in natural populations, when genotypes are evaluated across relevant environments? Can individual plasticity produce phenotypic alternatives that obviate diversifying local selection or that provide adaptive rescue in the face of rapidly changing environments? Are broadly adaptive norms of reaction limited to certain well-studied types of organism, such as annual plants and bony fish, or are they widespread among life-histories and phylogenetic groups? Do niche-constructing effects of realized phenotypes substantially alter selection pressures on the organisms that express those phenotypes? The evolutionary importance of eco-devo responses – their role in maintaining or exposing genetic variation, buffering locally varying selection pressures, promoting adaptive divergence, or generating selective feedbacks – depends largely on the extent to which genotypic norms of reaction in various taxa (a) differ and (b) provide adaptively effective phenotypic diversity, in actual environments both now and in the future.

Addressing these fundamental issues calls for norm of reaction experiments designed around real-world systems: empirical studies that replace the single "control" environment of traditional genetics and development research with realistic levels of ecologically meaningful environmental variables, for genotypic samples drawn from natural populations (Gilbert 2001; Scheiner and Holt 2012). The critical step in experimental design is to identify environmental factors that influence phenotypic expression, and to recreate specific treatment levels of those factors that reflect their variability within and among field habitats. Combinatorial treatments can be even more ecologically meaningful than those that vary single environmental parameters. Such experimental design choices demand greater attention to field environments on the part of evolutionary biologists and a commitment to less arbitrary experimental treatments than in many previous norm of reaction studies. Tests of possible selective feedback from niche-constructing traits require that researchers measure environmental conditions as mediated by the organisms themselves, perhaps in conjunction with environmental parameters as they exist independently.

The recognition that phenotypic expression and consequently genetic variance are environmentally dependent calls for this renewed emphasis on ecological context, even for research programs that are carried out in highly controlled laboratory or growth chamber settings. Even more challenging, the potential for inherited environmental effects on phenotypic expression points to the need for trans-generational experiments to test for the possible roles of alternative parental or ancestral conditions on phenotypes and their realized variance. Eco-devo experiments are also challenging simply by virtue of their expanded size: characterizing norms of reaction requires replicating each genotype (through cloning or inbreeding) and raising replicate individuals in each of the various environments. When the timing of response is itself of interest as an adaptive trait that may differ among genotypes, repeated measures of each individual, or varying treatment durations or switching periods, may also be required, further expanding experimental demands. Although an eco-evo-devo research program is without doubt a challenging one, this integrated, ecologically informed approach can lead to a richer knowledge of development, variation, and evolution.

Cross-References

- ▶ Coevolution and Macroevolution
- ▶ Conrad Hal Waddington (1905–1975)
- ▶ Developmental Evolutionary Biology (Devo-Evo)
- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Developmental Plasticity and Evolution
- ▶ Evo-Devo and Niche Construction
- ▶ Ivan I. Schmalhausen (1884–1963)

References

Abouheif E, Favé MJ, Ibarrarán-Viniegra AS, Lesoway MP, Rafiqi AM, Rajakumar R (2014) Eco-evo-devo: the time has come. In: Landry CR, Aubin-Horth N (eds) *Ecological genomics: ecology and the evolution of genes and genomes*. Advances in experimental medicine & biology, vol 781. Springer, Netherlands, pp 107–125

Barton NH, Turelli M (1989) Evolutionary quantitative genetics: how little do we know? Annu Rev Genet 23:337–370

Bateson P, Gluckman P (2011) *Plasticity, robustness, development and evolution*. Cambridge University Press, New York

Baythavong BS (2011) Linking the spatial scale of environmental variation and the evolution of phenotypic plasticity: selection favors adaptive plasticity in fine-grained environments. Am Nat 178:75–87

Blake GE, Watson ED (2016) Unravelling the complex mechanisms of transgenerational epigenetic inheritance. Curr Opin Chem Biol 33:101–107

Des Marais DL, Hernandez KM, Juenger TE (2013) Genotype-by-environment interaction and plasticity: exploring genomic responses of plants to the abiotic environment. Annu Rev Ecol Evol Syst 44:5–29

DeWitt TJ, Scheiner SM (eds) (2004) *Phenotypic plasticity: functional and conceptual approaches*. Oxford University Press, Oxford

Ehrenreich IM, Pfennig DW (2016) Genetic assimilation: a review of its potential proximate causes and evolutionary consequences. Ann Bot 117:769–779

Gienapp P, Teplitsky C, Alho JS, Mills JA, Merilä J (2008) Climate change and evolution: disentangling environmental and genetic responses. Mol Ecol 17:167–178

Gilbert SF (2001) Ecological developmental biology: developmental biology meets the real world. Dev Biol 233:1–12

Gilbert SF, Epel D (2015) *Ecological developmental biology: integrating epigenetics, medicine, and evolution*, 2nd edn. Sinauer Associates, Sunderland

Jablonka E, Raz G (2009) Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. Q Rev Biol 84:131–176

Knight H, Knight MR (2001) Abiotic stress signaling pathways: specificity and cross-talk. Trends Plant Sci 6:262–267

Kingsolver JG, Diamond SE, Siepielski AM, Carlson SM (2012) Synthetic analyses of phenotypic selection in natural populations: lessons, limitations and future directions. Evol Ecol 26:1101–1118

Laland KN, Uller T, Feldman MW, Sterelny K, Müller GB, Moczek A, Jablonka E, Odling-Smee J (2015) The extended evolutionary synthesis: its structure, assumptions and predictions. Proc R Soc B 282:20151019

Leimar O, McNamara JM (2015) The evolution of transgenerational integration of information in heterogeneous environments. Am Nat 185:E55–E69

Lema SC, Kitano J (2013) Hormones and phenotypic plasticity: implications for the evolution of integrated adaptive phenotypes. Curr Zool 59:506–525
Lewontin RC (2000) *The triple helix: gene, organism and environment*. Harvard University Press, Cambridge
Moczek AP, Sultan SE, Foster S, Ledón-Rettig C, Dworkin I, Nijhout HF, Abouheif E, Pfennig DW (2011) The role of developmental plasticity in evolutionary innovation. Proc R Soc B 278:2705–2713
Nijhout HF (2003) Development and evolution of adaptive polymorphisms. Evol Dev 5:9–18
Odling-Smee FJ, Laland KN, Feldman MW (2003) *Niche construction: the neglected process in evolution*. Princeton University Press, Princeton
Paaby AB, Rockman MV (2014) Cryptic genetic variation: evolution's hidden substrate. Nat Rev Genet 15:247–258
Scheiner SM (1993) Genetics and evolution of phenotypic plasticity. Annu Rev Ecol Syst 24:35–68
Scheiner SM, Holt RD (2012) The genetics of phenotypic plasticity. X. Variation versus uncertainty. Ecol Evol 2:751–767
Soubry A, Hoyo C, Jirtle RL, Murphy SK (2014) A paternal environmental legacy: evidence for epigenetic inheritance through the male germ line. BioEssays 36:359–371
Stearns SC (1989) The evolutionary significance of phenotypic plasticity. Bioscience 39:436–445
Sultan SE (2007) Development in context: the timely emergence of eco-devo. Trends Ecol Evol 22:575–582
Sultan SE (2015) *Organism and environment: ecological development, niche construction and adaptation*. Oxford University Press, London
Sultan SE, Spencer HG (2002) Metapopulation structure favors plasticity over local adaptation. Am Nat 160:271–283
Van Dyken JD, Wade MJ (2010) The genetic signature of conditional expression. Genetics 184:557–570
West-Eberhard MJ (2003) *Developmental plasticity and evolution*. Oxford University Press, New York

Evo-Devo and Niche Construction

Daniel B. Schwab and Armin P. Moczek

Contents

Abstract

Much thinking in evo-devo is dominated by a mindset that views traits and trait variants as emergent properties of genes and genomes, and environments as strictly external to and separable from the organisms that develop within them. Growing evidence accumulating across diverse fields is increasingly questioning the continued usefulness of this framework, resulting in calls for a more explicit recognition and integration of the interdependencies between development, environment, and phenotypic evolution. In the first section of this chapter, we review the ubiquitous and diverse roles that environmental conditions play in instructing developmental outcomes, as well as how failure to provide proper environmental signals can disrupt development or lead to the expression of novel phenotypic variants. In the second section, we discuss how the environmental conditions that

D. B. Schwab (✉) · A. P. Moczek
Department of Biology, Indiana University, Bloomington, IN, USA
e-mail: schwabd@umail.iu.edu; armin@indiana.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_46

organisms experience are often modified by the organisms themselves, how these interactions can reciprocally shape development, and how their study is best advanced within the context of niche construction theory. In the final section, we address how the integration of niche construction theory with five research programs central to evo-devo (i.e., evolutionary innovation and diversification, developmental bias, developmental plasticity, genetic accommodation, and inclusive inheritance) can lead to a more holistic and complete understanding of development and developmental evolution.

Keywords

Ecological development · Developmental plasticity · Developmental bias · Genetic accommodation · Non-genetic inheritance

Introduction

Evolutionary biologists seek to understand how and why biological evolution unfolds the way it does, including the origins of adaptations and the mechanisms that shape short- and long-term patterns of diversification. Historically, such questions have been approached through the framework of the Modern Synthesis, which integrated Darwinian natural selection, population-level thinking, and Mendelian inheritance in the mid-twentieth century. Application of this framework enabled the rigorous, quantitative examination of important evolutionary processes and, through its successes over many decades, deeply ingrained dichotomies that now characterize the way we conceptualize organismal evolution. For example, phenotypes are generally viewed as rooted in genes and genomes, and environments – though increasingly recognized as an important source of developmental information – nevertheless remain conceived as passive, external to, and separable from the organisms responding to them and the selective pressures that they impose. Without question, this conceptual framework has enabled countless advances in our understanding of the nature of biological evolution; however, a subset of foundational objectives of evolutionary biology have stubbornly resisted productive investigation through conventional approaches. For example, the processes that enable, shape, or bias the origin of novel, complex traits and the corresponding major transitions in evolution that they facilitated are of fundamental interest to the discipline. But because of their entrenchment in deep time, and the lack of phenotypic variation accessible to quantitative and population genetic approaches, contemporary evolutionary biology has thus not been able to provide satisfactory resolution to these challenges. However, here as well as in other contexts, the increasing integration of evolutionary developmental biology (evo-devo) with conventional approaches has begun to significantly enhance the explanatory reach of evolutionary biology.

Traditional evolutionary biology treats developmental processes as proximal, that is, development translates genotype into phenotype, but by itself does not influence or direct evolutionary outcomes beyond simply excluding those that are developmentally inaccessible. Consequently, understanding how development works is generally not considered necessary for understanding the evolutionary process. However, a large body of evidence has now accumulated that thoroughly challenges this assumption. Evo-devo has shown that evolution frequently proceeds through changes in the genetic and physiological regulation of developmental processes, taking advantage of the highly modular organization of organismal function at all levels of biological organization, from gene regulation to organ systems. As such, innovation and diversification in evolution are enabled through – and channeled or biased by – the differential reuse and recombination of otherwise conserved developmental building blocks. This focus on how phenotypes are constructed in development and re-assembled in evolution has illuminated many issues traditional evolutionary biology had to leave unresolved, such as the origin of phenotypic novelty, the maintenance of homology, or the frequent, independent re-use of the "same" developmental pathways to build fully or partly convergent traits in unrelated organisms. More generally, thanks to these efforts we now understand that the nature of development may itself be a source of evolutionary innovation, encouraging change in certain directions over others. Understanding the nature of development has thus emerged as critical to understand why evolutionary change unfolds the way it does.

At the same time, several key perspectives in evo-devo have remained remarkably traditional. Specifically, much research in evo-devo continues to view developmental evolution as ultimately rooted in genes and genomes, a mindset that has critically shaped concepts and terminology in the field, such as the postulate of a genetic toolkit or the notion of selector genes. Secondly, while practitioners of evo-devo increasingly recognize the environment as an important source of information needed to instruct normal development, it remains viewed as an agent that is separable from the organism, whose role in development is passive and whose role in evolution is restricted to shaping selective conditions.

In this chapter, we discuss the value of both perspectives in the light of accumulating evidence. We first review the pervasive role environmental conditions play in enabling and instructing developmental processes, and in shaping developmental outcomes. Secondly, we explore the notion that rather than viewing environments solely as external and passive, they may better be understood as at least in part shaped by organisms themselves. We then present how integrating the field of niche construction, a conceptual framework that has emerged independently in evolutionary ecology, provides promising opportunity to incorporate these revised perspectives, thereby expanding the explanatory power of evo-devo in particular, and evolutionary biology in general. Throughout, we highlight examples of evo-devo case studies that, by integrating a revised evaluation of the role of the environment into their research programs, have the potential to advance long-standing and critical questions in the biological sciences.

Organism, Environment, and the Promise of Niche Construction Theory

To Develop is to Interact with the Environment

Recent decades have seen a growing appreciation for the environmentally responsive nature of developmental processes and their outcomes (e.g., see chapter ▶ "Eco-Evo-Devo"). This increased focus is due in part to an understanding that environmental signals, acting across multiple levels of biological organization, are often necessary for the completion of normal development. For instance, the neuroendocrine systems of many developing organisms are primed to receive environmental signals that relate information on nutritional conditions, temperature, and season (Gilbert and Epel 2015). The reception of these signals is subsequently integrated to generate changes in gene expression, physiology, morphogenesis, and behavior, in extreme cases specifying discrete, alternative phenotypes such as the nutritionally responsive horned and hornless morphs of *Onthophagus* dung beetles, the temperature-dependent sex determination of some amphibian and reptile species, and the summer to winter changes in coat color and texture that are characteristic of arctic foxes. However, these obligate environmental signals are not comprised solely of external, abiotic factors, but are increasingly recognized as encompassing other developing and evolving organisms that reside internally within responsive individuals. This includes contributions from microbial symbionts, which are often responsible for providing crucial signals for normal host development. For instance, microbiota contribute to pre-embryonic development by mediating cytoplasmic incompatibility across most major arthropod taxa, establish the anterior-posterior axis in embryonic nematodes, influence tissue and organ development in a wide array of invertebrate and vertebrate taxa, and induce settlement and metamorphosis in marine invertebrate larvae (reviewed in Gilbert et al. 2012; Landmann et al. 2014; Shikuma et al. 2014). Environmental conditions, however generated, therefore play an instructive role in normal development in a wide range of taxa and in diverse circumstances.

Just as the significance of environmental conditions in instructing the developmental trajectory of organisms is now broadly recognized, ecologists and evolutionary biologists have also begun to appreciate that these interactions need not point unidirectionally from environment to developmental system. Rather, organism-environment interactions may more commonly be reciprocal, with developmental systems constructing environmental conditions that then allow the next phase of environment-responsive development to take place. For instance, the growth and differentiation of a given cell is influenced by the prevailing cellular and physiological environment in which it finds itself, characterized by the presence or absence of nutrients, morphogens, and paracrine or endocrine factors. In response to this internal environment, cells specify patterns of gene expression that affect not only the growth and differentiation of future cells, but also their own developmental context at later points in development. At higher levels of organization, diet for

instance has long been known to shape pharyngeal jaw morphology in cichlids (Greenwood 1965), as well as gut formation and differentiation from invertebrates to vertebrates (e.g., Agrawal et al. 2002; Ledon-Rettig et al. 2008), in each case affecting future foraging and dietary environments experienced throughout the remainder of development. In sum, organisms execute their development in tight interdependence with the environment, with significant aspects of the ontogenetic environment being both cause and effect of organismal development.

At the same time, a growing literature demonstrates that the environment-constructing nature of organismal function need not be limited to developmental processes per se or take place only within the boundaries of the organism. Instead, even external environments may be shaped in profound ways by organisms, including the same organisms whose development, physiology, and behavior is then subsequently affected by these same, constructed environments. Understanding the causes, nature, and consequences of these interactions are the central objectives of niche construction theory, as introduced next.

Organism and Environment as Cause and Effect of Each Other

First acknowledged by Richard Lewontin (1983) and subsequently refined into a theoretical framework by others (reviewed in, e.g., Odling-Smee et al. 2003), niche construction theory states that organisms, via their physiology and behaviors, modify their own and each other's niches in nonrandom and often systematic ways. A substantial literature now exists that documents the nature and scope of these modifications, which can range from the construction of physical structures such as nests, dams, and burrows to alterations of chemical states in the surrounding environment (i.e., perturbational niche construction), to the selection of alternative habitats and social environments (i.e., relocational niche construction; reviewed in Odling-Smee et al. 2003). The nature and scope of these modifications can range from being active and nonrandom changes to the local environment to byproducts of organismal physiology and behavior. For instance, the digging and tunneling behaviors of earthworms modify the surrounding soil in ways that increase its ability to capture and retain rain water, reduce its clay fraction, facilitate gas exchange, and increase its nutrient content due in part to the concentration of nitrogen and phosphorus found in worm excrement (discussed in Odling-Smee et al. 2003). At the same time that these modifications aid individual worms in maintaining osmotic balance, they also alter the soil ecosystem in ways that benefit other worms, soil macroinvertebrates, and plants. Therefore, the developmental, ecological, and evolutionary consequences of these niche constructing traits need not be limited to the niche constructor itself, but can generate important byproducts that scale-up to shape the ecology of other species and even influence ecosystem-level processes (Erwin 2008). Additional well-studied examples that illustrate the same features include the niche constructing activities of beavers (leading to wetlands), hippopotami (transforming savannahs), or corals (enabling reef formation).

By actively constructing and modifying their biotic and abiotic environments, niche constructing organisms have the potential to alter the developmental and selective environments acting upon them in directional ways that can (but do not necessarily have to) increase organism-environment fit. These effects may most commonly manifest in nature when niche construction buffers the developmental and evolutionary responses of populations against stressful environmental conditions. For instance, larvae of the goldenrod gallfly, *Eurosta solidaginis*, secrete factors that induce galls to form on goldenrod plants, providing the gallfly with a constant source of nutrition as well as protection from parasitoids and avian predators (Abrahamson et al. 1989). In this case, niche construction imposes stabilizing selection and decreases the range of environmental conditions experienced by the organism. Ecological and population-genetic models provide further evidence that niche construction can significantly alter the rate and direction of evolution, generate eco-evolutionary feedbacks, and influence whether genetic variants are maintained or lost (Laland et al. 1999; Silver and Di Paolo 2006; Kylafis and Loreau 2008). Therefore, niche construction may contribute in diverse ways to the complementarity between organisms and their environments, i.e., to adaptation. Furthermore, by systematically biasing selection pressures, niche construction may rightly be considered an evolutionary process (alongside natural selection, drift, etc.), one that allows organisms to be both the object and creator of the conditions under which natural selection occurs.

Because most organisms are thought to engage in some form of niche construction during ontogeny (Laland et al. 2008), environmental modifications may be fundamental to the normal development of the organisms that generate and experience them. At the same time, clear parallels exist between the constructive and reciprocal nature of developmental processes that are increasingly the focus of evo-devo studies and those organism-environment interactions that are characteristic of niche construction. Yet although such linkages have been acknowledged previously (e.g., Laland et al. 2008), the field of niche construction remains as of yet poorly assimilated with contemporary evo-devo. Below, we highlight several objectives and conceptual foci of contemporary evo-devo that we believe to be especially well aligned with niche construction theory.

Advancing Evo-Devo Through the Study of Niche Construction

We conclude this chapter by briefly highlighting five interrelated topic areas that feature prominently within contemporary evo-devo research programs and discuss how they are conceptually aligned with, and empirically approachable through, niche construction theory. We then consider how the integration of niche construction into these long-standing research programs may allow evo-devo to overcome important conceptual roadblocks, thereby enhancing its explanatory reach as an integrative discipline.

Evolutionary Innovation and Diversification

One of the most celebrated contributions of evo-devo to evolutionary biology has been the realization that much of the innovation and diversification in evolution is enabled through the modular nature of developmental processes and the re-use and rearrangement of otherwise conserved developmental building blocks. More recent work, often placed in the realm of ecological developmental biology (eco-evo-devo), has added to this the realization that selectable phenotypic variation contributed by development is itself a function of environmental context within which developmental systems operate: for example, environmental conditions influence the presence and degree of genetic and phenotypic correlations and determine whether genetic variation will remain cryptic or manifest in selectable phenotypes (reviewed in Paaby and Rockman 2014). Niche construction theory heavily emphasizes this latter relationship as well: ecological conditions feedback on patterns of phenotype expression, often across generations, and in the case of ecosystem engineering, across many taxa. Indeed, examples of ecosystem engineering ranging from the construction of coral reefs and beaver dams to the oxygenation of soils by bioturbators all possess the property of generating novel ecological niches and opportunities for evolutionary innovation and diversification to occur (Erwin 2008).

