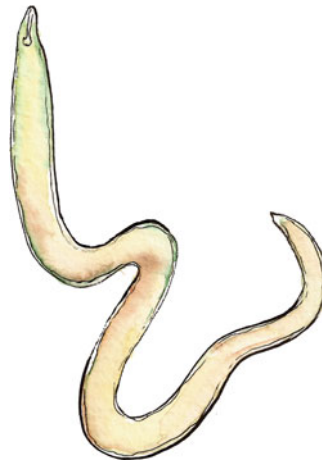


Ralf J. Sommer



Although commonly considered a subtaxon of Cycloneuralia, the Nematoda are covered separately in this chapter.

Chapter vignette artwork by Brigitte Baldrian.
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INTRODUCTION

Nematoda (roundworms) are mostly small animals in the range of only millimeters. While they are hard to see without a microscope, nematodes represent the largest animal phylum with an estimated number in the range of one to ten million species (Lambshhead 1993). Nematodes are characterized by three general features. Besides species richness, these are numerical abundance and ecological omnipresence because they usually occur in high numbers and they are found in most ecosystems. For example, in some soil samples, nematodes can occur in excess of one million individuals per square meter (Floyd et al. 2002). The highest diversity of nematodes is found in marine environments and in terrestrial settings, often in association with arthropods or other invertebrates. Some nematodes are important parasites of plants, livestock, and humans. In the last 15 years, molecular phylogenetics has resulted in a comprehensive understanding of the relationships among nematodes that can serve as the basis for evolutionary considerations (van

Megen et al. 2009). For example, molecular phylogenetics convincingly showed that parasitism has evolved at least seven times independently in nematodes, involving both plant and animal parasitism (Fig. 2.1; Blaxter et al. 1998). By now, many parasitic nematodes have their genome sequenced (Fig. 2.1), representing a promising starting point to understand associated biological processes (for a review, see Sommer and Streit 2011).

One particular nematode species, *Caenorhabditis elegans*, serves as an important model organism for both basic and applied research and plays a pivotal role in the elucidation of basic principles of biology and biomedical research. Not surprisingly, therefore, the knowledge available from *C. elegans* also served as a starting point for studies in evolutionary biology and EvoDevo. The success of *C. elegans* as a model results from the easiness of its culture in the laboratory (Brenner 1974). With a life cycle of only 3 days (20 °C), *Escherichia coli* as food source, and self-fertilization as the typical mode of reproduction, *C. elegans* can be cultured indefinitely in large numbers.

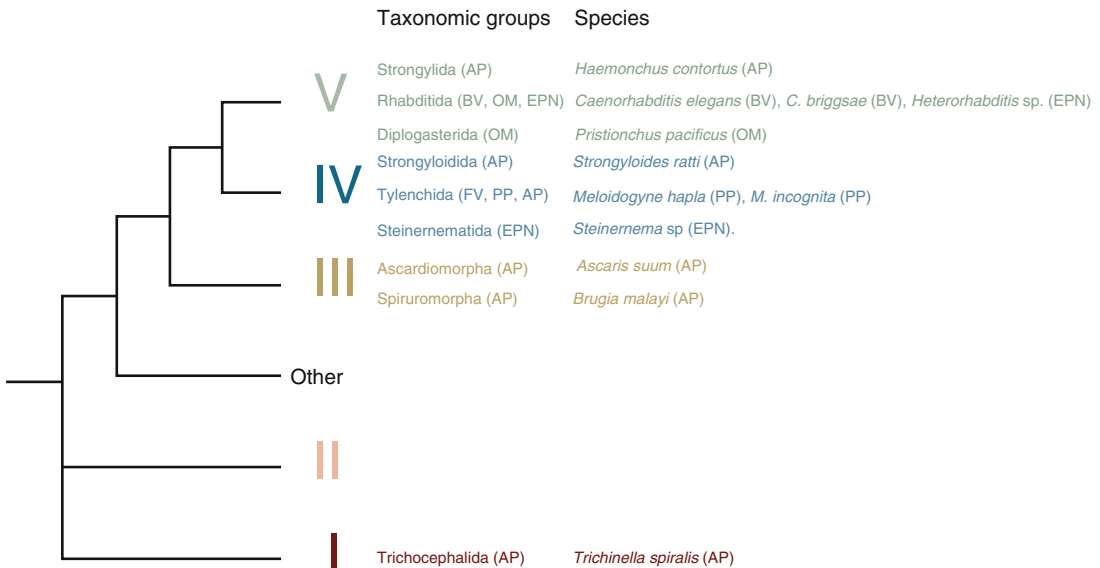


Fig. 2.1 Phylogenetic relationship of nematodes and their associations. *Roman numerals* indicate the distinction of five clades according to Blaxter et al. (1998). Species with a published genome sequence are indicated

in **bold**. *AP* animal parasite, *BV* bacteriovore, *EPN* entomopathogenic nematode, *FV* fungivore, *OM* omnivore, *PP* plant parasite (Redrawn from Sommer and Streit (2011). © Ralf J. Sommer 2015. All Rights Reserved)

C. elegans has a typical nematode life cycle. It undergoes embryonic development within an egg shell, and postembryonic development consists of four stages, called juvenile stages (J1–J4), separated by molts (Fig. 2.2; Wood 1988). In nematode evolution, particularly the evolution of parasitic forms, this general life cycle has been modified in numerous ways. As nematode life cycles are described in numerous textbooks, the following just gives a very brief summary of the different ecologies and life cycles of nematodes.

Saprobiotic, bacterial-feeding nematodes are common in the family Rhabditidae to which *C. elegans* belongs, but also in the Diplogastridae, including another nematode model, *Pristionchus pacificus*. Under favorable conditions, the development of *C. elegans* takes as little as 3 days,

and the adult reproductive life span is also in the order of several days. In contrast, under unfavorable conditions, these worms form a non-feeding but motile alternative J3, called a dauer juvenile, which will be discussed below in greater detail (Fig. 2.2). In the Rhabditidae and Diplogastridae families, an androdioecious mating system has evolved multiple times independently (Denver et al. 2011). Hermaphrodites can mate with males but can also self-fertilize. In both families, the best-studied representatives, i.e., *C. elegans* and *P. pacificus*, are hermaphroditic.

However, other ecologies and life cycles have evolved repeatedly. The genera *Steinernema* and *Heterorhabditis* are two phylogenetically unrelated groups of entomopathogenic nematodes (Fig. 2.1; Gaugler 2002). These nematodes form

