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A new era for evolutionary developmental biology in non-model organisms

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Since the publication of *The Origin of Species* by Charles Darwin, evolutionary biology has developed into a key discipline of biology and experienced several important expansions contributed by the sequential integration of Mendelian genetics, Morgan's chromosome heredity mechanism, and Crick's genetic central dogma theory. Especially in recent decades, next-generation sequencing has developed rapidly and provides a powerful tool for identifying candidate targets that govern the evolutionary changes, greatly promoting the study of evolutionary biology toward understanding the molecular mechanisms. More attention has been paid and funding has been devoted to this field especially in China, such as the Microevolution Project supported by the National Natural Science Foundation of China and Complex Traits Evolution Project supported by the Chinese Academy of Sciences. These initiatives greatly motivated research into evolutionary biology and a big leap has been achieved in China.

However, the evolutionary mechanisms of organismal diversification over time still remain largely unresolved. Evolutionary changes can occur not only by the direct transmission from ancestors into descendants, but also in the developmental processes to produce innovative traits. The developmental process also constrains evolutionary directions. Therefore, evolution cannot be comprehensively understood without the knowledge of development biology. Numerous studies have been devoted to understanding the relationships between developmental processes and phenotypic changes during evolution, promoting the development and maturation of the subdiscipline of evolutionary developmental biology (evo-devo).

Evo-devo is a subdiscipline combining evolutionary biology with developmental biology, seeking to explain the influence of developmental process on evolutionary changes in phenotype. It focuses on discovering and understanding the genetic and developmental mechanisms of the origin and evolution of key innovative traits (Hall, 2003). Its central scientific question is to reveal the molecular basis underlying morphological diversity and phenotypic changes from an evo-devo perspective. From my point of view, evo-devo has experienced three leapfrogging developmental stages. The first was marked by the identification of conserved set of gene toolkits that were commonly found among most metazoans. The second was to uncover how morphological diversity evolved and developed through the regulation of gene networks. The ongoing third stage is the introduction of multi-omics techniques, gene editing and experimental functional validation to advance our understanding of evo-devo mechanisms.

As a subdiscipline, evo-devo seeks to complete the conceptual framework of modern evolutionary biology. Three primary forces significantly drove the start of a new era for evo-devo studies. Firstly, integration of multiple disciplines in evolutionary research has been an overarching trend in the

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21st century. The innovative techniques and approaches of genetics, systematic biology, biogeography, and ecology will provide powerful impetus as well as the means to profoundly understand the molecular mechanisms of origin and evolution of complex phenotypes in ever-greater detail. Secondly, with the development of next-generation sequencing, high-quality reference genomes and re-sequencing have been available to reveal a more comprehensive picture of genetic diversity. Large-scale candidate gene sets have been created by high-throughput sequencing to understand the genetic mechanisms of innovative traits such as unique pseudo-thumbs of the giant and red pandas (Hu et al., 2017). Especially recently significant advances in multi-omics technologies have allowed evolutionary biologists to efficiently answer more challenging evo-devo questions in different scales, including genes, mRNAs, proteins, modifications, and metabolites of different taxa. Last but not least, genome editing tools, especially the CRISPR-Cas system, have become increasingly available (Luo et al., 2018; Sun and Zhu, 2019; Hong et al., 2019) to facilitate non-model organisms' genome editing. Gene editing combined with targeted transgenesis-based experimental validation integrated into evo-devo studies will enable us to better understand molecular mechanisms of phenotypic plasticity and morphological diversity.

Recently, more high-profile researches attempted to reveal the evo-devo mechanisms of complex traits, both in animals and plants (Table 1). In animals, evolution of the tetrapod limb from fish fin is one of the important evolutionary events that enable aquatic vertebrates to conquer land and begin to adapt to diverse terrestrial habitats. Early fossils provide solid evidence that fish fins evolved progressively distal structures from ~400 million years ago. The evolution of tetrapod limbs from fish fins is evident as striking similarities in morphogenesis architecture and gene expression module. The integration of 'omics' approaches and gene editing-based experimental validation has advanced our understanding of the evo-devo mechanisms underlying limb organogenesis. Recent studies have proven that a complex gene network including *Shh* and *Hox* gene clusters plays an important role in the fin-to-limb transition (Nowoshilow et al., 2018). Comparative genomic analyses of a wide range of vertebrates including snakes and fishes show an increased evolutionary rate and progressive function loss of a critical limb enhancer ZRS in snakes which explains morphological disappearance of limbs (Kvon et al., 2016). CRISPR/Cas9-based gene editing enabling enhancer replacement in the mouse model makes it possible to reconstruct the limb reduction across evolution through *in vivo* experiments.