What niche construction theory adds, and contemporary evo-devo lacks, is the reciprocal view: the emphasis on environmental conditions as something that is at least in part actively created and shaped by the organism itself. Like other phenotypes, niche constructing abilities themselves are contributed to by developmental (and physiological, behavioral, etc.) systems and, depending on their respective heritable variation in natural populations, may contribute to population divergence, ecological radiations, and niche innovation. The greatest value of better integrating evo-devo, eco-devo, and niche construction perspectives on organism-environment interactions may thus lay in how these extend each other's explanatory power: evo-devo and eco-devo may inform niche construction theory by offering possible developmental, physiological, neurobiological, or microbiological mechanisms that have enabled or constrained the origin and diversification of niche constructing abilities. In return, niche construction theory forces evo-devo and eco-devo to move beyond a mindset that views environments solely as an external agent of selection and to formulate hypotheses that also take into account that environmental conditions may themselves evolve and that this may contribute significant heritable variation biasing the processes of developmental innovation and diversification in natural populations.

Developmental Bias

Among its central aims, research in evo-devo seeks to clarify the causal-mechanistic basis by which phenotypes and phenotypic variants arise during development and how these processes of phenotype construction in turn interact with evolutionary

processes. One emerging observation from these efforts is that phenotypic variation is systematically biased by the process of development, with some phenotypes more likely to be generated than others. This bias emerges on a variety of organizational levels. As highlighted in the introduction, the highly modular nature of development, from cis-regulatory elements, genes, and signal transduction pathways to morphogenetic processes and organ systems, has enabled phenotypic evolution to proceed via rearrangements of its component parts in developmental space, time, and context. Here, bias results from the re-use of the same developmental modules and causes what should be independent evolutionary events to instead unfold as parallelisms, yielding nonhomologous traits made up of deeply homologous components in the process. At the same time, this developmental bias does not just limit or constrain what may be allowed to evolve, but instead also facilitates the emergence of novel and functionally integrated traits through the re-use of pre-existing components already selected to work well together.

A related but also distinct form of bias emerges through the exploratory, demand-based, and self-regulatory nature of many developmental processes. For instance, the formation of muscular-skeletal attachments is highly reliant on the exploratory behavior of muscle precursor cells, which migrate randomly during early development and are only maintained into later stages if they manage to innervate muscles. This combination of exploratory behavior followed by somatic selection, which is seen in many other contexts, demonstrates how developmental processes may be inherently biased towards producing functional, well-integrated states, even in the face of environmental or genetic perturbations.

Similar kinds of developmental biases may also be inherent in the ways in which organisms engage with and alter their external environment through the process of niche construction, resulting in qualitatively similar developmental as well as evolutionary consequences. For instance, on a general level, niche constructors predictably bias environmental states towards those that best suit the traits of the initial niche constructor or its descendants. Like developmental bias, niche construction thus allows organisms to channel development preferentially towards functional phenotypes that adaptively fit their environment, thereby imposing some directionality on evolution. Further, the traits organisms rely upon to construct their environments, from the chemicals they secrete to the nests they build to the gut endosymbionts they harbor, endow lineages with a sort of *niche construction toolbox*, one that can be engaged over and over independently in different taxa and possibly developmental contexts, thereby on one side biasing the way niche constructors may alter their environments, while on the other facilitating diversification through the use, re-use, and recombination of effective nice constructing activities already honed through previous rounds of selection.

Integrating evo-devo and niche construction perspectives on developmental bias promises a more complete and holistic understanding of the interdependencies of organismal development, phenotype function, environmental conditions, and adaptive evolution. In particular, in the numerous cases in which parental niche constructing behaviors affect early developmental environments of offspring, understanding why and how developmental evolution unfolds the way it does in a given

lineage will require an understanding of the ontogenetic basis of both niche construction and offspring embryogenesis, as well as their interactions. The already well-established importance of maternal transcripts in early zygotic differentiation or maternal transmission of antibodies and symbionts illustrate the significance of these interactions, which are likely to exist on many other levels of biological organization as well.

Developmental Plasticity

Developmental plasticity refers to the ability of developing organisms to adjust their phenotypes in response to environmental conditions (see chapter ▶ "Developmental Plasticity and Evolution"). The field of plasticity research emerged with a focus on plasticity as an exceptional case, one that can be parameterized in quantitative genetic models in the form of G × E interactions and best studied through reaction norm approaches. In recent years, it has become clear that plasticity is a normal feature of development, rather than an exception, and that organisms ranging from the earliest embryos to mature adults are primed to integrate and plastically respond to diverse environmental signals. Furthermore, research from the field of evo-devo has provided a causal-mechanistic understanding of developmental plasticity, elucidating the gene regulatory and physiological bases of plasticity, as well as how such environmental sensitivity evolves across populations and species (Schlichting and Pigliucci 1998).

Both conceptual and mechanistic links exist between developmental plasticity and niche construction: on one level, niche construction necessitates environmental responsiveness in those aspects of development, physiology, and behavior that enable niche constructors to sense, evaluate, and respond adaptively to the often complex and heterogeneous environments that they face. Niche construction is therefore facilitated by developmental plasticity, which can be thought of as providing the mechanistic scaffolding for environmental modifications to occur. At the same time, organismal plasticity and niche construction emphasize opposite causations regarding adaptation: whereas the former allows organisms to maintain high fitness by adjusting their traits to their environments, the latter allows organisms to maintain high fitness by adjusting their environments to their traits. As a result, niche construction may simultaneously be seen as a source of robustness by reliably and systematically buffering developing organisms against stressful environmental conditions. This is illustrated, for instance, by the gall formation of goldenrod gallflies (see above), or the widespread construction of nests, mounds, burrows, etc., across diverse animal taxa. Therefore, developmental plasticity and niche construction may be thought of as reciprocal and interdependent processes.

Despite these obvious linkages, both the evo-devo community and the field of developmental plasticity research are poised to benefit from a more rigorous theoretical and empirical assimilation of niche construction. For instance, it will be critical to experimentally evaluate the extent to which developing organisms are

plastically responsive to the environments that they, themselves, have generated through niche construction. Such plasticity will not only influence phenotype formation as expressed through modified norms of reaction, but may also alter the heritability and response to selection of plastic traits, including the extent to which genetic variation may be maintained or lost (Saltz and Nuzhdin 2014). In parallel, theoretical and conceptual models will be necessary to fully understand how and under what circumstances these complex feedbacks influence evolutionary trajectories. Throughout these efforts, it will be important to acknowledge the diverse forms through which plasticity (e.g., morphological, behavioral, learning) and niche construction (e.g., perturbational, relocational) are expressed, as each may influence the outcomes of theoretical models and experimental studies in different ways, thereby illustrating the potentially diverse consequences of these phenomena for development and developmental evolution.

Genetic Accommodation

Over the last few decades, evolutionary ecologists have generated a robust body of empirical and theoretical work investigating the ecological conditions under which selection favors (or disfavors) the evolution of plasticity (Schlichting and Pigliucci 1998). More recent work has begun to emphasize that plasticity may not just be a product of phenotypic evolution, but may itself also be a factor in shaping subsequent evolutionary trajectories: for instance, plasticity has the potential to facilitate the colonization of and persistence in novel environments (reviewed in Pfennig et al. 2010). Further, a growing number of studies have raised the possibility that plastic changes may precede genetic changes during the process of adaptation (reviewed in Pfennig et al. 2010). If correct, developmental systems may readily be able to integrate novel environmental inputs through the coordinated and functional adjustment of their morphology and physiology. This phenomenon, known as phenotypic accommodation, has the potential to enhance fitness by closely aligning organismal phenotypes with their prevailing selective environments (West-Eberhard 2005). For instance, when reared within a terrestrial environment, basal ray-finned fishes in the genus *Polypterus* plastically develop skeletal features and locomotive behaviors consistent with those of stem tetrapods, suggesting that phenotypic accommodation may have acted as a first step in the evolutionary transition of limbed vertebrates from water to land (Standen et al. 2014). Such responses may act as a precursor to phenotypic evolution through the process of genetic accommodation, in which environmentally induced phenotypes become refined and stabilized over many generations via selection on standing genetic variation, formerly cryptic genetic variation, or *de novo* mutation. Evolution by genetic accommodation received its strongest empirical support initially through laboratory experiments, but is now increasingly validated by field studies on diverse taxa such as water fleas (Scoville and Pfrender 2010), sticklebacks (Wund et al. 2008), blind cave fish (Rohner et al. 2013), and house finches (Badyaev 2009).

Although genetic accommodation remains controversial among many evolutionary biologists (e.g., Laland et al. 2014), studies of this phenomenon have historically considered environments through the lens of conventional evolutionary thought: as being an inducer of phenotypes that is both separable from and external to the organism. By contrast, niche construction theory focuses explicitly on the environment-generating capacity of organisms and promotes the argument that environments and organisms are both cause and effect of each other. Yet genetic accommodation and niche construction are at least partially congruent: both focus on the organism, through its development, physiology, and behavior, to act as a sieve through which environmental variation is transduced into phenotypic variation. In so doing, both concepts highlight the largely contingent nature of development and developmental evolution. Similarly, the nature and extent of genetic accommodation has the potential to be modified by the niche constructing activities of organisms in novel environments. For instance, because niche construction can buffer organisms, diminishing the range of environments that they experience, the nature and magnitude of morphological and physiological accommodation that must occur in a novel environment may be lessened or may be directed down some routes and not others. At the same time, because niche construction can generate strong covariance between niche constructing organisms and their environment, constructed selective environments can increase in frequency alongside the traits that construct them, thereby potentially accelerating the rate of genetic accommodation. Empirical and theoretical work is now needed to evaluate the extent to which niche construction can initiate and promote genetic accommodation in natural populations.

Inclusive Inheritance

Because evolutionary biology has long treated biological information as being rooted largely in genes and genomes, it is not surprising that many evolutionary biologists and even evo-devo advocates treat transmission genetics as the only general (and evolutionarily relevant) inheritance system. Yet there is growing recognition that heredity is not simply a genetic construct, but that the construction of developmental environments may be inclusive of multiple interacting mechanisms of nongenetic inheritance that span multiple levels of biological organization. These mechanisms are commonly thought to include (i) *epigenetic transmission*, such as the posttranslational methylation or acetylation of histone proteins, the methylation of cytosines in DNA, and the inheritance of microRNAs, (ii) *parental effects*, ranging from the behavioral interactions between parents and offspring (i.e., parental care), to the germline and environmental transmission of key nutrients and microbiota, and (iii) *cultural inheritance*, such as the transmission of acquired group behaviors via learning (Danchin et al. 2011). In each case, a growing literature has begun to document the causal role of these mechanisms in modifying development to enable the maintenance of parent-offspring similarity (i.e., heritability) and in the adaptive fitting of organisms to prevailing environmental conditions (Gilbert and Epel 2015). As a result, these additional inheritance systems have the potential to

bias both the rate and direction of evolution, though this effect may be attenuated by their stability and effect size across generations (Danchin et al. 2011).

Importantly, niche construction provides an additional route for nongenetic inheritance. While the niche constructing activities of individuals may modify ecological conditions and the selective pressures that they generate, the developmental and fitness consequences incurred by these activities need not be limited to a single generation or constructor, but can additionally span multiple generations in the form of *ecological inheritance*. Ecological inheritance occurs when organisms bequeath their modified selective environments to descendant offspring, exemplified by the dams of beavers, the mounds of termites, or the modification of soil nutrients by earthworms and plants, all of which can substantially outlast the lifetime of an individual niche constructor. The nature of ecological inheritance is unique from other sources of nongenetic inheritance, in that the products of niche construction may not only be consequential for an individual's offspring, but can scale-up to affect the structure and functioning of whole populations, communities, and ecosystems. Consequently, ecological inheritance has the potential to influence not only parent-offspring similarity, but can also strongly affect long-term ecological and evolutionary dynamics (e.g., see Laland et al. 1999).

The incorporation of niche construction and ecological inheritance into standard views of inheritance offers significant novel explanatory power to evo-devo practitioners who seek to understand the developmental origins of organismal phenotypes. Much of this promise is already being realized in closely related fields. For instance, it is now clear that gut development is often incomplete without signals from maternal microbiota, that the function of the immune system is informed by maternally acquired antibodies, and that social environments substantially influence cognitive and behavioral development in diverse organisms. Therefore, failure to evaluate the degree to which the ecological inheritance of organisms contributes to parent-offspring similarity and phenotypic variation risks ignoring valuable sources of heritable variation.

Conclusions

In this chapter, we have argued that diverse aspects of normal development necessitate close interactions with the environment, that traditional views regarding the nature and separability of environments and organisms are challenged and extended by niche construction, and that features of contemporary evo-devo are highly complementary to, and may benefit from, a more pronounced integration with niche construction theory. Indeed, we posit that the further synthesis of these two presently disjointed fields promises a deeper and biologically more realistic understanding of the nature of organism-environment interactions and their consequences for phenotypic evolution. Already, advances from both fields have independently led to and reinforced two emergent, unifying themes of organismal development. First, organismal development is a highly *constructive* process: organisms shape their developmental trajectory by constantly responding to internal and external states

via diverse developmental processes (as illustrated by evo-devo) or through modifications to ecological niches (as illustrated through niche construction). Second, organismal development is *reciprocally causal*: organisms shape, and are in turn shaped by, their selective and developmental environments. What remains now is to assess in diverse taxa the extent to which an understanding of these interdependencies facilitates a more full accounting of the origins of phenotypic variation, and how they shape, bias, enable, or constrain the evolution of development.

Cross-References

- ▶ Developmental Plasticity and Evolution
- ▶ Eco-Evo-Devo
- ▶ Evolvability
- ▶ Inherency

References

Abrahamson WG, Sattler JF, McCrea KD, Weis AE (1989) Variation in selection pressures on the goldenrod gall fly and the competitive interactions of its natural enemies. Oecologia 79:15–22

Agrawal AA, Vala F, Sabelis MW (2002) Induction of preference and performance after acclimation to novel hosts in a phytophagous spider mite: adaptive plasticity? Am Nat 159:553–565

Badyaev AV (2009) Evolutionary significance of phenotypic accommodation in novel environments: an empirical test of the Baldwin effect. Philos Trans R Soc Lond B 364:1125–1141

Danchin É, Charmantier A, Champagne FA, Mesoudi A, Pujol B, Blanchet S (2011) Beyond DNA: integrating inclusive inheritance into an extended theory of evolution. Nat Rev Genet 12:475–486

Erwin DH (2008) Macroevolution of ecosystem engineering, niche construction and diversity. Trends Ecol Evol 23:304–310

Gilbert SF, Epel D (2015) Ecological developmental biology: the environmental regulation of development, health, and evolution. Sinauer Associates, Incorporated, Sunderland

Gilbert SF, Sapp J, Tauber AI (2012) A symbiotic view of life: we have never been individuals. Q Rev Biol 87:325–341

Greenwood PH (1965) Environmental effects on the pharyngeal mill of a cichlid fish, *Astatoreochromis alluaudi*, and their taxonomic implications. Proc Linnean Soc Lond 176:1–10

Kylafis G, Loreau M (2008) Ecological and evolutionary consequences of niche construction for its agent. Ecol Lett 11:1072–1081

Laland KN, Odling-Smee FJ, Feldman MW (1999) Evolutionary consequences of niche construction and their implications for ecology. Proc Natl Acad Sci 96:10242–10247

Laland KN, Odling-Smee J, Gilbert SF (2008) EvoDevo and niche construction: building bridges. J Exp Zool B Mol Dev Evol 310:549–566

Laland K, Wray GA, Hoekstra HE (2014) Does evolutionary theory need a rethink? Nature 514:161

Landmann F, Foster JM, Michalski ML, Slatko BE, Sullivan W (2014) Co-evolution between an endosymbiont and its nematode host: *Wolbachia* asymmetric posterior localization and AP polarity establishment. PLoS Negl Trop Dis 8:e3096

Ledon-Rettig CC, Pfennig DW, Nascone-Yoder N (2008) Ancestral variation and the potential for genetic accommodation in larval amphibians: implications for the evolution of novel feeding strategies. Evol Dev 10:316–325

Lewontin RC (1983) The organism as the subject and object of evolution. Scientia 118:63–82

Odling-Smee FJ, Laland KN, Feldman MW (2003) Niche construction: the neglected process in evolution. Princeton University Press, Princeton

Paaby AB, Rockman MV (2014) Cryptic genetic variation: evolution's hidden substrate. Nat Rev Genet 15:247–258

Pfennig D, Wund MA, Snell-Rood EC, Cruickshank T, Schlichting CD, Moczek AP (2010) Phenotypic plasticity's impacts on diversification and speciation. Trends Ecol Evol 25:459–467

Rohner N, Jarosz DF, Kowalko JE, Yoshizawa M, Jeffery WR, Borowsky RL, Lindquist S, Tabin CJ (2013) Cryptic variation in morphological evolution: HSP90 as a capacitor for loss of eyes in cavefish. Science 342:1372–1375

Saltz JB, Nuzhdin SV (2014) Genetic variation in niche construction: implications for development and evolutionary genetics. Trends Ecol Evol 29:8–14

Schlichting CD, Pigliucci M (1998) Phenotypic evolution: a reaction norm perspective. Sinauer Associates Incorporated, Sunderland

Scoville AG, Pfrender ME (2010) Phenotypic plasticity facilitates recurrent rapid adaptation to introduced predators. Proc Natl Acad Sci 107:4260–4263

Shikuma NJ, Pilhofer M, Weiss GL, Hadfield MG, Jensen GJ, Newman DK (2014) Marine tubeworm metamorphosis induced by arrays of bacterial phage tail–like structures. Science 343:529–533

Silver M, Di Paolo E (2006) Spatial effects favour the evolution of niche construction. Theor Popul Biol 70:387–400

Standen EM, TY D, Larsson HC (2014) Developmental plasticity and the origin of tetrapods. Nature 513:54–58

West-Eberhard MJ (2005) Phenotypic accommodation: adaptive innovation due to developmental plasticity. J Exp Zool B Mol Dev Evol 304:610–618

Wund MA, Baker JA, Clancy B, Golub JL, Foster SA (2008) A test of the "flexible stem" model of evolution: ancestral plasticity, genetic accommodation, and morphological divergence in the threespine stickleback radiation. Am Nat 172:449–462

Evo-Devo of Social Behavior

Kate E. Ihle, Gro V. Amdam, and Adam G. Dolezal

Contents

K. E. Ihle (✉)
Department of Evolutionary Biology and Environmental Science, University of Zurich, Zurich, Switzerland
e-mail: kateihle@gmail.com

G. V. Amdam
School of Life Sciences, Arizona State University, Tempe, AZ, USA

Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, Aas, Norway
e-mail: Gro.Amdam@asu.edu

A. G. Dolezal
Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA, USA
e-mail: adolezal@iastate.edu

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_45

Abstract

The interdisciplinary field of evolutionary developmental biology (evo-devo) combined the strengths of developmental biology with insights from molecular evolutionary biology to rapidly advance our understanding of morphological differentiation and the evolution of diverse multicellular life-forms. The same concepts have been applied to the study of the evolution of social behavior to good effect. By treating correlated behavioral and physiological states as units, similar to the developmental modules defined by more traditional evo-devo approaches, evolutionary developmental biologists have identified common "genetic toolkits" involved in the transition from solitary to social and eusocial life-histories across independent evolutionary origins of social behavior. Data from the rapidly expanding library of high-quality genomes from organisms across the tree of life has enabled direct tests of the predictions of an evo-devo approach to the evolution of social behavior, demonstrating both the power and limitations of such an approach to explain the evolution of behavioral traits as well as generating new insights into the often shared proximate mechanisms underlying the evolution of social behavior.

Keywords

Social evolution · Phenotypic plasticity · Developmental plasticity

Introduction

The origin of eusocial behavior is one of the major transitions in evolution (Maynard Smith and Szathmáry 1997), and a critical ongoing challenge for biologists is to understand the genetic basis of such major transitions in complex traits. The advances made by the field of evolutionary development in our understanding of morphological evolution suggest a template for the investigation of the proximate evolutionary pathways to social behavior (Dolezal et al. 2014; Toth and Robinson 2007). The advances in traditional evolutionary developmental biology were closely linked to the technical advances in the fields of genetics and molecular biology to identify how evolution has acted on common molecular networks to give rise to incredibly diverse body plans (Carroll 2008) Similarly, a rapidly expanding array of sequenced genomes from organisms with varied social behavior within and between lineages has begun to yield exciting insights into the shared proximate pathways to social behavior (see Kapheim et al. 2015 and references therein).