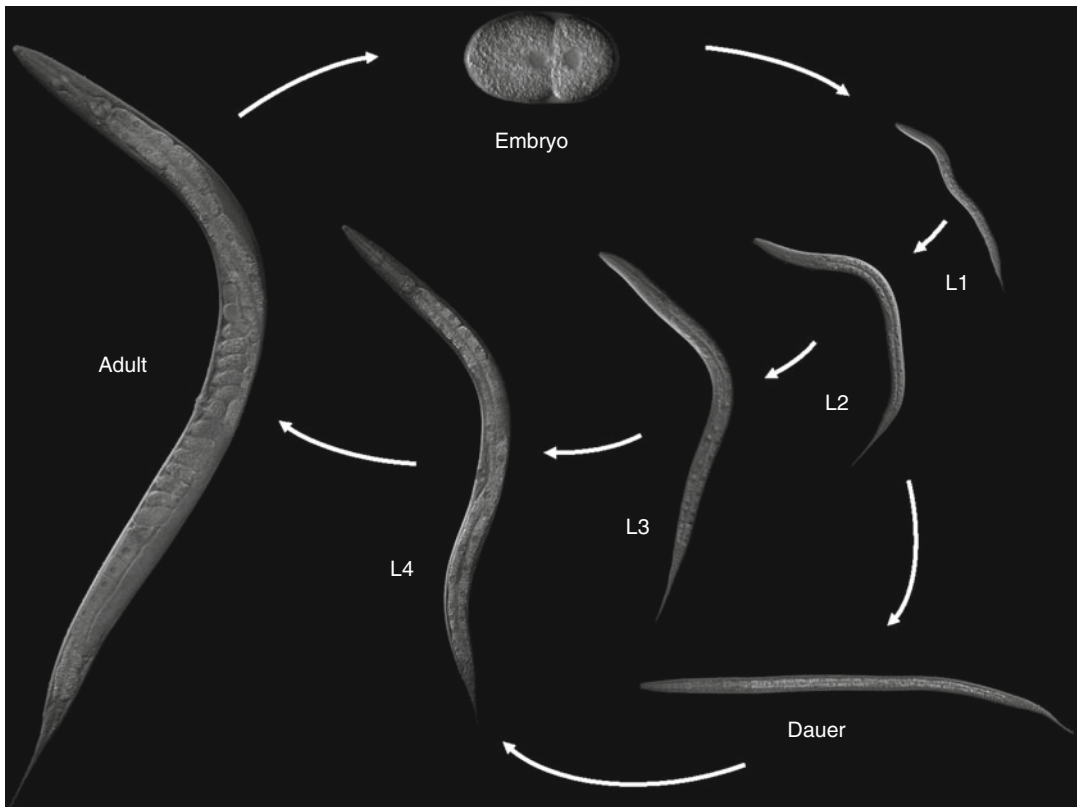


Fig. 2.2 The basic nematode life cycle exemplified with *C. elegans*. *C. elegans* has a simple life cycle that can be completed in 3 days in the laboratory. The self-fertilizing hermaphrodite lays eggs which hatch into an L1 larva. Four larval stages are separated by molt. Larvae grow primarily by the enormous increase in the reproductive

organ, the gonad. Adults can lay more than 200 eggs and can live for several weeks. When conditions become harsh, animals will redirect their development and form an arrested dauer larva, an alternative L3 stage (© Ralf J. Sommer 2015. All Rights Reserved)

infective larvae that carry a species individual of symbiotic bacteria, *Xenorhabdus* spp. in the case of *Steinernema* spp. and *Photorhabdus* spp. in that of *Heterorhabditis* spp. Worms invade insect larvae and release the bacterium, which reproduces very rapidly and kills the insect by the secretion of a number of toxins providing the food for nematodes to grow on the insect carcass. Other nematodes are gastrointestinal parasites of vertebrates, i.e., *Haemonchus contortus* living in the abomasum of sheep and goats, *Ascaris suum* in the small intestine of pigs, and members of the genus *Strongyloides* in the small intestine of mammals. In contrast to these cases, *Trichinella spiralis* is an intracellular parasite. Finally, nematodes are equally successful parasites of plants, with members of the genus *Meloidogyne* spp. being among the most important agricultural pests.

C. elegans and nematode anatomy, gene sequences and expression patterns have been extensively studied in the last two decades, providing detailed insight into the comparative biology of these organisms. Detailed ONLINE platforms (see paragraph below) have been established that summarize and review these topics in form of “living,” regularly updated, chapters. Therefore, the reader is referred to these contributions for a comprehensive summary and state-of-the-art description of expression patterns and gene sequence similarities. Instead, the following text provides an overview on EvoDevo studies in free-living nematodes with the aim to highlight important conceptual findings, such as developmental systems drift. Also, the author wants to highlight the need for integrative research programs, which in nematodes can be very fruitful by combining laboratory studies with fieldwork. Throughout the text, review articles focusing on specific aspects of nematode EvoDevo are mentioned in the respective paragraphs. For example, phylogeny, genomics, and the evolution of reproductive systems are not covered here, and the reader is referred to other recent review articles (Denver et al. 2011; Sommer and Streit 2011; Schierenberg and Sommer 2014).

NEMATODE ONLINE PLATFORMS: WORMBASE, WORMBOOK, AND WORMATLAS

C. elegans is one of the most important model organisms in the modern life sciences and provided detailed mechanistic insight into many fields of biology, including developmental biology and neurobiology. Building on the cellular understanding of embryonic and postembryonic development with the formation of 959 cells in the adult hermaphrodite, generations of scientists have studied many developmental, cellular, and physiological processes in great detail. Fortunately, *C. elegans* has not only been at the forefront of basic research but has also launched several ONLINE platforms for the transparent and open access-driven distribution of knowledge (Table 2.1) that can serve as road model for other research communities. The three most important ONLINE platforms are discussed below.

WormBase (www.wormbase.org) represents a searchable database for all aspects of worm biology. WormBase covers the anatomy and cellular composition of *C. elegans* and contains detailed information on all genes. Gene data include gene classes, ontology, and potentially related human disease genes. Similarly, WormBase covers mutant phenotypes including those obtained by reverse genetic tools, such as RNA interference and the “Million Mutation Project.” The latter represents a mutagenesis project, in which a large number of viable mutants have been generated and all resulting lines have been whole genome sequenced and can be distributed upon request. In WormBase, the pages on individual genes also cover the expression patterns, sequence similarity to other nematodes and other model organisms, information about transgenic strains, and links to all related papers. WormBase is regularly updated, and at the time of writing (August 2014) is in version WS243.

WormBook (www.wormbook.org) represents an ONLINE review of *C. elegans* biology that is regularly updated. WormBook covers all areas of worm biology, including development, genetics,

Table 2.1 ONLINE platforms for *Caenorhabditis elegans* and nematode biology

1.	WormBase	www.wormbase.org	Genetic, biochemical and molecular information, expression patterns, sequence information, complete reference list, link to researchers	<i>C. elegans</i> , other nematodes, other model organisms
2.	WormBook	www.wormbook.org	Worm (<i>C. elegans</i>) biology, methodology, evolution	<i>C. elegans</i> and other nematodes
3.	WormAtlas	www.wormatlas.org	<i>C. elegans</i> anatomy with focus on electron microscopy	<i>C. elegans</i>

cell biology, and biochemistry. It contains a WormMethods section and covers other nematodes, making it an important tool for evolutionary biologists.

WormAtlas (www.wormatlas.org) represents a database featuring the behavioral and structural anatomy of *C. elegans*, making use of the unique serial reconstruction of the worm body by transmission electron microscopy. It contains handbooks for the hermaphrodite, the male, and the dauer stage and as resources provides worm images, a slidable worm, and worm wiring diagrams. Together, these three comprehensive ONLINE platforms make *C. elegans* and worm biology available for scientists in all research fields.

Finally, the *C. elegans* Genetics Center (CGC) distributes all type of reagents necessary to research throughout the world. All of the above-mentioned platforms as well as CGC are funded through the National Institute of Health (NIH) in the United States of America.

NEMATODE EVODEVO

Building on the detailed knowledge about embryonic and postembryonic processes in *C. elegans*, nematode EvoDevo studies cover a variety of developmental patterns and processes. For embryonic developmental processes, the reader is referred to a recent review describing in detail many aspects of early and late embryogenesis throughout the Nematoda (Schierenberg and Sommer 2014). In the following, a brief overview about the evolution of selected embryonic and postembryonic processes is provided. These studies have focused largely on members of the

Rhabditidae and Diplogastridae family because nematodes in these groups can often be cultured in the lab similar to *C. elegans*. Also, in a selected group of nematodes, a functional toolkit was developed that provides mechanistic insight into the evolution of developmental processes.

