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Toward mechanisms underlying the developmental hourglass: evolvability and phylotypic conservation

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Abstract
Understanding the general relationship between animal ontogeny and phylogeny remains a long-standing issue in evolutionary developmental biology. Historically, observation of these two phenomena, which involve increasing complexity, led to a series of hypotheses culminating in Haeckel's recapitulation theory in the 1860s. These classical theories posited the conservation of earlier developmental stages in what is sometimes referred to as the 'funnel model' of development. However, in the 21st century, a body of evidence accumulated that robustly supported the "developmental hourglass model", first proposed by Duboule, in which the mid-embryonic phylotypic period is highly conserved. Despite this empirical progress, the mechanisms underlying such conservation have remained elusive. Recent empirical studies have begun to indicate that this conservation may stem from the reduced potential of embryos at this stage to create phenotypic variations. Nevertheless, the precise mechanisms underlying this lower evolutionary potential remain unclear. In this review, we outline historical and recent perspectives and findings on the relationship between ontogeny and phylogeny, contrasting historical assumptions with emerging evidence concerning the mechanisms of embryonic evolution. We particularly highlight the possible role of lower evolvability, referring to intrinsic properties of developmental systems that pontentially constrain phenotypic diversification.

Keywords Developmental hourglass model, Developmental stability, Evolutionary conservation, Comparative transcriptomics, Recapitulation theory

General relationship between ontogeny and phylogeny

The exploration of the general relationship between evolution and development is one of the central themes in biology. In development, the processes by which a fertilized egg transforms into a mature organism generally involves temporal progression from simplicity to complexity. This pattern of increasing morphological complexity over time is not only evident in ontogeny, but is also observed over evolutionary timescales (phylogeny) prompting scientists to propose parallelism between these two processes from as early as the eighteenth century [1–4]. In the late 18th and early 19th centuries, when

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natural philosophy (*Naturphilosophie*) reached its height, it was often posited that both ontogeny and phylogeny were governed by the same fundamental law. Prior to Darwin, biologists such as Oken [5], Meckel and Serres [6] proposed parallels between the hierarchical order of species and embryonic development [1]. Ernst Haeckel proposed the recapitulation theory at the time when Darwin's evolutionary theory was gaining ground, claiming that ontogeny represents a condensed reiteration of phylogeny, and hypothesizing that the embryonic stage of various animals sequentially mirrors the adult stages of their evolutionary ancestors [7]. These classical theories of evolutionary and developmental parallelism [5–7] generally share the idea that more derived characters tend to emerge later in embryogenesis, forming a key conceptual basis for the "funnel model" of ontogeny. The validity of such models has often been argued through experimental and theoretical examination of the underlying mechanisms. For example, changes occurring at earlier developmental stages are expected to exert a profound impact on later stages, including embryonic lethality, and consequently, that early stages were less likely to accumulate evolutionary changes (examples of such thinking include the stepping-stone model [8], developmental burden [9], and generative entrenchment [10]).

Over more than a century of debate, several alternative models have been proposed [11, 12], most notably the "developmental hourglass model" [13, 14]. In contrast to the aforementioned funnel model, which assumed that the earliest embryonic stages would be the most evolutionarily conserved, the developmental hourglass model posited that the mid-developmental phase (such as pharyngula stage, extended germ-band stage, etc.) would be the most highly conserved, and that applies to species in each animal phylum. The hourglass model arose from taking note of the appearance of similarities in morphology and Hox cluster expression during mid-developmental (organogenesis) period in animal embryos, whereas earlier and later developmental stages appear more phenotypically diverse. This conserved mid-embryonic stage is often termed the "phylotypic stage" [15] or "phylotypic period" [16], since the period was expected to show the "phylotype" which defines the basic anatomical pattern, or body plan of animals in the same phylum [15, 17].

Support for the hourglass model

To explore the general relationship between ontogeny and phylogeny, researchers have employed various approaches. Some pioneering studies attempted to assess the strength of developmental constraints during embryogenesis, or potential mechanisms that limit phenotypic diversification [18]. One major difficulty in measuring the evolutionary conservation of embryonic stages using morphological data lies in determining which embryonic features at different developmental stages should be considered equivalent or comparable [11, 12], leading to confusion in the debates. Some studies [19–23] have tried to overcome this by measuring the proportion of ancestral genes expressed at each stage, such as by Ancestor Index [24], Transcriptome Age Index (TAI) [25], or Transcriptome Divergence Index (TDI) [20]. These single-species approaches, however, do not provide a direct measure of the evolutionary conservation of developmental stages, since they do not evaluate shared features between developmental stages of different species but rather reflect the ancestrality (TAI) or sequence divergence (TDI) of expressed genes. This is because, even if a certain developmental stage is enriched with ancestral genes or their transcripts, it does not necessarily mean that their homologous genes are expressed in the same or similar manner in different species, and thus cannot serve as a direct indicator of the conservation of that developmental stage. In short, akin to a Gene Ontology analysis, Ancestor Index, TAI and TDI are essentially ways to evaluate the properties of genes expressed at each developmental stage within a given species, rather than measures of the evolutionary conservation of developmental stages.