Social behavior is a complex trait and can be found in many forms in many different taxa (Gadau et al. 2009). In this chapter, we focus on the social behaviors that take place within permanent associations of conspecific individuals. These forms of social behavior range from simple communal associations in which independently reproducing females share a nest site to the vast advanced eusocial societies composed of morphologically distinct queens that dominate reproduction

and sterile workers that perform all other tasks required by the colony. With increasing levels of social complexity come associated increasing costs to an individual in terms of direct fitness. For example, in communal groups, the interaction between the size of the nest and number of resident females may constrain the number of reproductive opportunities for the individual females. In primitively social and advanced eusocial societies, workers have either very limited or no possibilities for direct reproduction. The latter, most extreme form of social behavior is found primarily within the social insects: ants, bees, wasps, and termites, and so they will be the primary subjects of this chapter.

How does complex social behavior evolve, if it comes with such rising direct fitness costs? To address this question, the evolutionary biologist William D. Hamilton proposed the concept of "inclusive fitness" to explain how workers may gain in overall fitness by giving up direct reproduction (Hamilton 1964). According to this hypothesis, the fitness of an individual is composed of both direct fitness, individual reproductive output, and indirect fitness, the reproductive output of related individuals with shared genetic makeup. Here, workers may increase their overall fitness by helping their mother or sister produce fertile offspring. Inclusive fitness theory predicts that altruistic helping behaviors can be favored by selection when the fitness benefits to helping outweigh the costs of foregoing reproduction. This hypothesis can be summed up by Hamilton's Rule: $b\ x\ r > c$ where b = the benefit to the receiver of helping behavior, r = the genetic relatedness between the helper and the receiver of helping behavior, and c = the cost to the altruistic helper (Hamilton 1964). Recent work has challenged the importance of inclusive fitness in the evolution of social behavior, arguing that group rather than kin selection is likely to be the greater evolutionary force (Nowak et al. 2010). This position remains controversial, with many scientists defending the foundations of Hamilton's hypotheses.

While ultimate explanations for the evolution of social behavior aim to understand *why* social behavior evolved, they can tell us little about *how* selection acting on individual phenotypes gives rise to social behavior. These *how* questions are particularly amenable to an evolutionary developmental biology approach. By identifying the basic modules of behavior that combine to form complex behavioral phenotypes within and across species, the evolutionary developmental biology of social behavior uses a comparative approach to identify the physiological and molecular pathways that were targeted by selection for the regulation of social phenotypes.

Behavioral Modules of Social Evolution

Eusocial insect societies represent the most complex social organization in the animal kingdom. A major obstacle to studying the evolution of eusocial behavior had been the fact that many eusocial species diverge long ago from solitary relatives. An evo-devo approach to the evolution of social behavior uses a broad comparative

method to identify and characterize behavior modules in the life-cycles of the solitary relatives of social species. These behavioral modules are then hypothesized to form the building blocks of the complex social behavioral phenotypes that make up a eusocial society. The guiding principle of an evo-devo approach to the evolution of social behavior is the understanding that selection can only act on the expressed phenotypic variance within a population and that of the phenotypic correlations between physiology and behavior present in extant solitary relatives of eusocial species is reflective of their shared solitary ancestors.

From the Individual to High Society

Solitary Ancestors

Eusocial behavior evolved from the variation inherent in the presumably solitary ancestors of today's "super organisms." Biologists use a comparative approach to build hypotheses about the behavior of these ancient insects by studying the extant solitary relatives of eusocial species. The life-cycle of a typical solitary female bee in the temperate zone begins in summer, when she either emerges from a winter-long diapause or ecloses as a new adult, after which she will leave the nest and mate. A period of adult development then begins, fueled by the carbohydrates in collected floral nectars. Female bees require a protein rich food source to activate their ovaries for egg production and will begin foraging for pollen prior to brood cell construction and provisioning. When a brood cell is complete, the female will hoard pollen to form a small ball, consisting of pollen mixed with nectar onto which she will lay an egg before sealing the cell in the case of mass provisioning species. Progressively provisioning species will continue to feed the developing larvae before sealing the cell prior to pupation. After the female closes the brood cell, she begins the process again.

Communal Behavior

Communal behavior occurs when two or more females share a nest site but provision and care for their own offspring independently (Michener 1974). In communal species, the behavioral blueprint from solitary species is largely intact. Communally nesting females need not be related and communal associations can occur when multiple foundresses share the cost of establishing a nest. These associations are also often formed when sisters emerge and remain as adults at their natal nest to rear their own offspring. Communal behavior has been hypothesized as an evolutionary "stepping-stone" to eusocial behavior. In this scenario, alleles favoring cooperative behavior would be selected for when communal groups are able to outperform solitary competitors, likely through benefits to shared nest defense of decreased costs of nest founding (Hölldobler and Wilson 2009). However, the evidence for this hypothesis is mixed, with recent studies suggesting that communal behavior is a stable phenotype and a potential evolutionary alternative strategy to eusocial behavior as it occurs primarily in lineages that lack more complex forms of social organization (Gadau et al. 2009).

Primitive Eusociality

Females in primitively eusocial societies have a reproductive division of labor and participate in shared rearing of the colony's offspring (Michener 1974). In these societies, there is no morphological caste differentiation, but reproduction is monopolized by one or a few dominant individuals that function as queens. The behavioral life cycle of a solitary ancestor may be recognizable as a solitary queen founds a nest. However, when her offspring become adults, a behavioral caste system emerges in which the queen primarily specializes on egg production, while the workers perform tasks such as brood rearing, nest maintenance, and foraging. Unlike the workers from advanced eusocial societies, primitively eusocial workers are physically capable of mating and reproduction, and queens typically achieve reproductive skew through physical domination. Should the dominant queen die or become less competitive, a worker may usurp her position as the primary reproductive in the colony.

Advanced Eusociality

Animal social groups must meet three characteristic criteria before they are considered to be advanced eusocial societies: (1) reproductive division of labor, (2) cooperative brood care, and (3) overlap of generations (Wilson 1971). Advanced eusocial societies are characterized by morphological castes with a near complete reproductive division of labor between the highly fecund queen and the often sterile workers. The queens of mature eusocial colonies focus entirely on egg production, while the workers, who are unable to mate and rarely reproduce, perform the rest of tasks required to maintain the colony. In many advanced eusocial species, behavioral divisions of labor between worker groups are also present. These behavior divisions can be related to morphological differences, as in species with specialized soldier castes, or they can be age-based, as exemplified by the temporal polyethism of the honey bee (Winston 1987). Honey bee workers pass through an age-associated progression of tasks known as beginning with tasks within the brood nest such as the tending of larvae and cell cleaning. Older workers leave the nest as foragers, collecting the food resources required by the colony. Despite the often huge morphological differences between the queen and worker castes, these phenotypes develop from the same genome and are triggered by worker-controlled nutritional rearing regimes rather than by genetic differences.

A Ground Plan for Social Living

The "ground plan" hypotheses on the evolution of eusocial behavior posits that the complex behavioral phenotypes that make up a eusocial insect colony are derived from associations between behavior and reproductive physiology present in the solitary ancestors of social species (West-Eberhard 1987; Amdam et al. 2004). The reproductive ground plan hypotheses expanded the methods of traditional evo-devo by treating the correlations between behavior and reproductive physiology that occur at different life-cycle stages as modules, similar to the developmental modules,

exemplified by the classical *Hox* genes model, that form the foundations of evolutionary developmental biology. These hypotheses argue that social behavioral phenotypes are not the product of newly evolved molecular pathways, but rather the result of developmental differences in the ancestral phenotype. Selection could then favor the divergent phenotypes, acting on the mechanisms that regulate the sequential phases of a solitary reproductive cycle to instead direct the social behaviors of queens and workers.

The Ovarian Ground Plan Hypothesis (OGPH)

In her work with *Polistes* paper wasps, Mary Jane West-Eberhard observed that ovarian development as well as hormone levels in queens as well as workers is correlated with behavior. The OGPH accounts for reproductive division of labor between queens and workers, and developmental differences influenced the cycle of reproduction and provisioning behavior tied to ovary development and reproductive physiology in solitary species. According to the OGPH, the selection acting on these developmentally derived differences segregated the reproduction and provisioning phases of a solitary female life cycle into the queen and worker phenotypes such that queens had developed ovaries and high titers of gonadotropic juvenile hormone (JH) while workers have undeveloped ovaries and lower-hormonal titers (Fig. 1). The OGPH was later expanded to include an explanatory mechanism for "age polyethism," a correlation between behavior and chronological age that is seen in the worker caste of many social insects in which young females remain on the nest while older workers leave the nest on foraging trips (West-Eberhard 1987, 1996). Here, behavioral maturation in workers is influenced by ovary development and JH. Younger workers who remain on the nest have slightly developed ovaries and the capacity for opportunistic reproduction should the queen fail. Older workers leave the nest as foragers as their ovaries regress and their potential as replacement reproductive declines (Fig. 1b, West-Eberhard 1996). The relatively high physical cost to foraging is then balanced by decreasing potential for direct reproduction.

The Reproductive Ground Plan Hypothesis

The reproductive ground plan hypothesis (RGPH) of Gro V. Amdam and Robert E. Page Jr. is an extension of the OGPH that relates specifically to the foraging behavior of workers (Amdam et al. 2004). The RGPH was built upon an extensive body of research on the high and low pollen-hoarding strains of honey bees developed by Robert Page and M. Kim Fondrk (Page 2013). The pollen-hoarding strains were divergently selected for the amount of pollen stored in the hive, a colony-level trait. This colony-level selection regime also resulted in large phenotypic differences between the workers, particularly in food collection behaviors and reproductive physiology (Table 1). High pollen-hoarding strain workers begin foraging at earlier ages than workers from the low pollen-hoarding strain. They also bias their food collection toward protein-rich pollen, while low pollen-hoarding workers bias their foraging toward nectar, the carbohydrate source for the colony. Amdam and Page noted that workers from high pollen-hoarding lines had larger ovaries (number of ovarian filaments, called ovarioles) than low-strain workers.

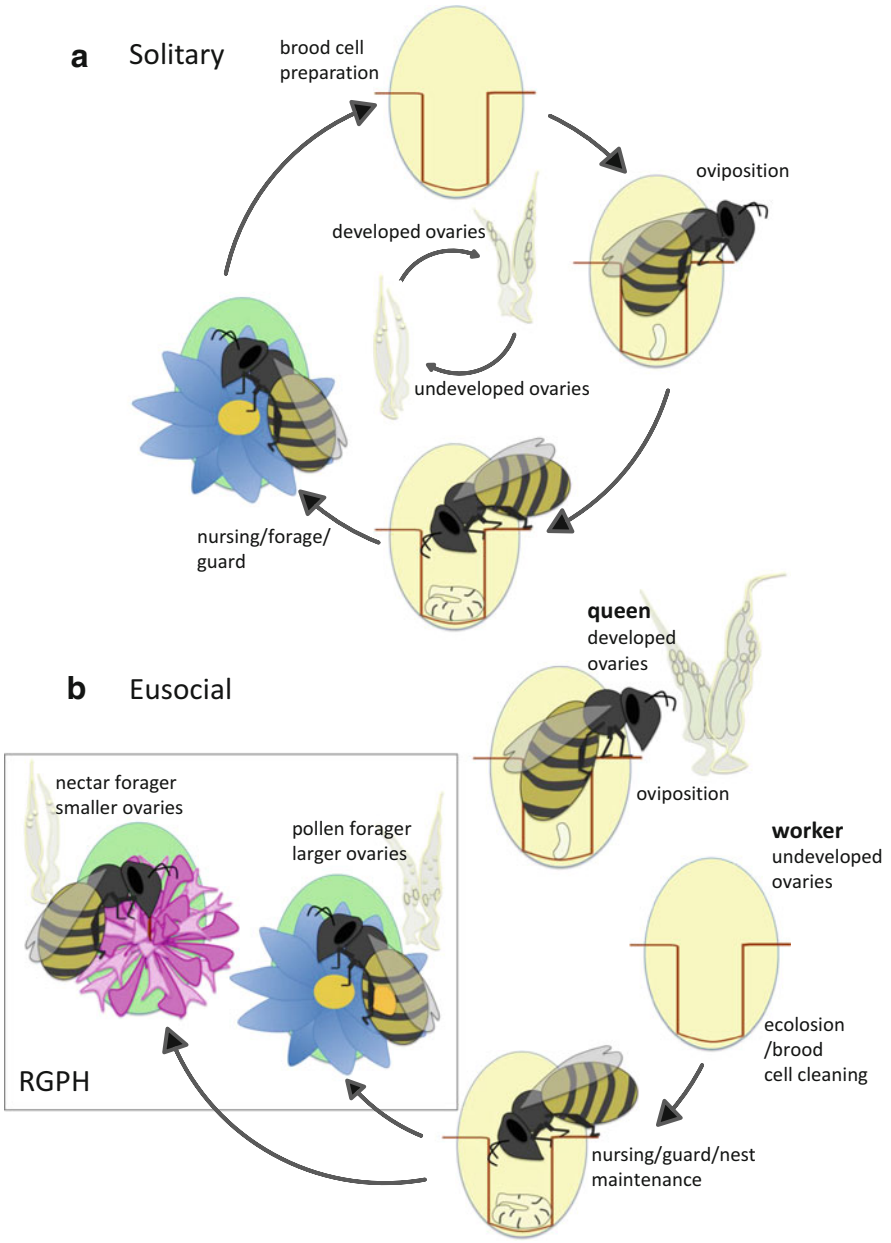


Fig. 1 The ovarian ground plan hypothesis (OGHP, West-Eberhard 1987, 1996) argues that the social phenotypes of queens and workers are developmentally derived from the ovarian cycle of a solitary ancestor (specifically, a progressively provisioning species with a long provisioning period). The reproductive ground plan hypothesis (RGPH, Amdam et al. 2004) extends the OGPH to explain the foraging division of labor for nectar versus pollen collection observed in honey bee workers. (**a**) A linked cycle of reproductive physiology and behavior frames the life cycle

Table 1 Many physiological and behavioral traits of individual bees were affected by the colony-level selection for high and low pollen hoarding. These divergent suites of traits inspired the reproductive ground plan hypothesis

	High pollen hoarding	Low pollen hoarding
Pollen stores	Larger	Smaller
Egg to adult development	Slower	Faster
Peak Vitellogenin titer	Higher	Lower
Vitellogenin/juvenile hormone feedback	Stronger	Weaker
Sucrose sensitivity	Higher	Lower
Ovary size	Larger	Smaller
Foraging onset	Earlier	Later
Foraging loading	Pollen bias	Nectar bias

These larger ovaries are accompanied by higher production of the yolk precursor protein, Vitellogenin. The RGPH argues that the variation in pollen versus nectar foraging observed in honey bee workers reflects and ancestral association between reproductive physiology and foraging behavior (Fig. 1b, Amdam et al. 2004).

In solitary insects, reproductive physiology is tied to nutritional input. Mosquitoes, for example, forage on nectar during periods of self-maintenance when their ovaries are inactive. Protein intake, in this case a blood meal, is required for female mosquitoes to activate their ovaries and fuel egg production. The RGPH posits that this ancestral association between protein foraging and reproduction is still present in the functionally sterile honey bee workers. Here, more reproductive-tuned workers, those with larger ovaries and higher titers of Vitellogenin will bias their foraging loading toward protein-rich pollen, while less reproductively tuned individuals will bias their collection toward carbohydrate-dense nectar (Amdam et al. 2004).

Fig. 1 (continued) of solitary females. As a female prepares a cell in which to deposit an egg, her ovaries are well developed. After oviposition, her ovaries remain undeveloped as she tends, guards, and forages for her developing larva. Her ovaries begin to swell with developed oocytes as she prepares the next brood cell. (**b**) In eusocial species, the OGPH posits that this cycle has been interrupted with the oviposition and brood care phases segregated into the queen and worker castes, respectively. Large, active ovaries support the sometimes enormous reproductive output of queens. Workers have underdeveloped ovaries and pass first through a stage of brood care, or nursing, behavior before transitioning to outside of the nest foraging behavior as they age and the likelihood of direct reproduction decreases. The RGPH argues that reproductive physiology regulates the division of foraging labor observed between honey bee workers for pollen versus nectar collection. Here more reproductively tuned workers with larger-ovaries (more ovarioles per ovary) and higher-peak titers of Vitellogenin bias their foraging efforts toward protein (pollen) collection while workers that are less reproductively tuned workers bias their foraging toward carbohydrate (nectar) collection

Food and Society

Nutrition status and food collection play an enormous role in shaping the life-history of any organism, and this is doubly true for the social insects where nutritional inputs during development determine the caste fate of a developing larva. The role of nutritional status in the triggering of developmental programs is especially clear in the advanced eusocial insects. In honey bees, for example, queen-destined larvae receive more and higher nutrient content food than do worker-destined larvae. In nature, these nutritional differences trigger discrete developmental programs that produce the morphologically distinct queen and workers castes. The workers tending the larvae, known as nurses, must tightly control the amount, quality, and timing of larval feedings in order to produce the distinct queen and worker phenotypes. In the lab, researchers routinely produce intercaste females, which have morphological features of both queens and workers, by varying the feeding regimes (Page 2013). There are no morphological castes in primitively eusocial species, but nutrition during development also plays a role in determining behavioral caste-fate in these species. Primitively eusocial queens are often bigger than workers, and large, newly emerged females are more likely to leave their natal nest as foundresses than their smaller sisters. The differences in body size are in part due to differential nutrition during development.

Nutritional status also plays a role in the pacing of behavioral maturation in social insects. Foraging is the final behavioral phase of life for the workers of many species. The foraging behaviors of solitary and social individuals of related species can be very similar. Bees gather floral nectars and pollens regardless of their social organization, but while solitary females gather food to increase their own fitness, social workers forage to increase the fitness of the colony. Nutritional physiology links the food collection behaviors of solitary and social species. Foragers often have depleted nutrient reserves relative to other behavioral castes, and experimental work in the honey bee demonstrated that reduction of lipid reserves in workers speeds their transition to foraging behavior (reviewed in Toth and Robinson 2007). Interestingly, it is not only individual nutrient status that can induce early foraging in honey bee workers. Reduction of the stored food within a colony also causes early foraging. These results suggest that the physiological mechanisms that regulate the foraging behaviors of solitary species have been co-opted by selection to regulate temporal polyethism in social species.

Genetic Toolkits Shape Social Life-Histories

After the discoveries showing that the reproductive and nutritional ground plans of solitary insects also regulated social insect life-history traits, researchers began using targeted approaches to understand the molecular underpinnings of social behavioral modules. Many of these studies initially targeted candidate genes identified from quantitative trait loci (QTLs), genomic regions statistically associated with a particular phenotype. While these QTLs were identified in the high and low pollen-

hoarding strains (Page 2013), many show shared brain gene expression patterns with *Polistes* wasps performing food collection behaviors (Toth et al. 2010).

A traditional evolutionary development approach suggests that changes in the regulation of genes and their expression, rather than functional changes in protein coding, is the predominant driver of evolutionary change (Carroll 2008). Amy L. Toth and Gene E. Robinson (2007) argued that gene expression differences may be particularly important for the evolution and regulation of social phenotypes. They proposed a model built from the concept of the eusocial insect colony as a "super organism," which could then be broken down analogously to the different cell types that make up an organism. Toth and Robinson (2007) extended this idea to hypothesize that, just as changes in the pattern of gene expression distinguish different cell and tissue types in a single organism, transcriptional differences also distinguish the behavioral and morphological phenotypes of the members (i.e., the "cells") of a social insect colony superorganism. Targeted molecular studies supported a role for reproductive and nutritional ground plans in the evolution of social behavior and identified potential "genetic toolkits" that regulate these associations between behavior and physiology across the spectrum of social complexity (reviewed in Toth and Robinson 2007; Dolezal and Toth 2014).

Molecular Pathways Associated with Reproduction

The dynamic differences observed in the Vitellogenin titers of the high and low pollen-hoarding honey bee strains were one factor that inspired the formation of the RGPH. Subsequent experimental work confirmed a regulatory role for Vitellogenin, not only in the decision to collect nectar versus pollen but also in the timing of the transition to foraging behaviors (Amdam and Page 2010), and demonstrated that Vitellogenin is one of the central players in a reproductive genetic toolkit that has evolved a regulatory role for non-reproductive social behaviors. Expression of the *vitellogenin gene* is higher in young, high pollen-hoarding strain workers, but the decline in *vitellogenin* expression with age also occurs earlier and more rapidly in this strain, corresponding to an earlier age of foraging behavior. Experimental reduction of *vitellogenin* expression via RNA-interference (RNAi) caused workers to collect heavier nectar loads and transition to foraging behavior at younger ages, confirming a regulatory role for Vitellogenin in both the onset of foraging as well as food collection decisions (Page 2013). A role for Vitellogenin in the regulation of social behaviors has since been confirmed in several species. For example, vitellogenin has recently been shown to be associated with parental care behaviors in burying beetles, suggesting that Vitellogenin and its link to both reproduction and provisioning behaviors is a powerful and flexible module in the evolution of behavioral phenotypes (Roy-Zokan et al. 2015).