Sex Determination

Sex in nematodes is most often genetically determined and involves sex chromosomes. Diploid species with sex chromosomes have females with an XX karyotype and males with an XY or an XO karyotype. *C. elegans* and its relatives have XX/XO karyotypes, and intense studies over the last two decades provided a detailed account of the genetics and the molecular biology of sex determination in *C. elegans* (Fig. 2.3; Zarkover 2006). Specifically, in *C. elegans*, the ratio of X chromosomes to autosomes controls a complex signaling pathway of negative-acting factors, and XO animals activate a master sex determination switch gene *xol-1*. In contrast, XX animals suppress *xol-1* activity, resulting in the activation of the zinc finger transcription factor TRA-1, thereby suppressing male development in hermaphrodites. Upstream of *tra-1* is a series of regulators consisting of *her-1*, *tra-2*, and three *fem* genes, with the FEM protein ubiquitin ligase complex eventually targeting TRA-1 for degradation (Fig. 2.3). In hermaphrodites, the absence of HER-1 results in TRA-2 inhibiting the ubiquitin ligase complex, and TRA-1 is finally active to repress male development.

Detailed comparative studies in different *Caenorhabditis* species revealed that sex determination appears to evolve rapidly (Haag et al.

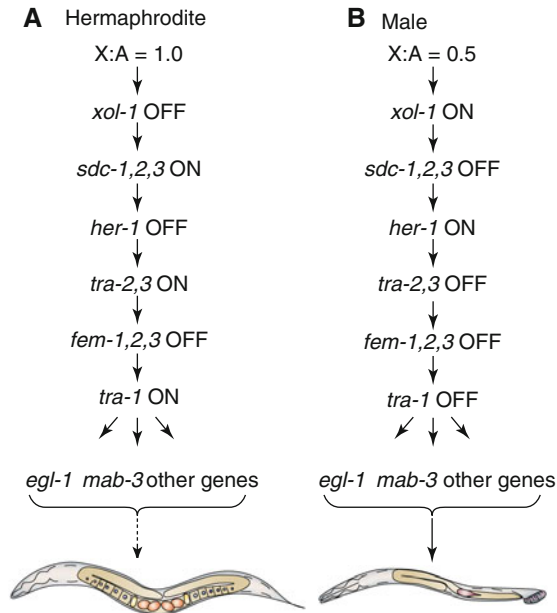


Fig. 2.3 Somatic sex determination in *C. elegans*. Genetic model for sex determination in hermaphrodites (A) and males (B). A series of negative regulatory interactions triggered by the X:A ratio (i.e., the ratio between sex chromosomes and autosomes) results in high TRA-1 activity in hermaphrodites and low TRA-1 activity in males. TRA-1 regulates transcription of various sex-spe-

cific genes, such as *egg-laying defective 1* (*egl-1*) and *male abnormal 3* (*mab-3*). Mutations in *C. elegans* sex determination genes result in distinct phenotypes: *tra* transformer of XX animals into males, *fem* feminization of XX and XO animals, *her* hermaphroditization of XO animals (Redrawn from Sommer and Bumbarger WIRE 2012. © Ralf J. Sommer 2015. All Rights Reserved)

2002). These studies also suggest that the more downstream players in the gene cascade are more conserved. For example, TRA-1 is highly conserved in evolution although the sex determination machinery is evolving rapidly. Interestingly, the regulation of TRA-1 seems to be highly species specific. In *C. elegans*, mutations in the genes *fem-2* and *fem-3* result in females that lack the short period of spermatogenesis typical for hermaphrodites. In contrast, these genes are dispensable for hermaphrodite development in the close relative *C. briggsae*, indicating that *C. elegans* and *C. briggsae* acquired their hermaphroditic mode of reproduction independently from male/female ancestors (Hill et al. 2006).

The gonochoristic *C. remanei* is a close relative of *C. briggsae*, representing the ancestral mode of reproduction. Generally, it is assumed that the evolution of self-fertilizing hermaphroditism, as observed in nematodes, is a late event in evolution and cannot be reverted, resulting in an evolutionary dead end. In the case of *C. remanei*

and *C. briggsae*, it was shown that mutations in just two genes are sufficient to allow the transition from females to hermaphrodites (Baldi et al. 2009). Specifically, lowering the activity of *tra-2* by gene knockdown via RNAi generated animals capable of making spermatids, but they remained dysfunctional unless a second mutation in the sperm activation gene *swm-1* was introduced.

Comparative Cell Lineage Analysis

Cell lineage analysis was the most important approach besides genetics that made *C. elegans* a unique model in developmental genetics. Due to its transparency and the small number of cells, the complete postembryonic cell lineage of *C. elegans* has been determined (Sulston and Horvitz 1977; Kimble and Hirsh 1979). The post-embryonic lineage was found to be invariant resulting in a final number of 959 cells for adult hermaphrodites and 1,031 cells in males.

The comparison between the complete post-embryonic cell lineages of *C. elegans* and the phylogenetically distant nematode *Panagrellus redivivus* revealed high similarities but also important differences that can be used to identify categories of lineage transformations (Fig. 2.4; Sternberg and Horvitz 1981, 1982; Sommer et al. 1994). Basically, five types of cell lineage transformations can be distinguished: (1) fate switch of a cell to a fate normally associated with another cell, (2) polarity reversal in the lineage generated by a blast cell, (3) alteration in the number of rounds of cell divisions, (4) changes in the relative timing of divisions, and (5) altered segregation of developmental potential, such that a fate normally associated with a specific cell instead becomes associated with its sister. Besides these general features, the postembryonic cell lineage of *C. elegans* and comparisons between different

nematodes provided a foundation for many experimental and functional investigations of postembryonic processes. In the following, a few landmark results from nematode EvoDevo studies are provided.

Gonad Development

In general, the structure of nematode gonads varies substantially between the two sexes and across species, with male gonads always being monodelphic (one armed), whereas female/hermaphrodite gonads can be didelphic (two armed) or monodelphic (Fig. 2.5; Chitwood and Chitwood 1977). In *C. elegans* hermaphrodites, the two gonadal arms develop nearly symmetrically from two somatic precursor cells, Z1 and Z4, which form the anterior and posterior arm, respectively (Kimble and Hirsh 1979). Z1 and Z4