The body plan has been defined as an important morphological feature for high-level taxa classification. Animals within the same phylum or the same phylogenetic group are considered to share similar basic body plan. The developmental hourglass model of embryonic evolution suggests that basic body plans are established with evolutionary constraints acting on mid-embryogenic (phylotypic) development. Multi-omics study of the chordates, including amphioxus and vertebrates, across multiple developmental stages, allowed investigation of the regulatory mechanisms of body plan, suggesting that paralogues derived from whole-genome duplications govern the chordate basic body plan (Marlétaz et al., 2018). A recent study showed that the dorso-ventral decoupling of Hox gene expression underpins the diversification of the molluscans body plan, providing a novel insight into the evo-devo mechanism of diverse body plans in the molluscan lineages (Huan et al., 2020).

Plant evo-devo studies largely focus on elucidating the genetic basis of organ diversity, especially floral diversity. The evolution of flowers is thought to have facilitated the extensive radiation of angiosperms through enhancing the efficiency of the interactions with pollinators to increase reproductive success. Comparative genomic analysis showed that whole-genome duplication events occurred before the diversification of angiosperms, and contributed to the emergence of many new genes including MADS-box genes, which are responsible for the origin and innovation of flowers. A combinatorial genetic model, the ABC model, as well as one of its derivatives, the ABCDE model, explains how MADS-box genes work together to specify organ identity in each whorl, assisting us to understand the evo-devo basis of flower diversity.

Evo-devo is an indispensable discipline that bridges the gap among macroevolution, microevolution and ultra-microevolution. It will provide solid support for the research into life science in all fields such as systematic biology, conservation biology, agriculture science and clinical medicine. The evo-devo mechanism of key innovative phenotypes, such as limbs, lung, placenta and wings of animals, as well as flowers and fruits of plants, which are vital for adaptation to terrestrial habitats, have not been completely clarified. Furthermore, much more attention should be paid to the evo-devo mechanism of non-model organisms, especially those representing major evolutionary lineages or possessing representative complex traits should be developed into model systems in future evo-devo studies.

Although accumulating genome-wide surveys across the tree of life have been used to screen candidate genes, considerable random noise may occur due to the neutral selection effect that would mask true positive selection signals. Comprehensive datasets will be created subsequently with the explosion of 'omic'-based studies. Thus, new algorithms and software should be developed to promote the understanding of the molecular mechanisms underlying key phenotypic innovations. New techniques and approaches such as single-cell sequencing, fossil genome or proteome sequencing, and artificial intelligence (AI) should be also in-

Table 1 A summary of recent representative advances of evo-devo study in non-model organisms

Organism	Key phenotype	Scientific issue	Methodology used	Ref
Mammals	Wing of bats	Genetic mechanism of bat wing development	Genomics, transcriptomics, epigenomics, and gene functional validation	Eckalbar et al., 2016
Birds	Flight loss of Galapagos cormorant	Genetic mechanism of flightlessness evolution	Genomics and CRISPR/Cas9-based experimental validation	Burga et al., 2017
Reptiles	Limb loss of snakes	Evolution of limb loss in snakes	Phylogenetic analysis and CRISPR/Cas9-based experimental validation	Kvon et al., 2016
Amphibians	Tail and limb regeneration of Salamander	Evolution of key tissue formation regulators	Genomics and CRISPR/Cas9-based experimental validation	Nowoshilow et al., 2018
Fishes	Pelvic-fin loss and male pregnancy of seahorse	Evolution of morphological innovations and phenotypic changes	Phylogenetic analysis and CRISPR/Cas9-based experimental validation	Lin et al., 2016
Cephalochorda	Body plan of amphioxus	Evolution of vertebrate morphological innovations	Genomics, epigenomics, transcriptomics and transgene experimental validation	Marlétaz et al., 2018
Crustaceans	Diversified appendages arthropods	Crustacean limb specification and evolution	CRISPR/Cas9 and RNAi-based gene functional validation	Martin et al., 2016
Dicotyledon	Leaf morphological diversity of *Cardamine hirsuta*	Development and diversity of leaf shape	Transgenes experiment validation, phenotypic analysis and modeling	Kierzkowski et al., 2019
	Parallel petal losses within Ranunculaceae	The genetic mechanism of parallel petal losses within Ranunculaceae	Phylogenetic analysis and gene functional validation	Zhang et al., 2013

troduced to answer more challenging questions in evo-devo fields. The conceptual framework of the developmental biology should be updated to provide a better complement for evolutionary biology, which is indispensable for the development and maturation of evo-devo in non-model organisms. Environment is a well-known and important factor for inducing species phenotypic variations. Integrating ecology into evo-devo as the ecological evolutionary development biology (eco-evo-devo) is also needed to elucidate the mechanism determining phenotypic plasticity. Therefore, profoundly understanding the origin and evolution of species diversity requires cross-disciplinary collaboration not only with the involvement of biologists but also with scientists from different fields.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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