The novel functions of *vitellogenin* in the regulation of social behaviors have likely evolved through several mechanisms. Most studies to date have focused on changes in the spatial and temporal expression of *vitellogenin* as underlying its roles in the evolution of novel phenotypes, but recent discoveries of gene duplications of

vitellogenin and *vitellogenin-like* genes in several species suggest that different or parallel mechanisms may be responsible for the novel functions of Vitellogenin. While Vitellogenin is known to regulate behaviors in many species, its mode of action is currently unknown. However, a growing body of research suggests that interactions with the systemic JH and the insulin/insulin-like signaling (IIS) pathway may mediate the effects of Vitellogenin on behavior.

Molecular Pathways Associated with Nutrition

From the moment a larvae hatches from the egg, nutrition and nutrient-sensing pathways dictate much of its future physiological and behavioral destiny. Nutrition during development plays a key role in caste-differentiation between workers and queens, as well as between morphological worker castes in some species (Dolezal et al. 2014). Later, these pathways also regulate the behavior of workers, either directly or through their effects on nutrient storage and metabolism (reviewed in Dolezal et al. 2014).

Signaling pathways and genes associated with nutrient sensing and balance comprise a second genetic toolkit that regulates social phenotypes (Toth and Robinson 2007). The nutrient sensing Target of Rapamycin (TOR) pathway plays a key role in the developmental switch between queen- and worker-destined larvae in honey bees. In larvae fed a diet that results in development as queens, experimental suppression of *TOR* instead channels them into the worker developmental pathway. Expression of *hexamerins,* genes encoding amino acid storing proteins differ in queen versus worker-destined larvae in several species. Additionally, experimental manipulation of *hexamerin* expression in termites demonstrated that they have a regulatory function in the differentiation of soldier versus worker phenotypes in termites (reviewed in Dolezal et al. 2014). Experimental work in other taxa is needed to test how universal is *hexamerin* regulation of social phenotypes.

Early targeted studies used comparisons with *Drosophila melanogaster* to test correlations between the expression of candidate genes and behavioral modules in social insects (reviewed in Toth and Robinson 2007). The *Drosophila foraging* (*for*) gene encodes the cyclic-GMP-dependent protein kinase G (PKG). Allelic variation in the *for* gene produces "sitter" and "rover" fly phenotypes. Rovers have higher expression of *for* and range farther to forage than sitters. The sitter and rover *Drosophila* phenotypes were reminiscent of the nurse versus forager behavioral modules in honey bees to Yehuda Ben-Shahar and colleagues, who demonstrated that foragers have elevated expression of *Amfor,* the honey bee orthologue of the *for* gene. Further, experimental elevation of PKG signaling induces early foraging onset, demonstrating a regulatory role for PKG in foraging behavior (reviewed in Toth and Robinson 2007). Intriguingly, this pattern is reversed in the harvester ant *Pogonomyrmex barbatus,* highlighting the flexibility of this regulatory module (reviewed in Dolezal et al. 2014).

The behaviorally associated differences in the expression of *for* genes are regulated in part by both *vitellogenin* and JH, highlighting the important connections

between reproductive and nutrient-sensing toolkits. The JH/Vitellogenin regulatory module is involved in many of the physiological changes that accompany the transition from nursing to foraging behavior in several social species (reviewed in Dolezal et al. 2014). The age-associated lipid loss in honey bee workers prior to foraging onset is influenced by both dietary and non-nutrient signaling. *Vitellogenin* expression can influence lipid storage and metabolism, independently of dietary factors (Ament et al. 2011). *Vitellogenin* has also been implicated in the mobilization of stored lipids and carbohydrates that fuels foraging behavior via its role as an upstream regulator of adipokinetic hormone (AKH). AKH is an insect glucagon equivalent, and the expression of its receptor differs between nurse and forager honey bee workers, suggesting that it may play a role in the regulation of social behavioral modules (reviewed in Dolezal et al. 2014).

Insulin/Insulin-Like Growth Factor Pathway

The insulin/insulin-like growth factor pathway (IIS) is a major regulatory pathway linking the reproductive and nutritional genetic toolkits. IIS is a conserved, central regulator of life-history with regulator roles in both nutrition and reproduction (Kenyon et al. 2004). In insects, IIS plays a crucial role in egg development, but it is best studied in its role as a regulator of resource allocation in adults. The IIS pathway helps to direct resources (i.e., nutrition) toward reproduction when nutrients are plentiful and toward self-maintenance when resources are scarce. In this capacity, the IIS pathway has ancient connections to both JH and Vitellogenin (reviewed in Toth and Robinson 2007).

These interacting regulatory mechanisms appear to be especially accessible to selection, both natural and artificial. IIS pathway genes are over-represented in QTLs for traits in the pollen-hoarding strains of honey bees (Page 2013), suggesting that the behavioral and physiological phenotypes affected by the artificial selection regime for the high and low strains are under pleiotropic control of this pathway. The *insulin receptor substrate* (IRS) gene encodes a membrane-associated protein that relays signals from the insulin receptor and was identified as a candidate gene for foraging behavior. Experimental reduction of *IRS* expression via RNAi confirmed its regulatory role in foraging preference, causing worker bees to collect less nectar (reviewed in Dolezal et al. 2014). *IRS* may affect behavior via downstream effects on JH signaling, as it does during honey bee development, but this hypothesis awaits direct testing. However, IRS can also act as a substrate for other receptors, and it is possible that some of its effects on behavior may be downstream of other pathways such as epidermal growth factor.

IIS pathway components are differentially expressed in developing queens versus workers in several species, suggesting that it may play a critical role in the ontogeny of social phenotypes. There is growing interest in the IIS pathway as common pathway for the evolution of social behavior, and future research will identify how the diverse molecules that form the IIS regulate both behavior and development in social species.

Genomic Approaches to Social Evo-Devo

Until recently, most studies examining the proximate pathways of social evolution used a targeted approach, examining one or a few candidate genes identified from QTL studies or comparisons with *Drosophila.* As more and more high-quality, well-annotated genomes of social organisms became available, the field is beginning to move toward whole-genome approaches, using large population studies to identify genomic regions under selection or a comparative approach using species with a variety of social structures to identify genomic correlates of social behaviors. Such studies have provided support for the evolutionary developmental approach to the study of social behavior (see Kapheim et al. 2015 and references therein). As the evo-devo model suggests, large-scale comparative genomic studies have found evidence that complex social behaviors are governed by conserved and pleiotropic regulatory modules also present in related species with different levels of social complexity (Woodard et al. 2011).

A prediction of genetic evo-devo is that evolution of gene regulatory mechanisms, rather than changes in protein coding genes, are likely to underlie the evolution of complex traits, especially at increasing levels of pleiotropy (Carroll 2008). In support of this hypothesis is the finding that with increased levels of social complexity come an increased genomic capacity for gene regulation (Kapheim et al. 2015). This increased regulatory capacity comes in several forms, including increased transcription factor pleiotropy, more transcription factor binding sites in cis-regulatory regions, and evidence for rapid evolution of signal transduction pathways (Kapheim et al. 2015). These authors also found evidence for an association between social complexity and constraints on protein evolution.

However, it is clear that changes in protein coding sequences are also key to the evolution of novel traits including social behavior (Jasper et al. 2015 and references therein), with so-called novel taxonomically restricted genes (TRG) contributing to the development of novel social tissues such as glands that produce social pheromones. The expression of TRG is often both spatially and temporally limited and regulated by conserved, pleiotropic regulatory pathways upstream (Jasper et al. 2015). The position of a gene in a molecular network is associated with its exposure to positive selection. Many recent studies have found that genes at the periphery of regulatory network modules are more likely to experience positive selection. Differences in the coding sequences of these genes are less likely to have large and possibly deleterious pleiotropic effects due to their peripheral positions. However, these rapidly evolving genes are likely regulated upstream by conserved regulatory elements with highly pleiotropic functions across tissues and developmental stages.

Epigenetics and Social Behavior

An additional level of gene regulation is at the epigenome. Epigenetic mechanisms are powerful regulators of phenotype, and evidence is mounting that a particular

form of epigenetic regulation, DNA methylation, is critical for regulating social phenotypes. DNA methylation can effect transcription of genes by affecting the ability of the transcriptional machinery to reach a methylated segment of DNA (reviewed in Dolezal et al. 2014). In a large comparative genomic study, Kapheim and colleagues (2015) found that the number of genes predicted to be methylated increases with social complexity in bees. In addition to species-level differences, caste-based methylation patterns are predicted to influence both developmental and behavioral programs. Developing honey bee queens and workers have different methylation patterns, and genetic knockdown of methylation enzyme DNMT3 disturbed caste-based development (reviewed in Dolezal et al. 2014). Methylation patterns also differ in the high and low pollen-hoarding strains of honey bee workers, demonstrating that DNA methylation can be affected by selection over short-time scales and suggesting that it may shape worker behavior. These finding make understanding the role of epigenetics in social behavior an important direction for future research.

Conclusion

The evolutionary development approach to the study of social behavior has helped shape the large, recent advances in our understanding of the proximate mechanisms that gave rise to social phenotypes. The theoretical foundations laid by the ground plan hypotheses of social evolution have provided a useful framework on which to build the current large-scale genomic studies. As more and more comparative and population genomics studies are done, we are able to begin to identify how selection shapes gene networks to shape complex novel behavioral phenotypes from comparatively simple substrates in conjunction with the evolution of novel taxonomically restricted genes.

Cross-References

- ▶ Developmental Plasticity and Evolution
- ▶ Evo-Devo Lessons from the Reproductive Division of Labor in Eusocial Hymenoptera
- ▶ Eco-Evo-Devo
- ▶ Evo-Devo's Contributions to the Extended Evolutionary Synthesis

References

Ament SA, Queenie WC, Marsha MW, Scott EN, Johnson SP, Sandra LR-Z, Leonard JF, Gene ER (2011) "Mechanisms of stable lipid loss in a social insect." Journal of Experimental Biology 214 (22):3808–3821

Amdam GV, Page RE Jr (2010) The developmental genetics and physiology of honeybee societies. Anim Behav 79:973–980

Amdam GV, Norberg K, Fondrk MK, Page REJ (2004) Reproductive ground plan may mediate colony-level selection effects on individual foraging behavior in honey bees. Proc Natl Acad Sci U S A 101:11350–11355

Carroll SB (2008) Evo-devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. Cell 134:25–36

Dolezal AG, Toth AL (2014) Honey bee sociogenomics: a genome-scale perspective on bee social behavior and health. Apidologie 45:375–395

Dolezal AG, Flores KB, Traynor KS, Amdam GV (2014) The evolution and development of Eusocial insect behavior. In: Steelman JT (ed) Advances in evolutionary developmental biology. Wiley, New York

Gadau J, Fewell J, Wilson EO (2009) Organization of insect societies: from genome to sociocomplexity. Harvard University Press, Cambridge

Hamilton WD (1964) Genetical evolution of social behaviour I. J Theor Biol 7:1–17

Hölldobler B, Wilson EO (2009) The superorganism: the beauty, elegance, and strangeness of insect societies. WW Norton, New York

Jasper WC, Linksvayer TA, Atallah J, Friedman D, Chiu JC, Johnson BR (2015) Large-scale coding sequence change underlies the evolution of postdevelopmental novelty in honey bees. Mol Biol Evol 32:334–346

Kapheim KM, Pan H, Li C, Salzberg SL, Puiu D, Magoc T, Robertson HM, Hudson ME, Venkat A, Fischman BJ (2015) Genomic signatures of evolutionary transitions from solitary to group living. Science 348:1139–1142

Kenyon C, Campisi J, Wallace D (2004) From worms to mammals: the regulation of lifespan by insulin/IGF-1 signaling. Mol Biol Cell 15:354A

Maynard Smith J, Szathmáry E (1997) The major transitions in evolution. Oxford University Press, New York

Michener CD (1974) The social behavior of the bees. Belknap, Cambridge

Nowak MA, Tarnita CE, Wilson EO (2010) The evolution of eusociality. Nature 466:1057–1062

Page RE Jr (2013) The spirit of the hive. Harvard University Press, Cambridge

Roy-Zokan EM, Cunningham CB, Hebb LE, McKinney EC, Moore AJ (2015) Vitellogenin and vitellogenin receptor gene expression is associated with male and female parenting in a subsocial insect. Proc R Soc B Biol Sci 282:20150787

Toth AL, Robinson GE (2007) Evo-devo and the evolution of social behavior. Trends Genet 23:334–341

Toth AL, Varala K, Henshaw MT, Rodriguez-Zas SL, Hudson ME, Robinson GE (2010) Brain transcriptomic analysis in paper wasps identifies genes associated with behaviour across social insect lineages. Proc R Soc B Biol Sci 277:2139–2148

West-Eberhard MJ (1987) Flexible strategy and social evolution. In: Itô Y, Brown JL, Kikkawa J (eds) Animal societies: theories and fact. Japan Scientific Societies Press, Tokyo

West-Eberhard MJ (1996) Wasp societies as microcosms for the study of development and evolution. In: Turillazzi S, West-Eberhard MJ (eds) Natural history and evolution of paper-wasps. Oxford University Press, New York

Wilson EO (1971) The insect societies. Harvard Belknap, Cambridge, MA

Winston ML (1987) The biology of the honey bee. Harvard University Press, Boston

Woodard SH, Fischman BJ, Venkat A, Hudson ME, Varala K, Cameron SA, Clark AG, Robinson GE (2011) Genes involved in convergent evolution of eusociality in bees. Proc Natl Acad Sci U S A 108:7472

Evo-Devo and Cognitive Science

Annemie Ploeger and Frietson Galis

Contents

Abstract

Evo-devo is an approach that integrates knowledge on evolution and development. Cognitive science is a research field that tries to unravel the functioning of the mind and the underlying processes. In this chapter, the main subfields within cognitive science that have contributed to a better understanding of the evolution and development of the mind are discussed. Highlighted are the subfields of evolutionary cognitive science, developmental systems theory, genes × environment interaction research, epigenetics, comparative cognitive science, and

A. Ploeger (✉)
Department of Psychology, University of Amsterdam, Amsterdam, The Netherlands
e-mail: a.ploeger@uva.nl

F. Galis (✉)
Naturalis Biodiversity Center, Leiden, The Netherlands
e-mail: frietson.galis@naturalis.nl

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_44

cognitive neuroscience. Finally, the question what cognitive scientist can learn from research in evolutionary developmental biology is addressed. Many evo-devo biologists study morphogenesis, which is relevant for cognitive development, but it is not always straightforward how to apply their knowledge to cognitive science research. Interdisciplinary research is strongly recommended, so scholars from different fields such as morphology, genetics, neuroscience, primatology, and psychology can learn from each other and contribute to the unraveling of the working of the mind.

Keywords

Evo-devo · Cognitive science · G×E interactions · Epigenetics · Developmental systems

Introduction

Cognitive science is a broad, interdisciplinary research field that tries to unravel the functioning of the mind and the underlying processes. It includes the study of perception, motor control, attention, consciousness, learning, memory, representation of knowledge, language, problem-solving, creativity, decision-making, reasoning, and intelligence (e.g., Newell 1990). The emergence of cognitive science has been called the cognitive revolution, which started in 1959. Linguist Noam Chomsky argued that language acquisition cannot be explained by simple stimulus–response associations proposed by behaviorism, which was the dominant paradigm in psychology at that time. Behaviorists, amongst others their famous proponents Ivan Pavlov, Edward Thorndike, John B. Watson, and B. F. Skinner, argued that mental processes cannot be scientifically studied and that psychologists should restrict their research to observable behavior. Chomsky's discussion preluded the rise of cognitive science.

Cognitive science has become a successful field, partly because of the development of new research tools, such as brain imaging techniques and computer simulations. Cognitive neuroscience is a rapidly growing subfield in which concepts such as attention and memory are linked to specific brain areas and neural activity. Artificial intelligence is another subfield that uses insights from cognitive research to create computer models of the mind. In huge programs such as the *Human Brain Project*, headed by physiologist Henry Markram (see Markram et al. 2011), knowledge on cognitive architectures, brain simulations, high-performance computing, neuroinformatics, neurorobotics, and other disciplines is combined with the aim to simulate the whole human brain. Results from this kind of projects show the fruitfulness of cognitive science and also how important technological progress has been.

The first aim of this chapter is to provide an introduction of the history of the attempts of cognitive scientists to integrate their work with developmental and evolutionary approaches. After the cognitive revolution, the study of cognitive

development started to grow. Jean Piaget, with his stage theory of cognitive development, became the major proponent of this field. Later, in the 1990s, the study of the evolution of cognition arose under the flag of evolutionary psychology. Major proponents of this field in the 1990s were cognitive psychologist Leda Cosmides, anthropologist John Tooby, linguist Steven Pinker, and social psychologist David Buss. In the first years of the new millennium, the first attempts were made to integrate developmental and evolutionary approaches to cognitive science by developmental psychologists David Bjorklund and David Geary, among others. Only lately, attempts have been made to integrate evo-devo biology and cognitive science.

The second aim is to show the importance of evo-devo research for cognitive science. Evo-devo is an approach that integrates knowledge on evolution and development. Evolutionary biologists study evolutionary change of organisms over generations; developmental biologists study the development of organisms within a single lifetime. Evo-devo researchers try to unravel the interaction between these two processes – evolution and development, to obtain a fuller understanding of each of these processes.

Before the contribution of evo-devo research to cognitive science is described, first the contributions of developmental cognitive science and evolutionary cognitive science are explained separately.

Developmental Cognitive Science

Developmental cognitive science is the study of cognitive development, from prenatal development to cognitive aging. The study of prenatal cognitive development is limited, because it is hard to study the cognition of fetuses in the womb. Most of this research is focused on the senses, especially the visual and auditory system. Vision research with premature infants revealed that they can distinguish between light and dark at 28 weeks after conception, and that they can distinguish different patterns at 30 weeks. Considering the auditory system, fetuses start to react with movement to acoustic stimulation between 23 and 25 weeks. It is also well known that newborns remember sounds they heard during their last month in the womb. In addition, newborns show a preference for their mother's voice compared to a stranger, and they can discriminate between their mother's and a foreign language.

In general, developmental cognitive scientists study the same topics as cognitive scientists, but they emphasize differences between children and adults by conducting longitudinal or cross-sectional studies. An example of a topic that recently has attracted a lot of attention is the development of executive functions. This is an umbrella term for all processes necessary for cognitive control, such as inhibition, task flexibility, planning, and working memory. Researchers generally agree that normal or good development of executive functions is required to be able to deal with daily-life problems in our complex society. Many common psychiatric

disorders, such as ADHD, autism, and anxiety, are associated with atypical development of executive functions.

As was mentioned in the introduction, the theory on cognitive development developed by Jean Piaget has been most influential. He argued that cognitive development proceeds stagewise, with children acquiring knowledge by changing their cognitive structures by the processes of assimilation and accommodation. When children encounter new situations, they try to assimilate their experiences into their existing cognitive structures. Only when they find out that the existing structures fail to explain the new situation, they will build new cognitive structures (a process called accommodation). New approaches that arose out of Piaget's theory are neuroconstructivism (e.g., Karmiloff-Smith 2006), which combines Piaget's theory with recent neuroscientific findings and dynamic systems theory (e.g., Thelen and Smith 1994). Adherents of the latter approach argue that development can be best described by differential equations, where development is modeled as a trajectory through state space. Following this approach, children learn by discovering that available patterns of knowledge are incomplete, leaving them in a state of disequilibrium, after which a new equilibrium can be reached, when new patterns of knowledge are formed. The process of breaking down old patterns and establishing new ones occurs by means of phase transitions. This process is considered to be self-organized, because there are no control parameters that govern it. This approach has been specifically successful in explaining motor development, but also other areas of cognitive development.