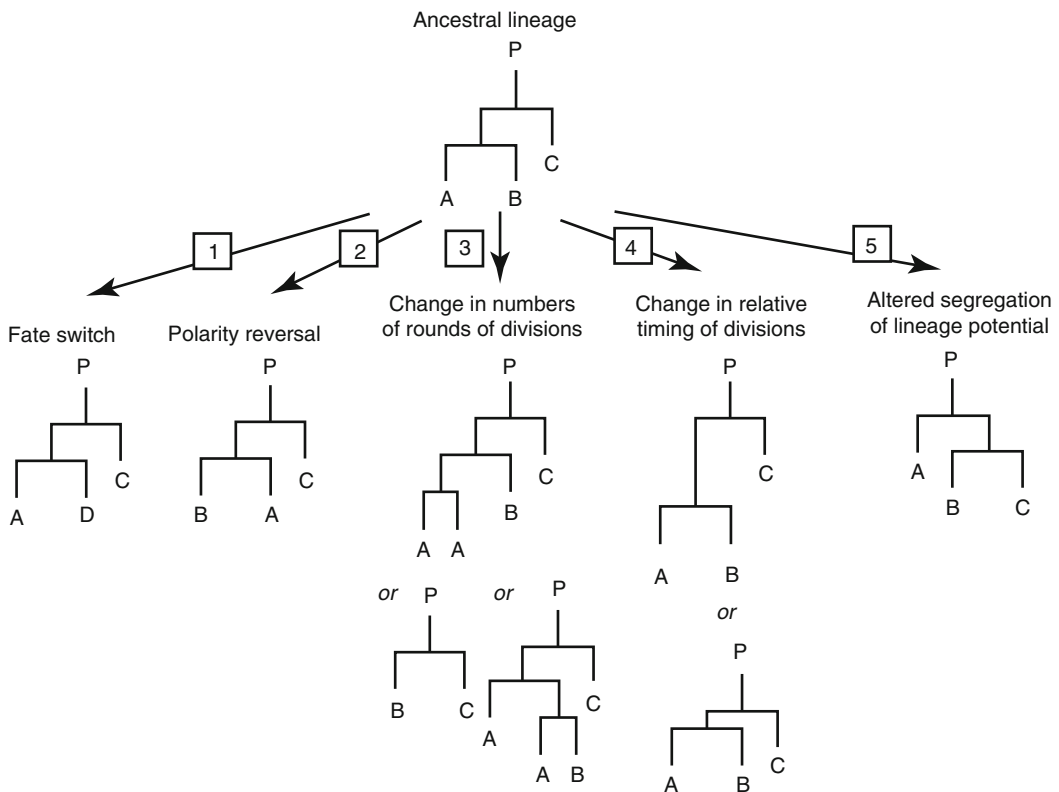


Fig. 2.4 Cell lineage transformations. Five possible kinds of changes in cell lineage pattern found in nematodes. *P*, precursor cell; *A–D*, different cell types (From

Fitch and Thomas (1997), after Sommer et al. (1994). © Ralf J. Sommer 2015. All Rights Reserved)

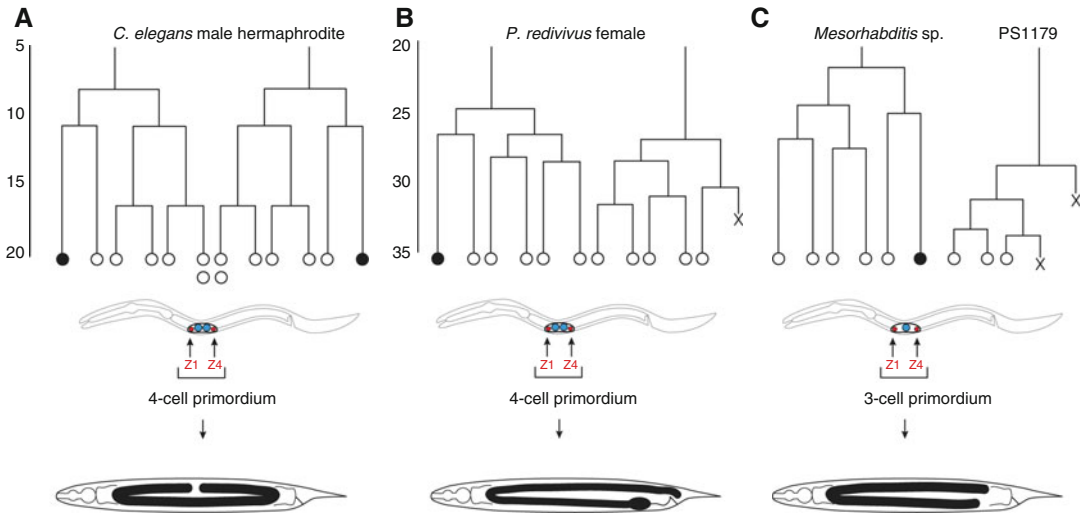


Fig. 2.5 The evolution of gonad development and schematic representation of nematode gonads and their evolutionary modifications. Cell lineage and gonad development of (A) *C. elegans* hermaphrodites, (B) *Panagrellus redivivus* females, and (C) *Mesorhabditis* sp. 1,179 females. Lineages: line length represents the relative timing of divisions. Terminal Xs at the lineage base represent cell deaths; black circles represent the distal tip cell; white circles represent all other fates. Thus, the fate of these par-

ticular cells is sex specific. *Mesorhabditis* starts with a three-cell primordium as a single germ line precursor is present; all others start with a four-cell primordium with Z1 and Z4 as precursor of the somatic gonad (red circles) and Z2 and Z3 as precursor of the germ line (blue circles). Lower images show final anatomy of the gonad. *C. elegans* hermaphrodites have a didelphic gonad, others a monodelphic gonad (Redrawn from Sommer and Bumbarger, WIRE 2012. © Ralf J. Sommer 2015. All Rights Reserved)

give rise to a distal tip cell (dte; Z1.aa and Z4.pp), which promote growth and shape of the gonadal arm (Fig. 2.5).

Panagrellus redivivus was the first species with a monodelphic female gonad to be studied in greater detail (Sternberg and Horvitz 1981). Cell lineage analysis revealed that gonad monodelphy resulted from the programmed cell death of the posterior dte Z4.pp, revealing an astonishing simple mechanism: the programmed cell death of a single cell has a major influence on the overall anatomy of the nematode – a monodelphic vs. didelphic gonadal structure (Fig. 2.5). Within the family Rhabditidae, monodelphy has evolved repeatedly, and in these cases, the first asymmetry between the anterior and posterior part of the gonad can be seen already at the time of Z1 and Z4 division (Fig. 2.5; Felix and Sternberg 1996). Thus, already the descriptive analysis of cell lineage patterns in nematodes can explain major morphological differences among species.

Comparative Gene Expression Studies

The development of powerful genomic and transcriptomic tools in recent years also helped advancing the understanding of comparative embryogenesis in nematodes, allowing for the first time a link between morphological stages of development and the underlying molecular activities (Levin et al. 2012). While comparative cell lineage studies focused largely on the early embryo, transcriptomic tools allow later stages to be analyzed in similar detail. Levin and coworkers (2012) defined the expression profile of *C. elegans* at a genome-wide level, distinguishing ten developmental stages from the four-cell embryo to the hatching L1 larva. Specifically, 6,790 *C. elegans* genes were clustered in 20 distinct developmental profiles based on their dynamic expression patterns. Comparing different stages revealed two drastic transitions in gene expression profiles associated with gastrulation (stage 3 of Levin et al. 2012) and ventral closure

(stage 7). Functional characterization indicated that gastrulation shows intestine-enriched gene expression, whereas ventral closure around stage 7 shows the expression of marker genes known to be involved in the specification and differentiation of neurons and muscles (Levin et al. 2012). Thus, a transcriptomic approach provides new inroads into the analysis of neurogenesis and myogenesis, which are complicated to study by cell lineage analysis given their late occurrence in the *C. elegans* embryo.

The same authors have added to their analysis of *C. elegans* four additional *Caenorhabditis* species, which differ in their length of embryogenesis. Interestingly, the analysis and the comparison with *C. remanei*, *C. briggsae*, *C. brenneri*, and *C. japonica* revealed qualitatively similar relationships in expression profiles to those found in *C. elegans*. These similarities were independent of timing, which differed up to 20 % between the five *Caenorhabditis* species. Together, these studies revealed the existence of two conserved “developmental milestones,” characterized by expression dynamics and the activation of key developmental regulators. Also, this study indicates that the use of transcriptomics in comparative cell lineage analysis and EvoDevo is a powerful tool, which in the future can hopefully be applied to postembryonic processes.

Conservation of Developmental Control Genes: A Slightly Different Perspective

EvoDevo research over the last two decades has resulted in the truism that developmental control genes are highly conserved throughout evolution. However, in nematodes, or at least *C. elegans*, many of these stories are slightly different. For example, *C. elegans* does not contain a typical Hox cluster. While six Hox-type genes have been found, only four of them are in a cluster-like region, and only one, *ceh-13*, the labial-like Hox gene of *C. elegans*, is involved in and is essential for embryogenesis (Aboobaker and Blaxter 2003). The other three classical Hox genes, *lin-39*, *mab-5*, and *egl-5*, play important roles in

postembryonic development, where they guide cell fate decisions in different body regions. Most of their defined roles are as transcription factors acting downstream of signaling pathways such as EGF/RAS and Wnt signaling. For example, vulva development, to be discussed below, requires double input of *lin-39* at different levels of the genetic hierarchy (Sternberg 2005). The functions of Hox genes in nematode development evolve rapidly, as reduction-of-function and loss-of-function mutants in homologous Hox genes in *C. elegans* and *P. pacificus* resulted in completely different phenotypes (Sommer 2008).

For other important developmental regulators, the story told in nematodes is even more different. For example, many studies have shown the transcription factors *groucho* and *hairy*, originally identified by genetic studies in flies, to be highly conserved in animal evolution (Rebeiz et al. 2005). However, there are no *groucho* and *hairy* genes in *C. elegans*, indicating that complex body plans can develop without this pair of genes. In contrast, genetic and molecular studies in *P. pacificus* revealed the existence of a *groucho* and *hairy* module with functions related to insects and vertebrates (Schlager et al. 2006). Thus, there can be major differences in the genetic composition of species within one phylum without these differences being associated with drastic changes of the body plan, as *P. pacificus* and *C. elegans* both look like typical free-living nematodes. The following paragraph will describe in greater detail one example of nematode EvoDevo and the conceptual conclusion of “developmental systems drift,” the notion that homologous structures can be specified by non-homologous molecular mechanisms.

NEMATODE VULVA DEVELOPMENT AND DEVELOPMENTAL SYSTEMS DRIFT

Comparative Vulva Development

The vulva is the egg-laying organ and mating structure of nematode females and hermaphrodites, and vulva formation in *C. elegans* represents one of the best-studied developmental

processes in animal development (Fig. 2.6; for a review, see Sternberg 2005). This precondition makes vulva development a unique paradigm and reference system for comparative EvoDevo studies with a particular emphasis on the underlying mechanisms. The vulva derives from the ventral epidermis in all nematodes studied to date, with the ventral epidermis consisting of 12 epidermal blast cells, called P1.p to P12.p from anterior to posterior (Fig. 2.6). Six of these 12 cells are set aside early in development, and a subset of 3 cells will later be induced to form the vulva.

Specifically, the six ventral epidermal cells P(3–8).p do not fuse with the hypodermis during early *C. elegans* larval development, like their anterior and posterior counterparts P(1,2,9–11).p (Fig. 2.6). They are named vulval precursor cells (VPCs) because they all have the potential to form part of the vulva. However, under unperturbed conditions, only three of these six VPCs, P(5–7).p, form vulval tissue due to an inductive signal from the gonadal anchor cell (AC; Fig. 2.6). P(5–7).p adopt a 2°-1°-2° fate pattern and form the vulva, whereas the three remaining VPCs, P(3,4,8).p, adopt an epidermal, so-called 3° fate (Fig. 2.6; 1°, 2°, 3° denotes a fate hierarchy; ablation experiments indicate that cells compete for a higher ranked fate). P5.p and P7.p have a 2° fate and produce seven progeny each, which form the anterior and posterior part of the vulva. In contrast, P6.p acquires the 1° fate and generates eight progeny forming the central part of this organ. Together, six VPCs adopt one of three alternative fates, resulting in a stereotypical 3°-3°-2°-1°-2°-3° pattern.

In *C. elegans*, vulva formation is induced by a signal from the anchor cell (AC), and the specification of vulva cell fate in *C. elegans* requires a complex network of signaling processes. The AC secretes an epidermal growth factor (EGF)-type ligand that is transmitted in the VPCs by RAS, a central member of various signal transduction pathways. A Notch-type lateral signaling process acts downstream or in parallel to EGF/RAS signaling to specify vulval fates. Once P6.p has been specified as the 1° cell by the AC-derived signal, it signals its two neighbors P5.p and P7.p to adopt a 2° fate via Notch signaling (Sternberg 2005).

Comparative studies on vulva development were initiated in the 1990s (Sommer et al. 1994). These studies revealed that the basic pattern of vulva formation is highly conserved in nematode evolution with P(5–7).p forming the vulva in most representatives studied to date, and the 2°-1°-2° pattern is found to be a basic principle of vulva formation (Kiontke et al. 2007). Surprisingly, however, there is substantial variation with respect to vulva induction. While *C. elegans* requires the AC for vulva induction, some nematodes which form their vulva in the posterior body region rely on cell-autonomous specification processes (Sommer and Sternberg 1994). Again other species require a continuous inductive signal, e.g., *P. pacificus* (Sigrist and Sommer 1999). For a detailed overview, the reader is referred to another recent review (Schierenberg and Sommer 2014). The following concentrates on molecular studies in one particular nematode, *P. pacificus* (see also boxed text), and the observed general phenomenon of developmental systems drift.

The Development of a Satellite Model System: *Pristionchus pacificus* vs. *Caenorhabditis elegans*

Caenorhabditis elegans is one of the most important model systems in modern biology (<http://www.wormbook.org>). As a point for detailed mechanistic and functional comparisons, *Pristionchus pacificus* was developed as a satellite model system. Since its description as a novel species in 1996, a functional toolkit has been generated in this species. *P. pacificus* is a member of the Diplogastridae family and thus only distantly related to the other nematode model system, *Caenorhabditis elegans*, which belongs to the Rhabditidae (Fig. 2.1). While *P. pacificus* and *C. elegans* differ in many developmental characteristics, they share many technical features, such as a short generation time, simple laboratory culture, self-fertilization as a mode of reproduction, and spontaneous male formation in laboratory cultures. In general,