Evolutionary Cognitive Science

Evolutionary cognitive science is the study of the evolution of cognition, with the expectation that knowledge about the evolution of cognition improves our understanding of the working of the human mind (Barkow et al. 1992). Evolutionary cognitive scientists try to discover cognitive adaptations that have been under natural selection or cognitive fitness indicators that were sexually selected. Well-studied examples of cognitive adaptations are language, face recognition, color perception, cheater detection, and spatial abilities that are related to hunter-gatherer skills (see chapter ▶ "Evo-Devo of Language and Cognition"). Geoffrey Miller (2000) has argued that many aspects of human cognition are sexually selected, such as art, music, humor, and science. Empirical support for this hypothesis is provided by studies that showed an association between these phenomena and measures of fitness, e.g., health and symmetry, and levels of estradiol (in females) and semen quality (in males). Other evolutionary cognitive scientists have used a comparative approach. For example, psychologist and primatologist Michael Tomasello (2014) has compared the cognition of human children and chimpanzees and concluded that their physical cognition (e.g., knowledge about quantities) is similar, but that 2-year-old humans already have a better developed social cognition (e.g., empathy) than adult chimpanzees.

Evo-Devo and Cognitive Science

Evo-devo research in cognitive science can be carried out in many different ways. Several subfields that combine an evolutionary and a developmental approach in the study of cognition are outlined. First, the work of evolutionary developmental cognitive scientists is discussed. Second, the work of developmental systems theorists is discussed. Third, studies on the interaction between genes and environment by cognitive scientists are discussed. Fourth, research on epigenetics in cognitive science are discussed. Fifth, comparative evo-devo studies by cognitive scientists are discussed. Sixth, the studies on cognition by evolutionary developmental neuroscientists are discussed. Finally, the work of evo-devo biologists extrapolating results on evolutionary developmental mechanisms to the field of cognition are discussed.

Evolutionary Developmental Cognitive Science

Evolutionary developmental cognitive scientists are usually trained as developmental psychologists and try to understand the origin of the behavior and cognitive abilities of children. Evo-devo psychologist David Bjorklund (2009) has studied the adaptive role of cognitive immaturity. Children are often viewed as "unfinished adults," but Bjorklund showed that children's failures on cognitive tests are sometimes adaptive. For example, young children often overestimate their cognitive abilities. It was found that this overestimation was associated with better cognitive performance at a later age, probably because overconfidence leads to more exploratory behavior. This suggests that overestimation is functional. Evo-devo psychologist David Geary (2005) has addressed educational psychology from an evo-devo perspective. He argued that children learn some abilities naturally, such as language and simple counting. Also children that are not stimulated by their parents or formally educated will learn these. He called these primary abilities. Other abilities are secondary, such as higher-order mathematics, which require formal instructions to be learned. He argued that children will be better motivated to learn secondary abilities when they are coupled to primary abilities.

Developmental Systems Theory

Developmental systems theorists argue that organisms do not only inherit the DNA of their parents but a whole developmental system, which includes the environment in its full spectrum – at the cellular, tissue, body, family, and ecological level (Oyama et al. 2001). They argue that the scope of some evolutionary cognitive scientists is too limited. For example, developmental psychologist Elizabeth Spelke has argued that human babies are born with core knowledge about objects, social agents, numbers, and geometry (Spelke and Kinzler 2007). Her conclusions are based on empirical results that revealed that newborns, even on the first day after birth, show

different responses (e.g., different looking times) to different situations, even though they have no prior experience with these situations. Spelke argues that, based on her data, the inevitable conclusion is that some basic knowledge is innate. Developmental systems theorists tend not to agree with this conclusion. Nonetheless, they agree that a functional perspective is necessary to get a better understanding of the mind, but they highlight the importance of ecologically relevant conditions to study cognition, in agreement with evolutionary ecologists.

Another illustration of the sometimes limited scope of evolutionary cognitive scientists was put forward by developmental psychologist Annette Karmiloff-Smith (2006). She criticized some of the arguments put forward by Steven Pinker in the discussion on language evolution. Pinker argued that the gene *FOXP2* is "the language gene." Research revealed that members of a family with severe language problems showed mutations in *FOXP2*, suggesting that it is a crucial gene in language development. Later research revealed that *FOXP2* is not specifically involved in language development but in multiple processes that are associated with producing sequential movements. Karmiloff-Smith argued that the effects of many genes are associated with general processes and that specific complex traits, such as language, are the result of several developmental pathways, with no simple relationship to a specific DNA sequence.

Some researchers have tried to bridge the gap between evolutionary cognitive science and developmental systems theory. For example, David Bjorklund (2015) has proposed the concept of an evolved probabilistic cognitive mechanism. He argued that it is inevitable that natural selection has selected cognitive adaptations to deal with problems related to survival and reproduction. However, these adaptations will not develop when individuals are placed in an environment that is not species typical (e.g., for humans an environment without spoken language). Therefore, he argued that all evolved cognitive mechanisms are probabilistic, with their development being dependent on the right environmental input.

Genes X Environment Interactions

The discussion on the interaction between the influence of genes and that of the environment on the development of phenotypes goes back to the seventeenth century when philosopher John Locke started the nature-versus-nurture debate. Nowadays most psychologists and biologists agree that both nature and nurture are important, with some researchers pointing to the importance of culture (e.g., Richerson and Boyd 2005; see chapter ▶ "Evo-Devo and Culture"), next to nature (genes), and nurture (parenting). Recently, a wide array of new discoveries has been made in studies that examine the interaction effects between specific genes and specific environmental input (i.e., G×E studies) on specific behavioral outcomes (e.g., aggression, depression, etc.). The paradigm case is a longitudinal study performed by Avshalom Caspi and colleagues (2002). They followed a large sample of boys from birth to adulthood in order to study the development of antisocial behavior. Data on individual differences at a polymorphism in the

promoter of the *MAOA* gene and maltreatment were collected. It was found that the interaction between maltreatment and having a genotype associated with low levels of *MAOA* expression resulted in a high risk of developing antisocial behavior. This was the first study that showed a significant G×E interaction. Many other studies followed.

Interesting theoretical contributions were made by psychologists Bruce Ellis, Jay Belsky, and colleagues. Ellis et al. (2011) reviewed several G×E studies and observed that some genotypes are associated with a vulnerability to develop psychopathology in interaction with a negative environment (such as maltreatment), but that the very same genotype is also associated with positive outcomes in interaction with a positive environment (such as the absence of maltreatment). They prefer to call these "plasticity genes," rather than "vulnerability genes." An appealing comparison with orchids and dandelions has been made. Orchids are beautiful flowers when they are taken good care off, but nothing but a boring empty stem remains when they are maltreated. In contrast, dandelions are arguably not that beautiful, but they can grow everywhere, even at a roadside or a dumping ground. Thus, some people are more susceptible to specific environmental influences – based on their genotype – and can be regarded as orchids, whereas other people are less susceptible to these influences and can be regarded as dandelions (although, naturally, plants that are more tolerant to environmental conditions are not necessarily less beautiful than those that are less tolerant). This is called differential susceptibility theory and has received considerable empirical support in the past decade.

A hypothesis derived from this theory is that orchids benefit more from psychotherapy than dandelions. It is well known that the success rate of psychotherapies is variable – some individuals improve considerably, while others do not. Where do these large individual differences come from? Part of the answer may be that individuals with a genotype that makes them more susceptible for environmental influences – the orchids – benefit more from specific therapies, because they are more susceptible to both positive *and* negative environmental influences than dandelions. A study on the interaction effect of an intervention and a genotype (the polymorphism in the promoter of the *DRD4* gene) on problem behaviors in children provided support for this hypothesis (for this and other interesting references, see Ellis et al. 2011). It was found that the positive effect of the intervention was largest in the group of children with *DRD4* genotypes associated with low levels of dopamine reception efficiency. This study provided experimental support for the hypothesis that children are differentially susceptible to intervention effects based on genetic differences. More studies on different types of differential susceptibility, age effects, and the relationship with therapy success are necessary.

In sum, this work shows the importance of studying both genetic and environmental differences to explain the development of phenotypical outcomes. The biological mechanisms underlying these processes remain unknown. Studies on epigenetics (see next section) should contribute to the understanding of these mechanisms.

Epigenetics

Recently, new discoveries have been made under the umbrella of epigenetics. Epigenetics refers to changes that influence how genes are expressed, other than changes in the DNA sequence (e.g., Masterpasque 2009). Two major epigenetic mechanisms are DNA methylation (the attachment of methyl molecules to cytosines, which switches off the expression of the gene) and histone modification (a change in the histone proteins around which DNA is wrapped, which causes the expression of the gene to switch on or off). Research relevant for cognitive science comes from two main sources: studies on the effects of early caregiving on later development and studies on the role of epigenetics in psychiatric disorders. Famous work was carried out on the effects of licking and grooming by the mother on later behavior, physiology, and epigenetics of newborn rats (for relevant references, see Masterpasque 2009). They found differences in the reactivity of the hypothalamic-pituitary-adrenal (HPA) axis of offspring raised by either high licking/grooming or low licking/grooming mothers. High HPA reactivity is associated with stress responses and psychiatric disorders in humans. The decrease in HPA reactivity in high licking/grooming offspring of rats is directly linked to decreased methylation of glucocorticoid receptor genes in the hippocampus. Cross-fostering experiments showed that the individual differences in reactivity were the result of licking and grooming patterns and not of differences in genotype of the mother. Performing similar experiments in humans is not possible, but postmortem studies with maltreated versus nonmaltreated humans revealed similar epigenetic patterns as were found in rats.

It is now well known that epigenetic processes play an important role in the development of psychiatric disorders. For example, research in mice has revealed that social stress leads to low levels of brain-derived neurotropic factor (BDNF) in the hippocampus due to histone modification. Similar patterns have been found in humans diagnosed with major depression disorder. Considering schizophrenia, postmortem studies revealed low concentrations of reelin in the brains of patients, which were associated with hypermethylation of the reelin gene. With regard to autism, studies were performed on a monozygotic twin, of which one was diagnosed with autism, while the other was not, despite their identical DNA sequence. Results revealed that the individual with autism showed methylation-dependent silencing of the *BCL-2* and the *RORA* gene.

Only a few of the many recent studies on epigenetics that are relevant for cognitive science have been described here. The studies on epigenetics are at the heart of evo-devo research: how do inherited DNA sequences interact with developmental processes that vary under the impact of environmental influences to form constant or novel phenotypes.

Evolutionary Developmental Comparative Cognitive Science

Evolutionary developmental comparative cognitive scientists compare the cognition of nonhuman primates and human children. The book *The Origins of Intelligence*

(Parker and McKinney 1999) is a hallmark in this field. In this book, the development of different primate species was compared, following the theory on cognitive development by Jean Piaget, as mentioned above. However, the choice to test children is usually not made because researchers are interested in a developmental perspective; often adult nonhuman primates and young human children (2–3-years-olds) are compared because their cognitive levels are similar. For example, the Primate Cognition Test Battery has been developed in order to have tests that both adult nonhuman primates and young human children can perform. Recently, a first large study was published where young chimpanzees and bonobos (together called *Pan* infants) and human children were followed longitudinally for 3 years with a new test battery, the Comparative Developmental Cognitive Battery (for relevant references, see Tomasello 2014). Individuals were tested on social cognition (e.g., gaze following, imitation, goal understanding), physical cognition (e.g., discriminating different quantities, tool use, understanding of object permanence), attention, and motivation. Results revealed that over all tasks, the rate of improvement is slower in *Pan* infants than in human children, and that abilities that require cooperative motivation do not emerge at all in *Pan* individuals. This is useful research, because it provides us insight in the differences and similarities between three closely related species, including two sister species of humans, and thereby also in the cognitive evolution of humans.

Evolutionary Developmental Cognitive Neuroscience

Evolutionary developmental cognitive neuroscientists study how brains change, both from an evolutionary and a developmental perspective. Two general models about brain evolution and development have emerged. One model emphasizes the relative independence and modularity of different brain structures, assuming that, for example, the auditory system requires different neural networks from the olfactory system. From an evolutionary perspective, it is argued that most brain areas are functionally specialized, and hence selection pressures will differentially affect brain areas (Barrett 2012). The other model assumes that the entire brain will change in response to selection pressures, and that architectural and functional constraints ensure that brain size as a whole will change.

Four types of brain growth have been observed in the evolution of mammals (Finlay et al. 2001). First, brain growth that is associated with body growth; when the body grows, the brain grows accordingly. Second, the brain can grow while body size remains constant; this kind of brain growth is associated with enhanced behavioral and cognitive capabilities in the course of evolution. Third, the limbic system, the part of the brain associated with emotion, motivation, memory and olfaction, grows independently of overall brain and body size. Fourth, other individual brain parts may vary in size independently of overall brain and body size. For example, prefrontal gray volumes are 4.8 times larger in humans compared to chimpanzees (for a review, see Schoenemann 2006). In addition, the relative size of the neocortex and striatum is positively correlated with tool use, innovation, and social learning. These four different types of growth indicate that both independent evolution of

different brain structures and size changes of the entire brain have been important for brain evolution and development.

Evolutionary Developmental Biology and Cognitive Science

The last subject addressed is the question what cognitive scientists can learn from evo-devo research in biology. Many evo-devo biologists study morphogenesis, and it is not immediately obvious how to relate their research findings to cognitive science. This issue has been addressed in an earlier paper (Ploeger and Galis 2011; relevant references can be found in this paper). A first conclusion was that some of the main issues in evo-devo biology and cognitive science overlap and that tools can be used profitably by both types of scientists. For example, modularity is a main topic in both evo-devo biology and cognitive science. Evo-devo biologists study the modularity of developmental and genetic pathways as well as that of body parts, whereas cognitive scientists study the modularity of the human mind. Evo-devo biologists have developed tools to study modularity that have been largely unnoticed by most cognitive scientists. It was argued that cognitive scientists can benefit from these tools. Another example is the issue of plasticity. Both evo-devo biologists and cognitive scientists are interested in the question how plasticity is important in an individual life time and how it evolved over generations. It was also argued in this case that evo-devo biologists have developed tools that should benefit cognitive scientists.

A second conclusion was that evo-devo biology research can provide new insights in the evolution and development of psychiatric disorders. One example is research on developmental constraints. For example, it was proposed that mutations that give rise to the positive aspects of the savant syndrome, i.e., the impressive memory capacity, cannot spread in the population, due to a developmental constraint that has its roots in low modularity. This developmental constraint is thought to result from the high interactivity (low modularity) among body parts during early organogenesis (i.e., the phylotypic stage). The interactivity during this stage involves all components of the embryo, and as a result mutations that affect one part of the embryo also affect other parts (pleiotropic effects or side-effects), with almost inevitably negative effects among them. As a result of the sheer unavoidable deleterious side-effects, there is strong selection against mutations with an effect on this stage, presumably leading to the extremely strong conservation of the entire stage. The low modularity of this embryonic stage has implications for the conservation of many traits of the body plan and is for example at the root of the strong developmental constraint against changes of the number of cervical vertebrae in mammals. The same hypothesis was proposed for the savant syndrome. Mutations, which give rise to the development of the positive aspects of the savant syndrome, i.e., an impressive memory capacity, will virtually always have deleterious side-effects on the development of other phenotypic traits. The support for such strong deleterious side-effects that are associated with the savant syndrome (e.g., autism and/or impaired motor coordination) was discussed. One of the new insights that

were reported is that psychiatric disorders that result from brain deviations usually appear to start to develop as early as during the phylotypic stage, due to the general instability and vulnerability of the stage that results from the intense inductive interactivity. Another example is research on epistatic interactions between the effects of different genes. It is a paradox why psychiatric disorders, such as schizophrenia and autism, are common, why they are highly heritable, and why they still persist. Why did natural selection not wipe out these disorders? The answer lies in the polygenic nature of most psychiatric disorders. When multiple genes are involved, the effects of these genes will interact during development, sometimes resulting in positive but sometimes in negative outcomes. Interdisciplinary approaches in which insights from evo-devo research on morphogenesis have shown to yield new hypotheses about the evolution and development of psychiatric disorders.

Conclusion

Evo-devo in cognitive science consists of a wide array of subfields, including evolutionary developmental cognitive research, developmental systems theory, genetic research (G×E interactions), epigenetic research, comparative research, neuroscientific research, and applications of evo-devo biology in cognitive science. Interdisciplinary research is strongly recommended, so that scholars from different fields such as theoretical biology, morphology, embryology, genetics/genomics, neuroscience, primatology, and psychology can learn from each other and contribute to the unraveling of the working of the mind.

Cross-References

- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Evo-Devo and Cognitive Science
- ▶ Evo-Devo and Culture
- ▶ Evo-Devo of Language and Cognition
- ▶ Evo-Devo of Social Behavior

References

Barkow JH, Cosmides C, Tooby J (eds) (1992) The adapted mind: evolutionary psychology and the generation of culture. Oxford University Press, New York

Barrett HC (2012) A hierarchical model of the evolution of human brain specializations. P Natl Acad Sci USA 109:10733–10740

Bjorklund DF (2009) Why youth is not wasted on the young: immaturity in human development. Wiley-Blackwell, Hoboken

Bjorklund DF (2015) Developing adaptations. Dev Rev 38:13–35

Caspi A, McClay J, Moffitt TE, Mill J, Martin J, Craig IW, Taylor A, Poulton R (2002) Role of genotype in the cycle of violence in maltreated children. Science 297:851–854
Ellis BJ, Boyce WT, Belsky J, Bakermans-Kranenburg MJ, van Ijzendoorn MH (2011) Differential susceptibility to the environment: an evolutionary–neurodevelopmental theory. Dev Psychopathol 23:7–28
Finlay BL, Darlington RB, Nicastro N (2001) Developmental structure in brain evolution. Behav Brain Sci 24:263–308
Geary DC (2005) The origin of mind: evolution of brain, cognition, and general intelligence. American Psychological Association, Washington, DC
Karmiloff-Smith A (2006) The tortuous route from genes to behavior: a neuroconstructivist approach. Cogn Affect Behav Neurosci 6:9–17
Markram H et al (2011) Introducing the human brain project. Procedia Comput Sci 7:39–42
Masterpasque F (2009) Psychology and epigenetics. Rev Gen Psychol 13:194–201
Miller G (2000) The mating mind: how sexual choice shaped the evolution of the human mind. Vintage, London
Newell A (1990) Unified theories of cognition. Harvard University Press, Cambridge, MA
Oyama S, Griffiths PE, Gray RD (eds) (2001) Cycles of contingency: developmental systems and evolution. MIT Press, Cambridge, MA
Parker ST, McKinney ML (1999) The origins of intelligence: the evolution of cognitive development in monkeys, apes, and humans. Johns Hopkins University Press, Baltimore
Ploeger A, Galis F (2011) Evo devo and cognitive science. WIREs Cogn Sci 2:429–440
Richerson PJ, Boyd R (2005) Not by genes alone: how culture transformed human evolution. University of Chicago Press, Chicago
Schoenemann PT (2006) Evolution of the size and functional areas of the human brain. Annu Rev Anthropol 35:379–406
Spelke ES, Kinzler KD (2007) Core knowledge. Dev Sci 10:89–96
Thelen E, Smith LB (1994) A dynamic systems approach to the development of cognition and action. The MIT Press, Cambridge, MA
Tomasello M (2014) A natural history of human thinking. Harvard University Press, Cambridge, MA

Evo-Devo of Language and Cognition

Sergio Balari and Guillermo Lorenzo

Contents

Abstract

Historically, the task of disentangling the evolutionary origins of language has been obscured by a number of difficulties that may be diagnosed as the *problem of ontology* (what the evolved phenotype is), the *problem of computation* (what kinds of cognitive processes subserve linguistic activity), the *problem of representation* (what is the nature of the objects of computation), the *problem of homology/novelty* (how language relates with animal cognition at large), and the *problem of selection* (how language has been fixed as a species-typical

S. Balari (✉)
Universitat Autònoma de Barcelona and Centre de Linguística Teòrica, Barcelona, Spain
e-mail: Sergi.Balari@uab.cat

G. Lorenzo
Universidad de Oviedo, Oviedo, Spain
e-mail: glorenzo@uniovi.es

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_43

trait). While assuming that facets of these problems remain as recalcitrant as ever, this chapter explains how the adoption of the developmental perspective offers the promise of gaining a degree of explanatory accuracy hitherto unknown in this field of specialization.

Keywords

Evolution of cognition · Evolutionary linguistics · Computational theory of mind

Introduction

The development of evolutionary ideas of mind and language started well before Darwin, with pre-Lamarckian thinkers and Lamarck himself, who had a strong influence on Darwin's own account of the evolution of human mental capacities. They shared the conviction that the mental capacities of human and nonhuman animals could be given a naturalistic explanation and that this explanation had a close connection with another natural phenomenon: the transformation of species. Within this general framework, however, they faced a paradox that emerged from the clash between their transformist ideas and their commitment with Locke's and Hume's epistemology.