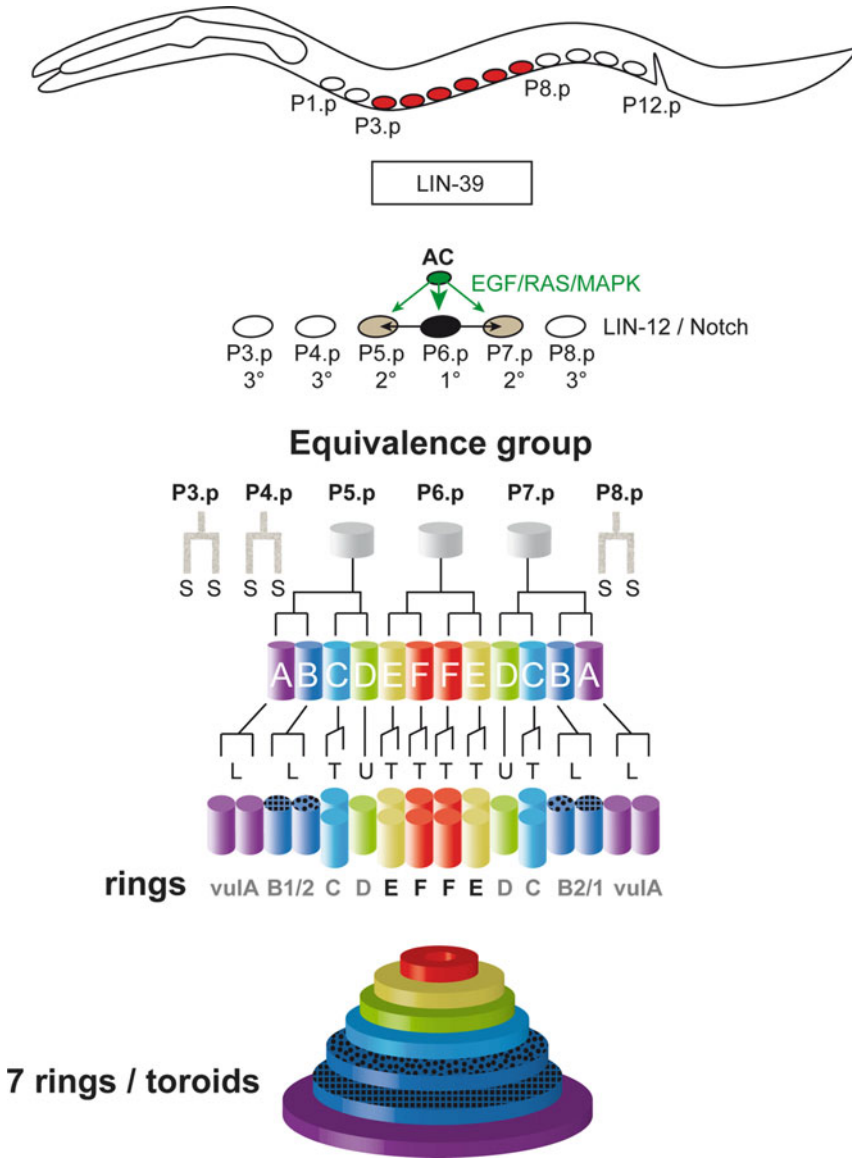


Fig. 2.6 Vulva development in *C. elegans*. *LIN-39* first determines the vulva equivalence group from P(3–8).p and acts as transcription factor downstream of EGF/RAS signaling. The midbody Pn.p cells P(3–8).p are set aside for vulva formation. Other Pn.p cells fuse in the L1 stage. P(3–8).p can adopt one of three alternative fates. P6.p adopts the inner vulval fate (1°, *black oval*), P(5,7).p the outer vulval fate (2°, *gray ovals*), and P(3,4,8).p a non-vulval fate (3°, *white ovals*). This spatial pattern of cell fates relies on an induction of vulval fates by the anchor cell (AC, *green oval*) (green arrows) and lateral signaling between P6.p and its neighbors through a Delta-Notch pathway (black arrows), which inhibits the 1° fate and activates the 2° fate in P(5,7).p. P(5–7).p forms vulval tissue by dividing three times and generating a total of 22 progeny. These 22 progeny partially fuse in

later development and form a total of seven rings, often called toroids, as indicated in the bottom part of the figure. These rings form the scaffold-like structure of the vulva, which connects the uterus to the outside environment. Each fate corresponds to a specific cell division pattern that is executed in the late L3 stage, with characteristic orientations of the third round of division: *T* transverse division (*left-right*), *L* longitudinal (anterior-posterior division), *U* undivided. In the L4 stage, the symmetric cells of the P5.p and P7.p lineages, and of the two daughters of P6.p, migrate toward each other, fuse, and form seven superimposed syncytial rings around a vulval invagination. The two sisters of the B granddaughter form two rings, vulB1 and vulB2; the progeny of all other granddaughters form a single ring (Modified and redrawn with permission from Kiontke et al. (2007). © Ralf J. Sommer 2015. All Rights Reserved)

hermaphrodites are modified females that produce sperm during a short period of larval development to become mature adult females. Hermaphrodites will use their self-sperm for fertilization. However, they can mate with males, the latter of which can easily be obtained and maintained under laboratory conditions as a result of meiotic nondisjunction of the X chromosome. These reproductive features of *P. pacificus* simplify a number of forward and reverse genetic tools, which facilitate mechanistic studies similar to *C. elegans*, *Drosophila* or *Arabidopsis*. In addition to forward and reverse genetics, DNA-mediated transformation is available in *P. pacificus*, providing an important tool for the manipulation of the organism under laboratory conditions.

Developmental Systems Drift

The theory of developmental systems drift was introduced by True and Haag in 2001 and provides a concept to explain the discrepancy between *macroscopic diversity* as seen in animals, plants, and fungi and *microscopic uniformity*, describing the general notion that animal life relies on a small number of signaling pathways that are conserved throughout the animal kingdom to regulate development in diverse organisms. True and Haag argued that the development of conserved morphological structures could involve large-scale modifications in their regulatory mechanisms and that developmental specification mechanisms might evolve rapidly and independent of the morphological structures they are specifying (True and Haag 2001). One example is the rapid evolution of sex determination in animals (see above).

The comparison of the molecular mechanisms involved in vulva formation in *C. elegans* and *P. pacificus* represents a second example for developmental systems drift. Vulva development in *P. pacificus* involves a set of evolutionary conservations and modifications when compared to *C.*

elegans. First, the vulva is a homologous organ because it is formed from homologous precursor cells in both species. In contrast, vulva induction in *P. pacificus* is a continuous process that requires multiple cells and extends over more than 10 h of larval development (Sigrist and Sommer 1999). Detailed genetic and molecular studies revealed that *P. pacificus* vulva induction relies on Wnt signaling rather than EGF signaling (Tian et al. 2008; Wang and Sommer 2011).

There are two astonishing aspects of the different regulatory input to vulva induction. First, the role of EGF vs. Wnt signaling in *C. elegans* and *P. pacificus* evolved in the absence of changes in the genomic composition of these two nematodes. Both species contain largely similar genes in their genome that encode for ligands, receptors, cytoplasmic regulators, and transcription factors of all essential signaling pathways (EGF, Wnt, FGF pathways), and all these genes are 1:1 orthologs. Thus, changes in vulva induction do not depend on gene duplications and/or losses. Second, the change between *C. elegans* and *P. pacificus* occurred without major changes in gene expression, as all studied EGF and Wnt pathway genes in *P. pacificus* show expression patterns similar to *C. elegans* (Tian et al. 2008; Wang and Sommer 2011). Thus, changes in vulva regulation do also not depend on expression pattern changes. Rather, the functional specificity of individual genes and their encoded proteins changed during the course of evolution. One prime example is the LIN-17 protein, which represents a Frizzled-type receptor in nematode Wnt signaling. Genetic studies indicate that *Cel-lin-17* functions as an agonist that transmits Wnt-ligand signaling information (Sawa and Korswagen 2013). In contrast, *Ppa-lin-17* acts as an antagonist of Wnt signaling, indicating that the readout and regulatory linkage of proteins in regulatory networks can change substantially during the course of evolution (Wang and Sommer 2011). These findings result in the major conceptual conclusion that homologous structures formed by homologous cells can nonetheless be specified by completely different and unrelated molecular mechanisms, an extreme and prime example of developmental system drift.

INTEGRATIVE EVOLUTIONARY BIOLOGY

Research in EvoDevo as described above for nematodes tries to identify general principles involved in the generation of biological diversity. The idea to compare the development of different organisms in an evolutionary and phylogenetic context basically goes back to Darwin's principle of modification. However, the observed phenomenon of developmental systems drift, which is emerging as a general principle in EvoDevo if studies are performed in a mechanistic and causative framework, indicates the limits of a pure EvoDevo approach. Consequently, several authors have argued for more integrative approaches to tackle the evolution of function and form by considering all those research areas involved in the regulation and evolution of phenotypes (Gerhart and Kirschner 1997; Schlichting and Pigliucci 1998; West-Eberhard 2003; Sommer 2009). This results from the general notion that at least three research areas are involved in the study of the generation of form and diversity.

First, developmental biology and EvoDevo try to elucidate how morphological structures are formed throughout the ontogeny of the individual and how genetic and molecular alterations result in phenotypic evolution. This monograph represents a testimony of the success of EvoDevo in this research paradigm. However, additional research disciplines not covered in EvoDevo are also important for the understanding of biological diversity. A second research area is ecology, which contributes to the understanding of diversity by indicating how the environment influences development, resulting in evolutionary change ("EcoEvoDevo"). An ecological perspective on developmental processes is crucial to understand the generation of novelty over evolutionary timescales (Gilbert and Epel 2009). Third, population genetics describes how modifications and novelty arise as "natural variation" in populations (Lynch 2007). A population genetic perspective is thus a prerequisite for obtaining a comprehensive understanding of phenotypic change. It has been argued previously that a comprehensive understanding of the evolutionary

forces that generate biological diversity requires integrative approaches that bring developmental biology and EvoDevo closer to evolutionary theory (Sommer 2009). Such integrative studies, when performed in the same organism to study the same patterns and processes, can be integrated into a comprehensive framework.

Developmental systems drift as observed between nematode species in sex determination and vulva development immediately results in the idea that distinct molecular mechanisms are recruited in response to the adaptation to different environments. Unfortunately, little is known about the exact ecology of many model systems in developmental and molecular biology. This was initially also true for the nematode model organisms. Recent years have seen major changes, resulting in the beginning of the description of the environment in which *C. elegans* and other nematodes can be found. *C. elegans* and other rhabditids but also *P. pacificus* can be found in soil samples, and interestingly, the strains used as "wild-type," *C. elegans* N2 and *P. pacificus* PS312, were indeed isolated from soil (Brenner 1974; Sommer et al. 1996).

However, work during the last decade has provided more detailed insight into the ecology of both species. *C. elegans* can most reliably be found in rotten fruits, often rotten apples (Felix and Duvéau 2012). It is most reasonable to assume that *C. elegans* arrives on the apple fruit with a vector, probably insects, although details about the propagation are currently unknown. For nematode dispersal, it is long known that many species contain a specific dispersal stage, the arrested dauer larva (for details, see below). The association of nematode dauer larvae with insects has been called "phoresy" and represents a behavior also known from other invertebrates as a dispersal strategy (Poulin 2007).

The ecological association of *P. pacificus* had also been unknown for a long time. Detailed studies over the last decade, however, have provided ample of evidence for *Pristionchus* nematodes to be associated with scarab beetles (Fig. 2.7; Herrmann et al. 2006, 2010). While most *Pristionchus* species have a nearly species-specific association with scarab beetles, *P. pacificus* is found in association with different



Fig. 2.7 Development of ecologically relevant traits. *P. pacificus* is found in tight association with scarab beetles, such as *Exomala orientalis* (A). It is an omnivorous feeder that can predate on nematode prey (B). For this to be achieved, *Pristionchus* and related nematode have evolved novel morphological structures in forms of teeth-

like denticles. The mouth form of *P. pacificus* is dimorphic and can be either eurystomatous (C) or stenostomatous (D). The mouth dimorphism represents an example of phenotypic plasticity. Scale bar equals 10 μm (© Ralf J. Sommer 2015. All Rights Reserved)

scarabs in different parts of the world. *Pristionchus* nematodes rest on the beetle in the dauer stage and can use their insect vectors also for dispersal (Weller et al. 2010). In addition to this phoretic behavior, however, *P. pacificus* often remains on the beetle and waits for its natural death to feed on the developing microbes on the beetle carcass. Such associations have been called “necromeny” and are often considered to represent an additional step beyond phoresy toward the evolution of parasitism (Poulin 2007).

The inroads into the ecology of *P. pacificus* and related organisms have resulted in new research avenues. The following two paragraphs of this chapter will summarize recent findings of the development and function of dauer larvae and the evolution and development of feeding structures that have evolved in the context of the nematode-beetle ecosystem. These studies indicate the importance of considering EvoDevo questions in the context of ecology and, ultimately, population genetics.

THE DEVELOPMENT AND EVOLUTION OF DAUER LARVAE

Optimal growth conditions in the laboratory are unlike the harsh conditions that nematodes are exposed to in their natural environment. To survive adverse conditions, some groups of nematodes have evolved an effective survival strategy; a specialized alternative larval stage that is resistant to various environmental stresses called “dauer larvae” (Fig. 2.2). Dauer larvae show morphological, physiological, and behavioral adaptations that are not observed in other stages. This includes a closed mouth, a remodeled pharynx, and a thicker cuticle. Usually, dauers are formed as an alternative to the third larval stage, representing an example of phenotypic plasticity. The facultative nature of dauer formation enables a boom-and-bust lifestyle of nematodes: worms reproduce as much as possible while food is available but form arrested dauer larvae after food depletion. Besides survival, dauer larvae are specifically adapted for dispersal, enhancing the chances of finding a new food source (see above). It should be noted, however, that the exact ratio of direct vs. indirect development of *C. elegans*, *P. pacificus*, or other free-living nematodes is currently unknown. These uncertainties represent important shortcomings, as knowledge on the total number of nematode generations per year would be important for the understanding of divergence times between species.

In *C. elegans*, dauer formation is regulated by at least three environmental cues, i.e., starvation, high temperature, and high population density (Hu 2005). Among the many aspects of dauer biology, the genetic mechanisms regulating the entry into the dauer stage are most extensively studied, mainly using *C. elegans* (Hu 2005). In short, the decision to enter direct or indirect development is controlled by multiple signaling pathways, including insulin and TGF- β signaling, which converge at the regulation of a hormone and the nuclear hormone receptor DAF-12. The transcription factor DAF-16 acts in parallel to DAF-12. Mutations in the *daf-12* and *daf-16*

genes result in similar phenotypes deficient in dauer formation (Kenyon 2010).

Upstream of these signaling pathways act a number of small molecules to sense environmental cues, in particular population density. High population density triggers dauer formation and was recently shown to depend on a class of glycosides called ascarosides (for a review, see Ludewig and Schroeder 2013). Ascarosides contain a dideoxyhexose, ascarylose, as the sugar moiety, and they act as pheromones in the regulation of aggregation, mating, and the control of dauer development. However, little is known about the factors involved in linking ascaroside signaling to the signaling processes involved in dauer regulation.

EvoDevo studies on nematode dauer development have also focused on *P. pacificus*. As in *C. elegans*, dauer larvae are formed in response to starvation and high population density, involving a pheromone. The elucidation of the chemical nature of these small molecules resulted in the surprising finding that this nematode produces small molecules of a different composition and a much higher complexity than *C. elegans* (Bose et al. 2012). While still in their infancy, these studies might indicate that nematodes in general are very diverse in their secondary metabolite production, an idea that would correlate with the enormous genomic diversity seen in comparative nematode genome projects (for a review, see Sommer and Streit 2011).

The dauer larval stage as survival and dispersal stage is of tremendous importance for nematode ecology and evolution. Evolutionary theory would predict that the ecological properties of dauer larvae are under strong selection, resulting in natural variation for various dauer traits. Recent studies have started to investigate natural variation for dauer development by comparing multiple isolates of *P. pacificus* from around the world. Indeed, experimental studies of 16 *P. pacificus* strains showed that all strains produced a dauer pheromone (Mayer and Sommer 2011). Surprisingly, however, 13 of these 16 pheromones induce the highest rate of dauer formation in individuals of other genotypes, rather than of their own genotype. This cross-preference might point toward neutral evolutionary processes or

might be a sign of intraspecific competition, a previously unconsidered aspect of dauer formation (Mayer and Sommer 2011).