At least since Aristotle, causal explanations of behavior are based on the assumption that a specific behavior is the product of some proximal, i.e., internal or mental, cause; mental causes may themselves be caused by some distal (external) cause, but this need not be necessarily so: the mind has the power to generate causes without the participation of external stimuli. For any account of cognition along these lines to work, two basic ingredients have customarily been assumed to be essential: nervous systems possess (i) some means to *represent* their environment and (ii) the ability to deal with manipulative *processes* in order to produce new representations and, eventually, cause some behavior. The Lockean/Humean tradition was no exception to this: *ideas* represent the world and these are connected through a process of *association*. The problem for these earlier evolutionists was that, in line with the empiricist epistemology they assumed, ideas could only be acquired through the senses, by direct interaction with the environment. This was difficult to reconcile with the observation that all animals were capable of producing more or less complex behaviors immediately at the time of birth, which seemingly supported the idea of innate instincts rather than that of acquired habits. Erasmus Darwin's and Pierre-Jean Cabanis' way out of this conundrum was quite an ingenious one, as they appealed to ontogenetic processes to explain the persistence of certain behavioral traits in organisms of the same species. Such early-acquired sensations and habits, most of them through the use of specific anatomical structures of each organism, would later become the basis of new or modified behaviors and anatomical structures in response to the changing conditions of the environment and could later be inherited by the organism's progeny.

Darwin's original account was not too different from the ones developed by his grandfather Erasmus or by Lamarck. To be sure, Darwin bought wholesale Hume's theory of ideas and their associations, which made its way virtually unchanged in the *Descent*, with the exception of Darwin's willingness to accept the existence of innate inherited social habits (Darwin 1879). The *Descent*'s first chapter is devoted to homology, and Darwin's strategy becomes immediately clear as soon as one reaches Chaps. 3 and 4. There, Darwin puts forward a detailed application of the comparative method but centered on the "mental powers of man and lower animals," from basic instincts to moral senses, through attention, memory, tool use, language, and consciousness, among others. Recall that Darwin redefined Richard Owen's original notion of homology by introducing a historical dimension that was never present in Owen's homology. In so doing, Darwin simply accommodated homology to his idea of evolution seen as descent with modification in such a way that any two traits would be classified as homologous to the extent that it could be shown that they were modified variants of the same trait in a common ancestor; hence the importance of innate inherited social habits in Darwin's model, as they constitute the bedrock on which a complete evolutionary story of the gradual modification of basic mental/behavioral traits is constructed, linking basic social habits with the most sophisticated human capacities.

One need not go into the details to identify in Darwin's account two critical problems shared with contemporary evolutionary accounts of cognition: the *problem of homology/novelty* and the *problem of ontology*. Both are somehow connected, since both have to do with the phenotypes one chooses to focus on when investigating the evolution of some cognitive trait. The criteria one applies to determine whether two phenotypic traits are homologous or not are crucial: should one pay attention only to form, only to function, or both? The question becomes particularly vexing when dealing with cognitive phenotypes: must one focus on the functional, externally observable features of some cognitive trait in order to determine exactly its phenotypic characteristics, or must one just study its neurophysiological underpinnings independently of the observable behaviors/functions these may cause? Particularly illuminating to appreciate the subtle questions that the problems of homology/novelty and of ontology pose for any naturalistic account of cognition is Darwin's brief treatment of the origins of language in Chap. 3 of the *Descent* (Darwin 1879: 106–114). Right from the outset, Darwin expresses his conviction that there is a strict functional continuity between the vocal and communicative behaviors of animals and human speech and that the apparent gap separating humans from other animals can be explained by the "high development of [the] mental powers" of the former (Darwin 1879: 108–109). Darwin's is thus a paradigmatic example of the typical evolutionary explanation (not uncommon still today) where functional/behavioral continuity is favored over homological analyses based only on structural considerations, with the consequence of turning the linguistic phenotype into a heterogeneous admixture of features, going from brain structures to grammatical properties like agreement, through vocalization organs and multifarious communicative behaviors, like gestures and facial expressions, for example.

Darwin strived to accommodate his account of the evolution of mind and behavior within the strict limits imposed by his gradualist vision where the main causal force was natural selection. But he was forced to make some concessions to that view, since he found difficulties in explaining the origins of certain traits by solely appealing to natural selection. Actually, the complementary mechanisms of community and sexual selection were Darwin's reaction to a number of objections raised by some of his closer allies in many other matters—especially Charles Lyell, Alfred Russel Wallace, and Herbert Spencer—on the power of natural selection as the adequate mechanism to explain the origins of humans' higher cognitive capacities. With the obvious differences in emphasis and preference for one or another alternative explanation, one could fairly contend that the debate on the *problem of selection* is still alive today.

In the following sections, the different problems identified in this historical introduction will be presented, adopting a contemporary stance and suggesting some possible solutions offered by an evo-devo perspective.

The Problem of Ontology

The task of disentangling the evolutionary origins of language suffers from the lack of a consensual view about what the evolved linguistic phenotype is supposed to be. In a nutshell, theoretical positions differ along the following two main coordinates:

1. Is language an external, socially shared code of sorts, which somehow gets accommodated within an a priori uncompromised neural substrate in the early experience of children? Or is language an internal, organ-like component of the human brain, ready to make sense of certain salient environmental stimuli that simply shape it into a language-particular condition?
2. Is language a self-contained component of the human brain, showing the signature of a well-defined form-function unit relatively to other similar brain specializations (face recognition, motor planning, early vision, and so on)? Or is language a composite of different brain specializations, jointly laying down an emergent functionality (as, say, vision), but which are, individually taken, unspecific as for their linguistic dedication?

A brief survey of the theoretical elaboration of the concept of "language" reveals that, as for coordinate (1), a strong shift took place in the mid-twentieth century from externalist views of language to a predominant internalist stance (Chomsky 1986). As for coordinate (2), a similar shift is presently on the way toward a composite or mosaic conception of language when conceived of from such an internalist perspective (Boeckx 2012), which demotes theses like strong domain specificity or encapsulation that were once part and parcel of Chomsky's internalism. In any event, evolutionary linguists do not consensually adhere to any

of the cardinal positions thus far commented, and many middle-ground positions also exist that complicate the picture.

A consequence of the situation described above is that evolutionary linguistics seems doomed to remain anchored to dualist stances that run against the aim of releasing the evolutionary explanation of language from explanatory recipes with assorted exceptionalist flavors. Not surprisingly, the field continues to be receptive to a strong "culture/biology" divide that keeps apart the behavioral/psychological dimension of language and its biologically proper underpinnings, as if they were different (somehow connected) realms for which a more integrative ontology appears to be a mistakenly reductionist project. Besides, in the aftermath of the influential Hauser et al. (2002) paper, a growing acceptance appears to exist of a new-wave dualism that keeps apart what is deemed properly biolinguistic (i.e., language specific) within the human organism and what is just biological. These kinds of questions relate to issues that have been the target of recent evo-devo-oriented philosophical analyses, from which evolutionary linguistics may draw inspiration.

One obvious point of reference for linguists may be the concept of "developmental hybrid" currently being applied to accommodate socially shared and traditionally transmitted practices to the evo-devo agenda (Caporael et al. 2014). The underlying idea is that organisms are capable of engaging in interactive dynamics with specific components of the environment that transform their internal constitution in ways that reinforce the reiteration of such dynamics and pave the way to further, eventually broader forms of environment-organism interactions. In as much as it makes sense to say that the organism attunes its own properties in the process to the corresponding properties of the environment, it also makes sense to conclude that what develops is a hybrid entity. Hybrids so constructed may act as developmental scaffolds to further complexifications of such entities. Moreover, the link that obtains between environment and organism through these hybridization processes favors their endurance and intergenerational transmissibility, which means that the entities of concern are exposed to standard evolutionary effects.

Another obvious point of reference for linguistics is the idea of "developmental modularity," which looks particularly apt to accommodate a mosaic view on language without the strictures of a Fodorian-style view on modularity. Modularity at the level of the generative underpinnings of a given organic structure does not necessarily compromise the fate of its component parts in the direction of strict domain specificity, which is the picture that fits better with a growing body of research on shared neural patterns of activity in linguistic and nonlinguistic tasks and on comorbidity of linguistic and nonlinguistic deficits. Moreover, developmental modularity is an idea particularly congenial with the characterization of language as a hybrid entity along the lines of the previous paragraph, for concurrently developing aspects of the hybrid on the way may straightforwardly exert scaffolding influences on each other.

Some well-documented observations appear to support the idea that the triad "hybrid-scaffolding-modularity" is particularly fit to offer new ontological foundations to the language faculty. For example, newborns acquire their language-

particular phonological competence from general sensory-motor, computational and statistical abilities. This milestone, together with aspects of social intelligence, grounds, first, the acquisition of sound-meaning associations and, next, the explosion of syntax (Kuhl 2004). On the other hand, the earliest, most abrupt, and less resilient maturational effects on language acquisition are actually observed in the phonological component, which according to the above picture acts as the ultimate foundation for all the rest (Meisel 2013).

The Problem of Computation(s)

An element of consensus within the cognitive science community is that there is some causal story to be told about cognition and behavior. The central element of this causal story is that both cognition and behavior are explained by the action of some internal (mental) *operations* that may themselves be caused by some external stimuli (e.g., through perception), although this latter step is not necessary or sufficient for a full account of cognitive processes. Of course, the appeal to operations hardly constitutes an explanation, as what one really needs is a precise characterization of the operations and, also, of the entities these operations apply to. Moreover, in the naturalistic context of contemporary cognitive science, one expects to have an account of operations and entities that agrees with the workings of the brain. As for the latter, there is a wide consensus that mental operations apply to *ideas* or, to use the contemporary terminology, *representations*. As for operations, things are much less clear. With the sole exception of Thomas Hobbes and possibly Leibniz too, who equated mental operations with some form of calculation, only in the twentieth century will take form the idea that cognitive processes are *computational* processes or, in other words, that nervous systems are natural systems of computation. But what is a natural system of computation?

Gualtiero Piccinini has warned us against loose characterizations of computational systems—verging into vacuous pancomputationalism—and has enumerated a number of constraints any system must obey in order for it to be classified as computational (Piccinini 2015). Along with the constraints of being a functionally organized input-output system—a property that brains share with other organ systems—perhaps the most salient constraint Piccinini identifies as the landmark of computation is the ability to process a special class of physical entities according to rules that are sensitive to certain formal properties of the said entities. Piccinini refers to the physical entities that are the objects of computation with the term *vehicles*. This terminological move is justified on the grounds that the objects of computation need not necessarily be discrete entities—like the so-called symbols of theoretical computer science—or carriers of semantic content—like the traditional conception of symbol implies. It is clear that brains are natural computational systems at least in this generic sense: neural processes manipulate voltage changes in the dendrites, neuronal spikes, neurotransmitters, and hormones, which all qualify as bona fide vehicles. The question is whether brains are computational systems in

other, more specific senses, hopefully coincident with the assumptions of computational cognitive science.

Computational cognitive science's version of cognition is epitomized by the so-called *computational theory of mind* (CTM), whose locus classicus is the *language of thought* (LOT) *hypothesis*. In a nutshell, what the CTM+LOT hypothesis amounts to is that mental computations are performed over data structures made up of discrete symbols, which moreover have semantic properties. Note that this conception of computation is much more restrictive than the generic one presented above in requiring that vehicles be both discrete and carriers of semantic content. It does in fact correspond to a specific subclass of generic computation that Piccinini defines as *semantic digital computation*. Semantic digital computation has traditionally been the most favored one among cognitive scientists because it offers a framework within which higher cognitive processes, with language being the paradigmatic case, are easily characterized as computations over complex symbolic data structures with a syntax and a semantics.

The problem of computation can be formulated in the following terms: is it really the case that the brain, qua generic system of computation, is also a digital system of computation? Piccinini and Behar's (2013:477) conclusion is a rather bleak one: "current evidence indicates that typical neural signals, such as spike trains, are graded like continuous signals but are constituted by discrete functional elements (spikes). Therefore, typical neural signals are neither continuous signals nor strings of digits: neural computation is sui generis." The corollary of this conclusion is that a new kind of cognitive neuroscience is needed. This new perspective is, indeed, emerging, and its more recent findings suggest that such a pessimistic view might well be premature.

Until fairly recently, the primary concern of neuroscience was functional neuroanatomy, essentially extending the work of Korbinian Brodmann by attaching some function or other to the cortical areas he described attending mostly to histological principles. This kind of research, relevant as it is especially on its comparative aspect, is relatively uninformative when it comes to brain dynamics. The elaboration of detailed functional brain maps has nevertheless made clear that even more important than brain regions and their functional specializations are the networks different areas conform when jointly engaged in some cognitive process—hence the terms *connectomics* to refer to the structural description of the brain seen as a network of nodes and edges and *functional connectomics* when this description is enhanced with information concerning brain activity. The inevitable next step in this chain of events has been the initiation of research on the dynamic aspect of networks: how and what regions are connected and what signals they convey and how they are acted upon. This last breakthrough in neuroscience research has been crucial for two reasons. Firstly, the level of analysis of brain dynamics (or the *dynome*, as it has come to be named) defines an intermediate level that smoothly allows for the linking of the macrolevel of brain regions with the microlevels defined by the neuron and below (Kopell et al. 2014). Secondly, recent research at the level of brain dynamics appears to support precisely what Piccinini and Behar (2013) denied in their analysis of neural oscillations (spikes and spike trains), namely, that neural computation

might be of the digital kind after all, with these discrete assembly sequences playing a central role in different cognitive processes, as well as in language processing. A relevant datum in this connection is the high preservation of brain oscillations, at least across all mammalian genera (Buzsáki et al. 2013). So, if neural oscillations are the key to understand neural computation, a central question is how differences in cognitive abilities are to be accounted for within this scenario of high preservation. Clearly, research in the development of cell types, connections, and neural rhythms, along with their correlates both at the molecular and cognitive levels, should occupy a central role in the understanding of the evolution of language and cognition.

The Problem of Representation(s)

As stated in the previous section, computations are input-output processes capable of manipulating certain entities according to a specific set of rules; in other words, computations can be characterized as functions. Few are those who doubt that minds/brains compute and that the inputs and outputs of cognitive functions are what generically may be identified as *representations*. Epistemological disputes may arise as to whether representations are innate or learned, or concerning their nature, their form, and their mode of representation, but ontology appears not to be an issue in this case: there are representations.

Symbols (in an extended sense of the term capable of also applying to *neurophysiological states*) are the most plausible candidates for being the kind of representation format needed by a computational theory of cognition. The problem with symbols is to explain how they represent and how they acquire representational content. The usual story with symbols is that they represent by convention, but conventions are public, social contracts, and mental representations are not public. What is good for cultural symbols is not necessary good for neurobiological ones. Locke—neurobiological considerations aside, of course—saw this and tried to develop a causal theory of representation, the idea that symbols represent through *covariance*. Representation by covariance is a cover term for a whole family of theories that are nonetheless based on the idea that the represented object and its representation stand in a causal relation (covariance) in the sense that any presentation of the object causes a corresponding token representation in the mind/brain. In other words, content is the pair formed by a vehicle and its extension, i.e., the set of things in the world that would cause its tokening by the brain. Alternatively, one may assume that representation is not a relation, but just a property of the vehicles of content, where the latter is seen as intrinsic or perhaps acquired through the vehicles' roles during cognitive processing—a contention that, according to Egan (2014), runs into serious difficulties. Crucially, however, neither alternative is necessarily committed to a notion of representational content that is in turn committed to the conceptual apparatus typically associated to what is generally associated with the notion of intentionality. To be sure, there is a growing consensus that the latter is not a proper part of a naturalistic theory of content (Fodor and Pylyshyn 2015).

Now, so far it may seem that the problem of representation(s) has little to do with either evolution or development, but nothing could be further from the truth. Indeed, taking language as a paradigmatic case, it is reasonable to suppose that representations are also subject to developmental processes, which immediately suggests an analysis in terms of the notion of developmental hybrid like the one sketched in section "Introduction." This would clearly favor a conception of representation along the lines of the covariance model set forth by Fodor and Pylyshyn (2015), but also would open new avenues of research on the development and evolution of representations along the lines of models of cultural evolution like the one outlined in Charbonneau (2015) or those developed within the paradigm of iterated learning (Kirby 2013).

The Problem of Homology/Novelty

There exists a relative consensus among linguists around the idea that language is a human-specific endowment with no known equivalents in other nonhuman organisms. Yet experts disagree about the extent to which language is unique, for many believe that it is a thoroughly innovative capacity (Pinker and Jackendoff 2005) and others claim that it may be decomposed into shared and unique component parts (Hauser et al. 2002). The debate on language uniqueness, however, is one in which discussions are not particularly clarifying, for contenders base their positions on different implicit, unclear, and intuitive ideas about what makes a particular organic structure an innovative one. This is therefore an aspect of evolutionary linguistics in which the injection of concepts and criteria from evo-devo has been claimed to be particularly urgent (Balari and Lorenzo 2015). The diversity of current evo-devo approaches becomes a real hindrace in this particular subject matter, however; as pointed out by Benítez-Burraco and Longa (2010), such diversity often favors the more relaxed gene-centric versions of evo-devo over other, more promising ones. For one, Chomsky's (2010) forays into evo-devo, for example, continue to be anchored in his classical gene-centric view on universal grammar, which has uncritically led to a strong species-specificity bias, as well as to exceptionalist theories of language evolution.

Again, the idea of "developmental modularity" may serve as an obvious bridge, at least in those aspects of the language mosaic for which reliable developmental information exists at a molecular level of analysis. Key questions like the following will become more and more accessible with the body of information that will predictably accumulate in this area of expertise in the near future:

1. Attending to independently established developmental criteria, can we homologize different components of human language with different candidate aspects of the cognitive makeup of other nonhuman organisms? Is the homologation exhaustive, or residues remain of language that resist the test?

2. Were language to be exhaustively put into correspondence with other nonhuman homologues, how could we still make sense of the fact that no other organisms learn, use, and can take advantage of languages in the way humans do?

One may advance toward a preliminarily take on these questions by briefly examining the case of the aspect of language that most students have pinpointed as the locus of the radical innovativeness of this allegedly human-unique capacity, namely, the computational procedure capable of recursively putting pieces together and thus of endowing languages with their never-ending expressive potential (Hauser et al. 2002). The standard specification of this procedure is that it works pair-wise, so it takes two units X and Y (two lexical items or two previously constructed syntactic objects), and it outputs a single unified syntactic unit {X, Y}. Moreover, the operation creates structure, so at each step, it must also somehow signal an inner asymmetry within each newly created unit, $\{_{X}$ Z, $\{_{X}$ X, Y$\}\}$, and so on. There exists a broad consensus around the idea that this operation is human specific (Berwick and Chomsky 2016). But note that while this may be true if the operation is focused with an extremely fine-grained lens, it also makes sense searching for affinities with some nonhuman practices in order to test the idea that a homological thread exists among them. For example, there exists a large body of literature on birdsong that offers grounds to the idea that it benefits from the "same" pairing procedure as language, "save" for the lack of the principle/operation responsible of creating asymmetries. As a consequence, sequences are generated that display a flatter character relatively to human phrases (Berwick et al. 2011). A large body of literature seems to give support to these "abstract" parallels from the point of view of developmental genetics (Pfenning et al. 2014). Are we authorized to conclude that a bona fide homological relation exists between the corresponding systems of computation?

In order to answer this question, we need (i) independently well-established criteria to sanction homological relations and (ii) a good sample of the kinds of organic materials to which said criteria make reference. These are hard issues that entail some prior nontrivial questions: do systems of computation qualify as bona fide organs? And, ultimately, what does qualify as a bona fide organ? Any promissory route leading to a complete answer to this chained collection of questions needs to start at a solid developmental theory of organ identity, like the one put forward in Wagner (2014). Its main contention is that "character identity networks" (ChINs) exist at a molecular level that may offer the basis for organ identity discriminations. Roughly speaking, ChINs are distinctive patterns of regulatory genes (and related products) and gene interactions that mediate between signaling information and realizer genes, ultimately responsible of the attainment of variants of the same organ thus circumscribed. Evidence of a putative ChIN that appears to justify the idea that birdsong and human language (at least) share the same organ of computation may be based on the existence of shared inductive signals (retinoic signaling pathway), transcription factors (FOXP2), and target genes (*SLIT1*, *NEUROD6*, etc.) (Balari and Lorenzo 2015). Complementarily to this conclusion, one may also proceed to ask what is it that nevertheless makes language a unique manifestation of that organ (or, for that matter, what is it that makes birdsong a unique ability). The

answer will require a better understanding of the organic basis of the "structured/flat" character of the corresponding computed strings, as well as of the pattern of interface relations that systems of computation establish in different organic contexts (Balari and Lorenzo 2013).