Indeed, more recent studies showed that the small molecule profiles of six sympatric and allopatric *P. pacificus* strains differ substantially from each other (Bose et al. 2014). Also, these strains differed in their dauer formation response to individual small molecules, and there was limited correlation between small-molecule production and sensing in individual strains. Finally, intraspecific competition was directly observed in a specifically designed competition assay between three sympatric strains from La Réunion Island and two allopatric strains from California and Ohio (Bose et al. 2014). Such studies begin to add novel, previously unconsidered aspects to our understanding of nematode dauer formation and its ecological relevance. Competitive interactions are part of evolutionary arm races that result in novelty and are predicted in an environment such as the scarab beetle ecosystem that *P. pacificus* lives in. *P. pacificus* dauer larvae of different haplotypes are often found on the same beetle individual, which might indicate that intraspecific competition is of relevance in the wild (Morgan et al. 2012).

THE EVOLUTION OF NOVELTY

EvoDevo research focuses primarily on two objectives: first, finding developmental regulators conserved during the course of evolution and, second, determining the changes resulting in the modification of development and ultimately morphology and form. These objectives are fully in line with Darwin's principle of common ancestry and "modification," resulting in the diversity of life seen all over the planet. However, besides modification, the evolution of novelty represents a second important objective necessary to understand how the diversity of form known today has been acquired. The evolution of novelty is often attributed to key innovations, which are defined as phenotypic traits that allow the subsequent radiation and success of a taxonomic unit (West-Eberhard 2003). One prime example of a key

innovation is the neural crest, which resulted in the radiation of the vertebrates (Hall 1999).

Some authors have argued that phenotypic plasticity plays a crucial role in the generation of morphological novelty, a hypothesis that has been put forward under the term "facilitator of phenotypic evolution" (West-Eberhard 2003). The shared genetic control of dauer formation in free-living nematodes and infective juveniles in parasitic nematodes has been discussed as an example supporting this hypothesis (Poulin 2007; Sommer and Ogawa 2011). The following provides another example of phenotypic plasticity in form of a mouth dimorphism, which is unique for the genus *Pristionchus* and some related genera of the Diplogastridae family (Kanzaki et al. 2012).

P. pacificus worms are omnivorous feeders. With their necromenic lifestyle, they can find bacteria, protozoa, fungi, and other nematodes on beetle carcasses (Bento et al. 2010). *P. pacificus* is equipped with versatile teeth-like denticles in its mouth, and it can assume two forms, the so-called eury stomatous (EU) and the stenostomatous (ST) form (Fig. 2.7). EU worms are distinguished by a bigger clawlike dorsal left denticle and an extra right ventral denticle not present in ST animals, in addition to quantitative differences in the shape of the buccal cavity in EU worms (Fig. 2.7). The mouth of an individual nematode is irreversibly determined and executed during larval development. Interestingly, selection line experiments of ST and EU worms over several generations have indicated that the development of the mouth form is phenotypically plastic, representing another example of phenotypic plasticity in nematodes (Bento et al. 2010).

Several environmental perturbations strongly influence the mouth-form decision, with starvation showing one of the strongest effects (Bento et al. 2010). Given that starvation also regulates dauer formation in *P. pacificus* and *C. elegans*, genetic experiments have shown that dauer and mouth-form formation are regulated in part by similar molecular mechanisms. Specifically, the mouth-form decision is controlled by the nuclear hormone receptor *Ppa-daf-12*, and application of one of its steroid hormone ligands, dafachronic

acid (DA), also influences the mouth-form ratio (Bento et al. 2010). Thus, endocrine signaling module DAF-12/DA has been independently co-opted to the mouth-form regulatory network.

In contrast, other studies showed that mouth-form regulation relies on a number of novel regulators. First, unbiased chemical studies using NMR and mass spectrometry indicated a number of complex and novel small molecules to be involved in the regulation of the mouth form. For example, the dimeric ascaroside derivative *dasc#1* specifically regulates mouth form but not dauer development in *P. pacificus* (Bose et al. 2012). More recent genetic studies indicated that a novel sulfatase gene is part of a developmental switch mechanism that regulates the mouth-form decision in *P. pacificus*. Mutations in the *eud-1*/sulfatase result in the absence of the Eu mouth form, whereas overexpression fixes this form (Ragsdale et al. 2013). Surprisingly, it was shown that natural variation in mouth-form frequencies among natural isolates of *P. pacificus* involved expression differences of *eud-1*. Thus, microevolutionary differences can be attributed to a gene originally identified in a genetic screen. Using the possibility to form hybrids between *P. pacificus* and its gonochoristic sister species *P. exspectatus* provided further evidence for a role of the *eud-1* sulfatase also in the macroevolution of the mouth dimorphism (Ragsdale et al. 2013). The role of *eud-1* as a developmental switch brings long-standing research of phenotypic plasticity into the realm of molecular biology. In particular, the confirmation of the prediction of developmental switches (West-Eberhard 2003) indicates how the combination of laboratory studies and fieldwork (population genetics and the result isolation of wild strains) can result in new insight, helping to better integrate knowledge in evolutionary biology.

OUTLOOK

Nematode EvoDevo is an attractive research area given the easiness with which multiple nematodes can be studied in the laboratory. Not surprisingly, therefore, work on nematodes has contributed to the important conceptual finding

of developmental systems drift, in particular with detailed studies on sex determination and vulva development.

Given some of the attractive technical features of nematodes, this group of animals might also play an important role for the next conceptual challenges in EvoDevo and, more generally, evolutionary biology. Specifically, the author of this chapter wants to highlight that detailed integrative case studies in a diversity of organisms, including insects, cnidarians, and nematodes and covering diverse approaches involving genetics, molecular biology, ecology, and population genetics, are necessary to obtain a broad and comprehensive picture about evolution and its underlying mechanisms and causes. Therefore, the most pressing open questions in nematode EvoDevo relate to the analysis of ecologically relevant traits. This involves a different perspective of research. One example that can highlight these novel needs and which has been outlined above is nematode dauer development. For a long time, researchers have focused purely on the developmental and genetic aspects of dauer formation. However, dauer development also represents an example of phenotypic plasticity, providing an important link to ecology and evolution. Any finding made for the regulation of dauer development has to be considered in the context of these disciplines. A population genetic (natural variation) perspective of dauer development can provide important new insights and questions, which will ultimately redirect research into new avenues (Mayer and Sommer 2011; Bose et al. 2014). Thus, modern EvoDevo research needs broad perspectives and open-minded researchers because development and organisms are linked to ecology and evolution (“EcoEvoDevo”). The time is ripe.

OPEN QUESTIONS

How Questions

- Development of reverse genetic tools for gene knockouts, i.e., TALEN and CRISPR
- Molecular phylogenetic framework for EvoDevo organisms

- Case studies I: The evolution of sex determination and the mode of reproduction
- Case studies II: The evolution of the nervous system in the context of the organism's ecology
- Case studies III: The evolution of embryogenesis in the context of the organism's ecology

Why Questions

- Isolation of strains for a natural variation (population genetic) perspective
- Knowledge on the environment of EvoDevo model organisms
- Analysis of adaptive and nonadaptive forces acting on EvoDevo traits (i.e., sex determination, mode of reproduction, gonad development)
- The molecular mechanisms of developmental systems drift

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