The Problem of Selection

The application of the Darwinian paradigm to the case of language has always been a contentious issue, mostly due to Chomsky's anti-selectionist arguments. In outline, these arguments boil down to the following claims: (1) most uses of language are organism internal, not environmentally oriented; (2) it seems to be impossible to identify a particular kind of adaptive pressure to which language could be said to adjust in the expected lock-and-key manner; (3) many linguistic properties are actually misadjusted in relation to any particular well-defined purpose (to begin with, the unbounded expressiveness of languages); and (4) many well-formed expressions are not usable (e.g., for being too long), and many ill-formed expressions could be used without taxing mutual comprehension (Chomsky 1968).

Within this traditional context, many proposals have however been made about the role of selection in the evolutionary shaping of language, which differ on its relevance in the launching of language as an innovative endowment of humans, on the complexification and fine-tuning of its component parts, and on the kinds of environmental influences capable of exerting the corresponding selective pressures (Bickerton 2013). If one takes the vantage point of a full-fledged theory on the origin and evolution of organs like Wagner's (2014), the premise may be adopted that the role of selection is negligible in the initiation of brand new ones but inescapable in the subsequent processes of radiation of variants. But if one also accepts that the component parts of language are exhaustively homologizable with other nonhuman cognitive organs, then the question still arises of how selection has acted in imprinting on language its distinctive characteristics. In this respect, views differ on whether components added at different stages were specifically selected, fixed for the selective value of language as a whole, or not selected at all.

The idea of "developmental modularity," in inviting to break language into component pieces on developmental grounds, certainly gives some justification to the possibility that selective effects operate on language in a piecemeal way. But also, and maybe more importantly, it justifies the necessity of taking apart different senses or different levels at which such effects may act: first of all, parts must be proved to be mutually compatible, thus selected in an organismic internal sense, and then, and only then, they may confront the pressures of a demanding environment, eventually leading to their fine-tuning and fixation within a population. An important corollary of this last statement is that aspects of the linguistic hybrid might have evolved just for their role in facilitating the development of other parts of the hybrid, i.e., for their being useful for development per se (Minelli 2003: 14).

Besides, modules may be intertwined in ways that compromise the developmental fate of each other, so why and how parts evolve may be due not to pressures directly exercised by the environment, but to hitchhiking effects of sorts of an

endogenous character. This is how, for example, Balari and Lorenzo (2013: Ch. 6) try to make sense of the interconnectedness of the architectural components of languages, the multimodal character of lexical items, and the complexity level of linguistic computations at once, as due to heterochronic effects after the acceleration of cortical growth in human evolution. A virtue of these kinds of suggestions is that they may offer grounds to the idea that language is a "variational modality" of an ancestral overarching organ (Balari and Lorenzo 2015), in which a set of peculiar properties are brought together that are not accessible from the developmental underpinnings of other homologous modalities (Wagner 2014).

Obviously enough, conjectures like the ones put forth to in the previous paragraphs do not invalidate the putative role of natural selection in particular aspects of the evolutionary shaping of language, difficult as it may be to establish the particularities of such a process in the realm of cognition. The idea bumps into troubles, for example, when one is confronted with Chomsky's suggestion that the computational system of languages is a fixed, uniform component of human brains, contrary to the expectations of the theory of natural selection (Chomsky 2001). Others have argued, however, that more variation than expected may be found in this aspect of language (Hurford 2012), a fact that encourages exploring the application of the selectionist paradigm even to such a refractory arena.

Cross-References

- ▶ Developmental Homology
- ▶ Developmental Innovation and Phenotypic Novelty
- ▶ Evo-Devo and Cognitive Science
- ▶ Evo-Devo and Culture
- ▶ Heterochrony

Acknowledgments This work has been partially supported by the Generalitat de Catalunya through grant 2014-SGR-1013 to the Centre de Lingüística Teòrica of the Universitat Autònoma de Barcelona (SB). We wish to thank Víctor M. Longa for helpful comments to an earlier draft of this paper. Any remaining errors are our own.

References

Balari S, Lorenzo G (2013) Computational phenotypes. Towards an evolutionary developmental biolinguistics. Oxford University Press, Oxford

Balari S, Lorenzo G (2015) It is an organ, it is new, but it is not a new organ. Conceptualizing language from a homological perspective. Front Ecol Evol 3:58. https://doi.org/10.3389/fevo.2015.00058

Benitez-Burraco A, Longa VML (2010) Evo-devo—of course, but which one? Some comments on Chomsky's analogies between the biolinguistic approach an evo-devo. Biolinguistics 4:308–323

Berwick RC, Chomsky N (2016) Why only us language and evolution. The MIT Press, Cambridge, MA

Berwick RC, Okanoya K, Bechers GJL, Bolhuis JJ (2011) Songs to syntax: the linguistics of birdsong. Trends Cogn Sci 15:113–121
Bickerton D (2013) Language and natural selection. In: Boeckx C, Grohmann KK (eds) The cambridge handbook of biolinguistics. Cambridge University Press, Cambridge, pp 478–488
Boeckx C (2012) The I-language mosaic. In: Boeckx C, Horno-Chéliz MC, Mendívil-Giró JL (eds) Language, from a biological point of view. Cambridge Scholars Publishing, Newcastle upon Tyne, pp 23–51
Buzsáki G, Logothetis N, Singer W (2013) Scaling brain size, keeping timing: evolutionary preservation of brain rhythms. Neuron 80:751–764
Caporael LR, Griessemer JR, Wimsatt WC (eds) (2014) Developing scaffolds in evolution, culture, and cognition. The MIT Press, Cambridge, MA
Charbonneau M (2015) Mapping complex social transmission: technical constraints on the evolution of cultures. Biol Philos 30:527–546
Chosmky N (1968) Language and mind. Harcourt Brace Jovanovich, New York
Chomsky N (1986) Knowledge of language. Its nature, origin and use. Praeger, New York
Chomsky N (2001) Derivation by phase. In: Kenstowicz K (ed) Ken Hale: a life in language. The MIT Press, Cambridge, MA, pp 1–52
Chomsky N (2010) Some simple evo devo theses: how true might they be for language. In: Larson RK, Déprez V, Yamakido H (eds) The evolution of language. Biolinguistic perspectives. Cambridge University Press, Cambridge, MA, pp 45–62
Darwin C (1879) The Descent of man, and selection in relation to sex, 2nd edn. John Murray, London
Egan F (2014) How to think about mental content. Philos Stud 170(1):115–135
Fodor JA, Pylyshyn ZW (2015) Minds without meanings. The MIT Press, Cambridge, MA
Hauser MD, Chomsky N, Fitch WT (2002) The faculty of language: what is it? Who has it? How did it evolve? Science 298:1569–1579
Hurford JR (2012) The origins of grammar. Language in the light of evolution. Oxford University Press, Oxford
Kirby S (2013) Language, culture, and computation: an adaptive systems approach to biolinguistics. In: Boeckx C, Grohmann KK (eds) The Cambridge handbook of biolinguistics. Cambridge University Press, Cambridge, MA, pp 460–477
Kopell NJ, Gritton HJ, Whittington MA, Kramer MA (2014) Beyond the connectome: the dynome. Neuron 83:1319–1328
Kuhl PK (2004) Early language acquisition: cracking the speech code. Nat Rev Neurosci 5:831–843
Meisel JM (2013) Sensitive phases in successive language acquisition: the critical period hypothesis revisited. In: Boeckx C, Grohmann KK (eds) The Cambridge handbook of biolinguistics. Cambridge University Press, Cambridge, MA, pp 69–85
Minelli A (2003) The development of animal form. Ontogeny, morphology, and evolution. Cambridge University Press, Cambridge, MA
Pfenning AR, Hara E, Whitney O, Rivas MV, Wang R, Roulhac PL, Howard JT, Wirthlin M, Lovell PV, Ganapathy G, Mouncastle J, Moseley MT, Thompson JW, Soderblom EJ, Iriki A, Kato M, Gilbert MT, Zhang G, Bakken T, Bongaarts A, Bernard A, Lein E, Mello CV, Hartemink AJ, Jarvis ED (2014) Convergent transcriptional specializations in the brains of humans and song-learning birds. Science 346:1333–1346
Piccinini G (2015) Physical computation A mechanistic account. Oxford University Press, Oxford
Piccinini G, Bahar S (2013) Neural computation and the computational theory of cognition. Cogn Sci 34:453–488
Pinker S, Jackendoff R (2005) The faculty of language: What's special about it. *Cognition* 95:201–236
Wagner GP (2014) Homology, genes, and evolutionary innovation. Princeton University Press, Princeton and Oxford

Evo-Devo and Culture

Mathieu Charbonneau

Contents

Abstract

What does evo-devo offer for a better understanding of cultural evolution? Cultural evolutionists with a biological bend typically focus on the relation between genetic evolution and cultural change, a research program referred to as gene-culture coevolution. Development of the human organism is usually left unattended by cultural evolutionists, and so are the processes involved in the production of cultural phenotypes. Moreover, evo-devo research has yet to have any marked impact on the social sciences. Examining how evo-devo can contribute to the study of cultural evolution means understanding how cultural evolution and development shape one another. However, it is necessary to first clarify just what sorts of developmental processes we are interested in. There are two albeit not mutually exclusive candidate answers to this question. First, we can be interested in the interactions between cultural evolution and biological development – how does the development of human individuals and the cultural evolutionary process shape one another? Alternatively, we can be interested in

M. Charbonneau (✉)
Science Studies Program, Departments of Philosophy and Cognitive Science, Central European University, Budapest, Hungary
e-mail: mathieu.charbonneau1@gmail.com

L. Nuño de la Rosa, G. B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6_47

the interactions between cultural evolution and the generative mechanisms involved in the production of cultural phenotypes. The objective of the present discussion is to address both understandings of the relations between evo-devo and cultural evolution.

Keywords

Cultural evolution · Evo-devo · Cultural development · Enculturation · Metaplasticity

Introduction

What does evo-devo offer for a better understanding of cultural evolution? Following Müller (2007), evo-devo's research agenda can be broadly characterized as the solving of two key problems: how does evolutionary mechanisms generate and modify organismal developmental processes and how does the structure of developmental processes shape back the patterns and processes of species evolution? In order to understand either evolution or development, we need to understand how they shape one another. Analogously, examining how evo-devo can contribute to the study of cultural evolution means understanding how cultural evolution and development shape one another. However, it is necessary to first clarify just what sorts of developmental processes we are interested in (Mesoudi et al. 2006). There are two albeit not mutually exclusive candidate answers to this question. First, we can be interested in the interactions between cultural evolution and *biological development* – how does the development of human individuals and the cultural evolutionary process shape one another? Alternatively, we can be interested in the interactions between cultural evolution and the *generative mechanisms involved in the production of cultural phenotypes*. We will refer to these two projects as an *evo-devo of culture* and a *cultural evo-devo*, respectively.

The objective of the present discussion is to address both understandings of the relations between evo-devo and cultural evolution. However, the reader should be aware from the onset that there is no such thing today as *an* evo-devo of culture or *a* cultural evo-devo. Cultural evolutionists with a biological bend typically focus on the relation between genetic evolution and cultural change, a research program referred to as gene-culture coevolution (Boyd & Richerson 1985). The development of the human organism is usually left unadressed by cultural evolutionists and so are the processes involved in the production of cultural phenotypes (Charbonneau 2015a; Wimsatt 1999). Moreover, evo-devo research has yet to have any marked impact on the social sciences. However, there is an abundance of existing research that can bridge these gaps, spanning from developmental psychology, ethnography, and/or the neurosciences. Concepts from evo-devo also promise to offer important insights for the study of cultural processes under a novel, insightful light. So while this entry does not aim at offering a bird's eye-view of an actual research program – there is no such research program to begin with – it will identify key intersections

between the evo-devo framework and its potentially relevant fields of application in the study of cultural evolution.

An Evo-Devo of Culture

Naturalizing Culture

The human capacity to transmit, maintain, and incrementally modify cultural traditions across generations has had major impacts on the survival and natural history of the human species. Think of the many techniques for producing, using, and improving tools and technologies or of the rich variety of belief systems observed in extant and lost civilizations. A great many of human behaviors are neither learned directly from the environment nor are they the product of genetic inheritance. Rather, such human behavioral phenotypes are acquired and maintained from one generation to the next by human individuals learning from one another. In analogy to genetic transmission, which sustains a biological evolutionary process, social learning – the ability to learn from others – sustains a cultural evolutionary process with important impacts on the natural history of our species, a cultural evolutionary process likely intertwined with the biological evolution of our own species (Boyd and Richerson 1985). Accordingly, cultural evolutionists understand a socially learned behavior as a cultural trait when the mental representations (such as beliefs, norms, etc.) or information involved in the production of the behavior has been acquired from others and is widely distributed in a population. Teaching, imitation, apprenticeship, etc., are all key social learning processes, and species devoid of such social learning capabilities will fail to sustain and improve any cultural tradition.

The research program adopted by contemporary cultural evolutionists is a naturalistic one. Adopting a naturalistic approach to the study of human culture means first and foremost understanding social and cultural processes and phenomena in continuity with processes and phenomena of other natural domains, such as cognitive processes, mental states, and biological processes. Accordingly, in addition to the many social sciences specifically devoted to the study of human cultures, such as anthropology, archaeology, and history, the scientific study of culture has now grown into a vast interdisciplinary field involving evolutionary and behavioral biology, the neurosciences, cognitive psychology, and biological anthropology. This does not mean that cultural phenomena are nothing more than psychological or biological processes. Rather, the naturalistic program understands cultural processes as material processes interacting with and partially composed of biological, psychological, and social processes. (Sperber 1996)

It is generally agreed upon that cultural transmission shares many key features with genetic inheritance, features enabling social learning to support an evolutionary process of human traditions. As far as it insures the transmission of behaviors across generations and participates in sustaining a cultural evolutionary process,

social learning serves as a nongenetic mechanism of inheritance, one with its very own (nongenetic) channels of transmission (Boyd and Richerson 1985; Jablonka and Lamb 2005). Accordingly, cultural evolutionists typically orient their research towards the transmission patterns of cultural traits and their distribution in human populations. On the basis of the similarities identified between cultural and genetic inheritance, many cultural evolutionists have adopted the modeling tools and strategies of population genetics in order to study the population-level effects of individual episodes of social learning (Boyd and Richerson 1985). However, there are enough differences between genetic and cultural transmission so that models of population genetics cannot be straightforwardly transferred to the study of cultural change. Whereas cultural evolutionists have borrowed modeling methods and assumptions from population genetics, they have spent a great deal of effort specifying how the modeling techniques should be adapted to the idiosyncrasies of cultural transmission and evolution. Central to cultural evolutionary theory then are questions such as who learns what from whom, whether cultural transmission is a high-fidelity replicative process or not (i.e., the rate of cultural "mutation"), and what sorts of biases are involved in the transmission of culture and how these shape the distribution of cultural variation. The disanalogies picked-up by cultural evolutionists thus mainly concern differences in the network and channels of genetic and cultural information transmission. Cultural evolutionary models consequently involve horizontal and oblique transmission – transmission among peers of the same generation and to unrelated individuals of the next generation, respectively – and learning biases, i.e., preferences to learn some behaviors instead of others on the basis of the preferred teachers, of the behavior's outcomes, how frequent the behavior is in the population, etc. Consequently, the differences also justify a nonreductionist approach to cultural evolution as genes and cultures can evolve relatively independently from one another (contra Lumsden and Wilson 1981).

However, there is an important set of disanalogies that cultural evolutionists rarely address, namely, the differences between the structure of genetic inheritance and that of the enculturation process, i.e. the process by which an individual acquires the typical cultural repertoire of its group. Indeed, one important difference between genetic inheritance and the enculturation process concerns the specific moment in the organism's life-cycle and the duration of the acquisition of genetic and cultural information. Simply put, we inherit the whole of ours genes at the moment of our parents' reproduction. In contrast, we inherit our local culture throughout our lifetime and do so in a piecemeal and sequential manner (see Fig. 1). Building up one's repertoire of cultural traits is a life-long process, taking place not before the development of the human organism – and thus, somewhat in isolation of it, as it is with genes – but *during* development. An individual's enculturation is thus sensitive to its biological development. Moreover, a culture's specific enculturation process is structured, and its structure is itself socially transmitted and open to evolutionary change. This seemingly banal fact has important consequences for the evolution and the study of human cultures.

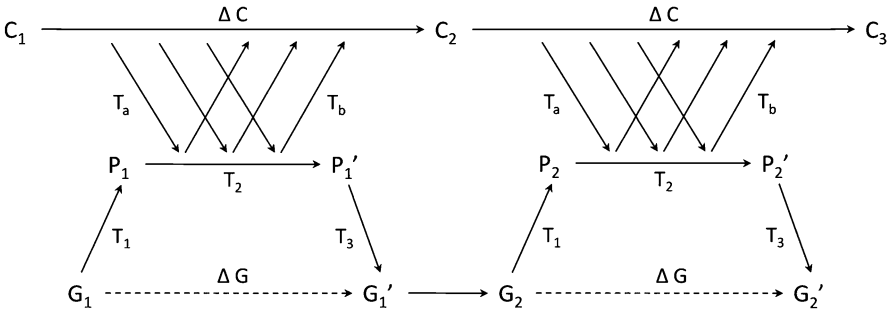


Fig. 1 Whereas organisms typically inherit their genome (G) at the moment of reproduction ($G_x' \rightarrow G_{x+1}$) and carry that same genome throughout their lifetime ($G_x \rightarrow G_x'$), an individual's cultural repertoire is constructed sequentially and in a piecemeal fashion (T_a) throughout its life time (T_2) (Adapted from Durham 1991, p. 186)

How Biological Development Shapes Enculturation

The enculturation process is sensitive to the cognitive and morphological development of the human individual. Perhaps most striking is the sensitivity to morphological development. Cultures adopting a sexual division of labor are nearly ubiquitous. Not only do females and males generally serve different ecological and social roles but more importantly they typically learn and transmit different cultural behaviors (e.g., foraging behaviors, clothing habits, etc.). Such division is based on the recognition by the group that one individual is of a certain gender, a recognition not based on observing the chromosomes individuals possess but on the perceived phenotype of the individual (e.g., recognition of sexual attributes), and often the learned and transmitted behaviors will also depend on the age of the individual (e.g., sexual maturity). The criteria used to distinguish genres vary from one culture to the next.

Cognitive and brain development of the human individual also constrains what can be learned at specific ages. An individual's first language is learned during childhood with little difficulty, whereas second languages learned at a later age will typically require much more cognitive efforts on the part of the learner. Additionally, more complex behaviors and abstract knowledge may only be learned when the proper cognitive capacities and motor skills have developed (Roux and Bril 2005). Just as with morphological development, a community may also adopt different norms for measuring cognitive maturity in order to decide if an individual will be allowed to learn some special skills (Rogoff 2003). Some behaviors and techniques also require a long time to master, whereas genes are all acquired quickly and at the same time. This means that one's developing expertise can interact and possibly be scaffolded with what other knowledge or skill one is learning at the same time.

How a community attests that the learner is ready for learning more about the techniques and who will take over such learning is also variable from one culture to

the next (Ruddle and Chesterfield 1977). For instance, this can mean that the network of interactions of an individual within the community may also vary according to the perceived maturity and gender of the developing individual (Rogoff 2003). In Western societies, youngsters will typically learn mostly from their parents but as individuals gain in age, they will enter the schooling system where oblique transmission is the rule. In other societies, youngsters may directly learn mostly from their aunts and uncles as parenting tasks are shared across the kinship and then as they get older join groups of other kids and learn mostly horizontally (e.g., through play).

Human populations vary in what individuals learn, at what age they learn it, and from whom they learn from. The cultural relativity of the enculturation process makes the use of population genetics tools problematic, as population genetics models typically assume a stable life-cycle, one that is generalizable across the species. In contrast, the variability of the structure of the enculturation process implies that models in cultural evolutionary theory may not assume some general cultural life-cycle as there may not be any stable, cross-cultural patterns of enculturation (Wimsatt 1999). Only by integrating the influence of biological development on cultural transmission and its impacts on structuring the enculturation process will cultural evolutionists be capable of articulating a general theory of cultural change.

The varying enculturation patterns are themselves maintained and inherited through social learning, with neither genetic nor environmental factors being capable of accounting alone for the patterns' diversity and stability. For instance, the Greco-Roman education structure strikingly exemplifies the inheritance of the enculturation process, with the Romans intentionally copying (with some adjustments) the education structure of Ancient Greece. In contrast, although they may lack any schooling institutions, traditional societies nevertheless exhibit highly structured, lasting enculturation patterns (Rogoff 2003; Ruddle and Chesterfield 1977). There are other studies in the developmental psychology, ethnography, and comparative pedagogy literatures that deal with the transmission of a culture's typical enculturation process. Unfortunately, there is little if any work on these topics that expressly adopt a cultural evolutionary framework.

Taking seriously the relation between individual development and cultural inheritance implies collecting data about how different cultures vary in the specifics of their enculturation process and examining how these differences enable and constrain the general evolution of cultural traditions. Moreover, this means to pay closer examination of how specific enculturation structures come about and how such structures can undergo evolutionary change. For instance, one key structuring constraint of many cultural traditions resides in the fact that for many cultural phenotypes, to acquire the trait one must already have learned some other cultural traits beforehand. Many complex cultural traits are in fact composed of simpler ones. So, for instance, in order to learn calculus, you already need to have somewhat mastered algebra, which in turns relies on you knowing the basics of arithmetic. These logical constitutive dependencies translate, in terms of the enculturation process, as a strict sequence of learning which must be respected if the enculturation process is to successfully allow the learning and further transmitting of these cultural traits (Wimsatt 1999). Moreover, each step in the sequential acquisition of a complex

trait may also depend on the specifics of the individual's cognitive maturity, where the cognitive capacities required to learn and master each trait in the sequence may not develop synchronously. (See Enquist et al. 2011 for a modeling effort of the sequentiality of the enculturation process).

The dependence relationships between cultural traits may have different impacts on the cultural evolutionary process. Wimsatt (1999) argues that enculturation, analogously to the genome, is subject to generative entrenchment. What one learns earlier in its life-cycle is less prone to change as any change risks having deleterious cascading effects on the acquisition of other traits depending on the earlier ones. So if we change the rules of arithmetic, these may not be coherent anymore with algebra or with calculus, leading to the failure of further learning the latter. Thus the stabilization and preservation of arithmetic is necessary for the successful transmission of algebra and calculus. Clarifying exactly at what level the entrenchment of cultural traits are located will prove to be an important part of the study of enculturation. Whereas possessing a language may be a necessary condition for both the invention and learning of arithmetic, acquiring any specific natural language is not. Again, contrary to gene inheritance, where the genome of the organism is typically inherited as a whole during reproduction, enculturation is a piecemeal, culturally variable process. This means that there are likely more chances that "pleiotropic" effects among cultural traits will agglomerate into relatively independent package of cultural traits (e.g.,. learning mathematics vs. learning fishing) rather than being distributed on the overall cultural repertoire of a population. A clear theory of the packaging of cultural traits and of their evolution together remains to be formulated.

How Enculturation Shapes Biological Development

Culture is a special form of phenotypic plasticity. Consider, for instance, the case of Padaung women, known through their touristic name of "giraffe-necked women," as a case of culture interacting with the developmental plasticity of the human organism (Fig. 2). The Padaung tradition of women wearing heavy brass neck-rings has effects spanning on many different levels of the human organism's phenotypic plasticity. The observed depression of shoulder girdle in Padaung women is due to the heavy brass rings they traditionally wear around their neck, thus giving the optical illusion of possessing a longer neck. These morphological changes also result in physiological problems such as increased blood pressure, and the older Padaung further risk to break their necks if the rings were to be removed, making them dependent on an artificial "exoskeleton." Perhaps most striking are the morphological effects, but for the cultural evolutionist, it is the brain's plastic capacity to learn a great variety of behaviors that is central. As a form of phenotypic plasticity, culture can be understood as a mechanism of phenotypic response to the behavioral displays of others. Social learning then is the capacity to reproduce behavioral phenotypes similar to those observed in other members of its population.

Central to culture, then, is the capacity of the human brain to change in response to the local culture. Not only do we learn from one another, but the structural and

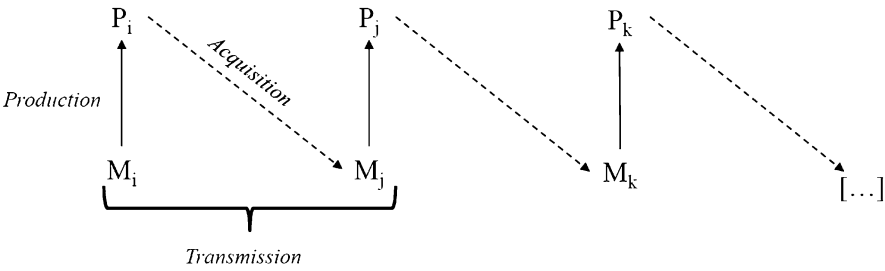


Fig. 2 Social transmission as a multistep process. A demonstrator's mental representation M_x is used to produce (*production*) some cultural phenotype P_x. For a tradition to be sustained, the learner acquires a similar mental representation M_{x+1} by observing the demonstrator's cultural phenotype P_x, and so on and so forth (Adapted from Charbonneau 2015a, p. 531)

functional organizations of our brain are greatly influenced by what we learn and the age at which we learn it. For instance, neuroimaging studies have shown cortical reorganization in professional musical skill training which has important impacts on tactile acuity. Moreover, cognitive changes induced by practice are sensitive to the age at which the skill is learned, with early learning leading to better expertise and with an increased ability to learn new tasks (Malafouris 2013). Thus different enculturation regimes may lead to cognitive differences between cultures without involving neither genetic nor noncultural environmental differences. In other words, the brain's plasticity exploited by culture can be altered with by what one learns. This plasticity of brain plasticity is generally referred to by the term "metaplasticity," a term coined to refer to changes in synaptic plasticity induced by synaptic activity (Abraham and Bear 1996). In the context of an evo-devo of culture, we can understand *cultural metaplasticity* as the capacity of culture to change the social learning processes and the cognitive capacities of the human individual. In other words, the metaplastic capabilities of the human brain makes it a cultural artifact *par excellence* (Mithen and Parsons 2008).

The study of the cultural metaplasticity of the human brain promises important consequences for the study of the relations between cultural evolution and biological development. Culture may not only serve as a behavioral inheritance system exploiting the brain's plasticity for memorization and behavior acquisition. It may also prove to be an important source of cognitive change altogether. In turn, culturally induced cognitive change can lead to cultural changes that would not have been possible otherwise (Malafouris 2009, 2010). For instance, increased tactile acuity in stone tool manufacture may lead individuals not only to learn novel, more demanding techniques of stone tool production, but also to discover novel sophisticated techniques altogether (Roux and Bril 2005). Cognitive change induced by cultural metaplasticity may lead to new possibilities of cultural innovations, with the novel innovations leading to novel cognitive capabilities, and so on and so forth. As individuals' cognitive capacities are partly shaped by culture, these can in turn impact further cultural change, creating an historical process of brain-culture coevolution that does not involve any genetic change. In other words, human

cognition may well have, in addition to a phylogeny, a cultural history. Little research has directly addressed the coevolution of cognition and culture from a cultural evolutionary and historical – rather than a phylogenetic – perspective. However, some work in neuroarchaeology is addressing how the material culture has co-developed with cognitive changes in early societies (Malafouris 2009, 2010). Some developmental psychologists have also addressed how social learning capacities are themselves the result of cultural evolution (Heyes 2012).

Cultural Evo-Devo

Cultural traditions are persisting causal chains of mental representations and public displays, such as behaviors (Boyd and Richerson 1985; Sperber 1996). The first link in these chains consists in producing some observable behavior from some mental representation. Whereas we may not directly access the mental representations of others, by effectively producing a learned behavior, an individual's private knowledge becomes publicly available for others to learn from. The second step consists in another individual perceiving the behavior and acquiring from it (or multiple repetitions of it) its very own private mental representation of the behavior. In future instances, the social learner will be able to reproduce the behavioral phenotype, which in turn will make it publicly available for another individual to acquire it, thus sustaining a cultural tradition (Fig. 2).

Cultural evolutionists typically emphasize the acquisition phase of social transmission, studying primarily the maintenance and transformation of the transmitted information. This practice can be illustrated by the use of terms like "cultural variants" or "cultural traits," referring indiscriminately to variant mental representations (e.g., beliefs, preferences, etc.) or to variant cultural phenotypes (e.g., practices, shape of artifacts, etc.), or both. Consequently, the production phase is generally black-boxed and the specific generative processes involved in the production of cultural phenotypes abstracted away. It is at this point that borrowing concepts from the evo-devo framework promises a better understanding of the interplay between the production of cultural phenotypes and their evolution in what is sometimes referred to as a cultural evo-devo (see Mesoudi et al. (2006, p. 367)).

There has been little work investigating this avenue mainly because there lacks a clear understanding of just what cultural development – in analogy to biological development – consists of (Mesoudi et al. 2006, p. 367). One possibility is to understand how variation in the socially transmitted mental representations maps onto variation in the cultural phenotypes they produce as a cultural analog to the genotype-phenotype map. Genes do not specify development such that variation of the phenotypes of organisms reduces to variation in their genetic material. Rather, genes and developmental processes interact with one another in complex ways such that the mapping between genotype and phenotype becomes itself a complex affair (Alberch 1991). In analogy, variation in cultural phenotypes would not reduce to variation in mental representations as the specific processes involved in producing

the cultural phenotypes may shape the latter's variation in complex ways. A cultural evo-devo would then consist in studying these complexities.

Most cultural evolutionists are in fact skeptic about using analogies with biological processes. However, perhaps there is no need to find a strong cultural analog to biological development. Indeed, if social learning does in fact serve as a nongenetic inheritance system, the socially transmitted mental representations taking part in cultural traditions can be conceptualized as generative factors participating in the production of behavioral phenotypes, not in analogy to genes, but as *alternative* developmental resources to genetic information. A similar logic would apply to other nongenetic inheritance systems (Jablonka and Lamb 2005). In the context of cultural evolution, we can thus understand the production phase as a form of cultural development, i.e., as the processes involved in the production of cultural phenotypes for inherited developmental resources, here socially acquired mental representations. A cultural evo-devo, then, would examine how the generative processes involved in the production of cultural phenotypes interact, shape, and are shaped by cultural evolution.

The first step in developing a cultural evo-devo should be to clarify just what the generative processes are made of. Following Mesoudi and O'Brien (2008), we can understand the structure of the generative processes involved in the production of cultural phenotypes through the concept of a *cultural recipe*. A cultural recipe is a hierarchically organized set of actions and decisions leading to the satisfaction of a specific, intended goal. The hierarchical structure of recipes can be decomposed into subassemblies of actions serving some subgoal that must be satisfied on the road to the intended end-product, i.e., the cultural phenotype. A subgoal consists of a measure of what conditions need to be satisfied and what to do next if the conditions are perceived as being satisfied and what to do when they are not. These subgoals can also be nested as intermediary steps in the realization of some other subgoals, thus generating a potentially complex structure of dependencies between action and decisions assemblies. Ultimately, all subgoals are ruled by a single master goal, that of the final intended end-result of the recipe. The hierarchical structure of recipes is typically depicted as a tree-like structure (see Fig. 3 for an example).

Cultural recipes are themselves transmitted from one generation to the next, and can vary, which confers them the capacity to evolve. Adequately, adopting explanatory concepts and tools from evo-devo will thus highly depend on whether the structure of cultural recipes can vary in ways similar to the development of an organism from its genetic material and environmental context of development. There have been some suggestions that the production of cultural phenotypes and the evolution of recipes are fit to adapt parts of the conceptual framework from evo-devo. In the remainder, we will discuss two of these – cultural modularity and that of a cultural genotype-phenotype map – and some of their consequences – such as cultural evolvability and cultural developmental constraints.

Mesoudi and O'Brien (2008) argue that complex recipes, ones possessing many levels of actions and decisions subassemblies, are likely to be decomposable into *cultural modules* given that recipe subassemblies tend to be more functionally integrated with one another than they are with the whole recipe. In other words, similarly to a modular genetic architecture, complex cultural recipes would be nearly

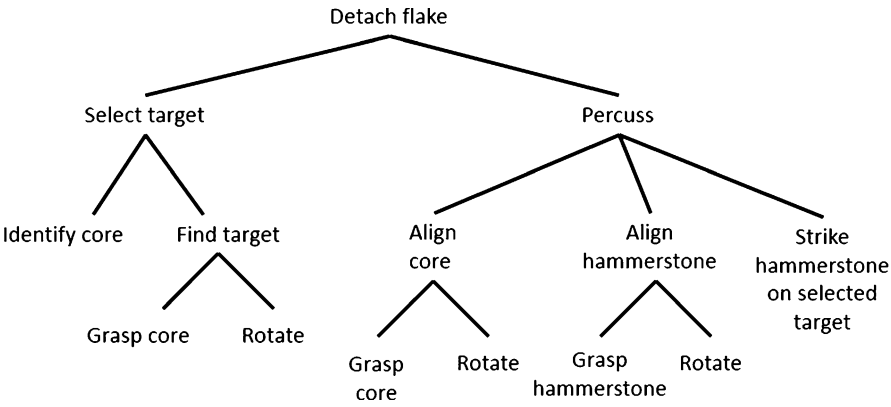


Fig. 3 The hierarchical structure of flake detachment. Early prehistoric stone tools were produced by detaching flakes off a core stone by hitting it with a hammerstone. The flake detachment behavior is composed of two main subbehaviors that of selecting a target on the core and that of percussion. Selecting a target consists in choosing a specific point on a core to hit with the hammerstone. Percussion consists in appropriately positioning the core, grasping the hammerstone, and striking the core on its target platform. Specific actions are represented at the lower end of the tree. Decisions are indicated as nodes (Adapted from Stout 2011, p. 1052)

decomposable. Moreover, such functionally modular subassemblies will tend to be transmitted as units as they can be learned as whole and relatively independently from one another or from the complex recipes in which they figure. Mesoudi and O'Brien (2008) models also suggest that the more modular cultural recipes are, the higher their chances of being transmitted. Modular recipes thus would have greater evolvability than more holistic (or less-modular) ones, as cultural modules, once learned, can be used in many different recipes (see also Charbonneau (forthcoming)).

Mesoudi and O'Brien (2008) offers a formal treatment of the structure of cultural recipes and do not address the different material and productive constraints involved in the cultural development process (Charbonneau forthcoming). Producing cultural phenotypes is a causal story starting with an individual's mental representations and ending in the public display of a specific cultural phenotype. This means that the production phase depends on cognitive, bodily, and ecological processes that are not necessarily involved in the acquisition phase. The production of cultural phenotypes may thus have its own enabling and constraining effects on social transmission and consequently on the evolution of cultures. The study of the generative mechanisms involved in the production of cultural phenotypes will thus be a complex endeavor, requiring the cultural evolutionist to address multiple mechanisms at different levels. Charbonneau (2015a) identifies four of these levels:

1. The cognitive processes and biases participating in the generation of public displays from mental representations (e.g., decision-making processes, mental imagery, motor control, etc.)
2. The external actions recruited in the production of the public displays (e.g., locomotion, prehension, manipulation, pronunciation, etc.), including the

affordances and constraints set by the particular body of the demonstrator (e.g., opposable thumb, flexibility, dexterity, body size, mass, etc.)
3. The specific tools and materials used to produce the public displays (if any)
4. The ecological processes engaged in the production of the public displays (e.g., chemical reactions, percussion effects, sound-wave propagation, etc.)

Charbonneau (2015a) argues that when these generative factors are taken into account, many assumptions typically adopted by cultural evolutionists may prove wrong. Once such assumption consist in the metrics used to assess gradual cultural evolution. Cultural evolutionists typically assume that errors in social transmission and even intentional transformations of cultural traditions tend to produce relatively similar cultural phenotypes. In other words, small changes in the transmitted information will result in small variations in the cultural phenotype, leading to a process of gradual cultural evolution that can be studied mainly at the level of the information being transmitted. However, taking into account both the complex structure of cultural recipes and the material processes involved in the production of cultural phenotypes shows that small modifications in the recipes can lead to large changes in the phenotype. Inversely, small changes in phenotypes may in fact depend on large changes in the structure of the recipes. For instance, Charbonneau (2015a) points out that in order to augment the width of lithic blades from 2.4 cm to 2.6 cm, stone knappers had to pass from a pressure-flaking technique to the use of the lever as only the latter could exert enough pressure to detach the wider blades. Whereas the blades are very similar (they have 0.2 cm of width difference), the underlying techniques and the set of behaviors and artifacts they depend on are radically different.

A closer look at how the productive processes constrain the variation of cultural phenotype may reveal further complicated cases of cultural genotype-phenotype mapping (or mental representation-public display mapping), challenging the typical cultural evolutionist's assumption of an isomorphic mapping. Moreover, investigating such mapping and the structure of cultural variational spaces can reveal which cultural forms of cultural phenotypes are possible and which ones are not (Charbonneau 2015b), suggesting that observed convergence in form in different cultures may be the result not so much of similar adaptations but of generative constraints on the development of cultural phenotypes.

Further work is required to make a more serious case for both an evo-devo of culture and a cultural evo-devo. Research in both directions is only at an embryonic stage. However, in the future we can expect further advances on the impact of enculturation and metaplasticity on cultural evolution, and also on issues pertaining to cultural modularity, evolvability, and constraints of cultural development. An important part of such work will consist in addressing what nonevolutionary social sciences already have to say about the cultural process and integrate such work into an evolutionary framework before any useful contribution from evo-devo can be productively harnessed.

Cross-References

- ▶ Evo-Devo and Cognitive Science
- ▶ Evo-Devo of Language and Cognition
- ▶ Evo-Devo of Social Behavior

References

Abraham WC, Bear MF (1996) Metaplasticity: the plasticity of synaptic plasticity. Trends Neurosci 19:126–130

Alberch P (1991) From genes to phenotype: dynamical systems and evolvability. Genetica 84:5–11

Boyd R, Richerson PJ (1985) Culture and the evolutionary process. University of Chicago Press, Chicago

Charbonneau M (2015a) All innovations are equal, but some more than others: (Re)integrating modification processes to the origins of cumulative culture. Biol Theory 10(4):322–335

Charbonneau M (2015b) Mapping complex social transmission: technical constraints on the evolution cultures. Biol Philos 30:527–546

Charbonneau M (forthcoming) Modularity and Recombination in Technological Evolution in. Philosophy & Technology

Durham WH (1991) Coevolution: genes, culture, and human diversity. Stanford University Press, Stanford

Enquist M, Ghirlanda S, Eriksson K (2011) Modelling the evolution and diversity of cumulative culture. Philos Trans R Soc B 366:412–423

Heyes CM (2012) Grist and mills: on the cultural origins of cultural learning. Philos Trans R Soc B 367:2181–2191

Jablonka E, Lamb MJ (2005) Evolution in four dimensions: genetic, epigenetic, behavioral, and symbolic variation in the history of life. MIT Press, Cambridge, MA

Lumsden CJ, Wilson EO (1981) Genes, mind, and culture. Harvard University Press, Cambridge, MA

Malafouris L (2009) "Neuroarchaeology": exploring the links between neural and cutlural plasticity. In: Chiao JY (ed) Cultural neuroscience: cultural influences on brain function. Elsevier, New York, pp 253–261

Malafouris L (2010) Metaplasticity and the human becoming: principles of neuroarchaeology. J Anthropol Sci 88:49–72

Malafouris L (2013) How things shape the mind: a theory of material engagement. MIT Press, Cambridge, MA

Mesoudi A, O'Brien MJ (2008) The learning and transmission of hierarchical cultural recipes. Biol Theory 3:63–72

Mesoudi A, Whiten A, Laland KN (2006) Towards a unified science of cultural evolution. Behav Brain Sci 29:329–383

Mithen S, Parsons L (2008) The brain as a cultural artefact. Camb Archaeol J 18(3):415–422

Müller GB (2007) Six Memos for Evo-devo. In: Laubichler MD, Maienschein J (eds) From embriology to Evo-devo. MIT Press, Cambridge, MA, pp 499–524

Rogoff B (2003) The cultural nature of human development. Oxford University Press, Oxford

Roux V, Bril B (eds) (2005) Stone knapping: the necessary conditions for a uniquely hominin behaviour. McDonald Institute for Archaeological Research, Cambridge

Ruddle K, Chesterfield R (1977) Education for traditional food procurement in the Orinoco Delta. University of California Press, Berkeley
Sperber D (1996) Explaining culture: a naturalistic approach. Blackwell Publishers, Oxford
Stout D (2011) Stone toolmaking and the evolution of human culture and cognition. Philos Trans R Soc B 366:1050–1059
Wimsatt WC (1999) Genes, memes, and cultural heredity. Biol Philos 14:279–310

Index

L. Nuño de la Rosa, G.B. Müller (eds.), *Evolutionary Developmental Biology*,
https://doi.org/10.1007/978-3-319-32979-6