Cross-species approaches may thus provide a better indicator for inferring the conservation of developmental stages among different species. For instance, cross-species comparison of whole-embryonic transcriptomes is widely used to evaluate evolutionary conservation of developmental stages. Although this approach does not necessarily imply morphological similarity between developmental stages in different species, it is assumed to reflect similarities in the proportions of homologous cell types. Meanwhile, it should be noted that whole-embryo bulk RNA-seq merely reflects an average similarity of overall transcriptome and does not necessarily mean that all cell types at that stage are equally ancestral or conserved. In fact, in some spiralian larvae, for example, conserved trochophore stages identified by whole-embryo comparative transcriptome analysis [26, 27] were later shown by single-cell analyses to contain evolutionarily novel cell types [28]. Nevertheless, results from comparative transcriptomic studies have lent experimental support to the hourglass model in various animal lineages. For example, cross-species transcriptomic studies have demonstrated hourglass-like conservation of gene expression in six *Drosophila* species [29], in four [30] and six [31] species of vertebrates, in two species of Mollusca [26], in five species of echinoderms [32], and in two species of Annelida [33].

Taxonomic breadth applicable to the hourglass-model and implications for phylum definition

Despite these accumulating studies and a prediction that hourglass-like conservation holds true for each animal phylum [14], the exact range of species to which the hourglass model can be applied remains to be elucidated [34]. If an "hourglass-like" conservation were shared across all animal phyla, the conserved features would account for a pan-animal "zootype" in Slack's sense [35], rather than phylum-specific foundations of body plans. In this light, one study performed cross-phylum comparisons using 10 species from 10 animal phyla and concluded that the hourglass model does not hold across phyla [36]; however, methodological concerns were raised about this study as the study did not take phylogenetic relationship into consideration in comparing embryonic transcriptomes [37]. Most studies have focused on limited taxa within individual phyla, yet only a limited number of studies have covered a wide range of species within an individual phylum (Table 1). Hu et al., for example, compared whole-embryonic transcriptomes of eight chordate species, including multiple vertebrates, a cephalochordate (amphioxus) and a urochordate (*C. intestinalis*); their cross-species analyses found hourglass-like conservation from smaller clades within a class to larger clades namely within phylum (persistent conservation) [31]. Nonetheless, the conserved stage in the tunicate *C. intestinalis* was rather obscure, for reasons unknown. Another study performed cross-species whole embryonic transcriptomic comparisons across five echinoderm species representing all five major classes in that phylum, and provided rare empirical support for the hourglass model in phylum-range species [32]. Interestingly, this study challenged the phylotype hypothesis, a subsidiary to the hourglass model which posits that morphologies characteristic of the conserved developmental stage serves as precursors to the adult body plan. While this study demonstrated the hourglass-like conservation in echinoderm embryos, the most conserved developmental stage (gastrula) did not match the stage when the adult body plan, or pentameral symmetry, emerges [32]. This discrepancy between the most conserved stage and the emergence of the body plan can serve as a potential counter-evidence for the phylotype hypothesis; however, alternative possibilities should also be considered. For example, it is possible that this represents a rare exceptional case arising from substantial morphological changes by developmental mode, namely, indirect development. In vertebrates and insects examined so far, metamorphosis does not appear to disrupt the basic anatomical pattern emerges at the mid-developmental stage, however, echinoderms exhibit a substantial decoupling between larval body plan (the bilateral symmetry) and the adult body plan (pentaradial symmetry). Further comparative studies in cnidarians would provide additional insights, as similar decoupling is observed in this phylum: the planula larva, which exhibits bilateral symmetry, develops into either the radially symmetrical polyp or medusa forms [38, 39].

Alternatively, the gap between the most conserved stage and the emergence of the body plan may indicate fundamental issues in the definitions of animal phyla and body plans; existing definitions are inherently circular, since a phylum is usually defined by a

Table 1 Cross-species comparative studies of the hourglass model

Phylum	Species	Conservation level	References
Arthropoda	*D. melanogaster* *D. simulans* *D. ananassae* *D. persimilis* *D. pseudoobscura* *D. virilis*	Genus (Drosophila)	[29]
Arthropoda	*D. melanogaster* *A. gambiae*	Order (Diptera)	[44]
Chordata	*M. musculus* *G. gallus* *X. laevis* *D. rerio*	Subphylum (Vertebrata)	[30]
Chordata	*X. laevis* *X. tropicalis*	Genus (*Xenopus*)	[41]
Chordata	*P. sinensis* *G. gallus*	Class (Reptilia)	[40]
Chordata	*D. rerio* *X. tropicalis*	Subphylum (Vertebrata)	[44]
Chordata	*M. musculus* *G. gallus* *P. sinensis* *X. laevis* *X. tropicalis* *D. rerio* *C. intestinalis* *B. floridae*	Subphylum (Vertebrata)	[31]
Chordata	*G. gallus* *X. tropicalis* *D. rerio* *O. latipes* *B. lanceolatum*	Phylum (Chordata)	[45]
Nematoda	*C. remanei* *C. briggsae* *C. brenneri* *C. elegans* *C. japonica*	Genus (Caenorhabditis)	[46]
Mollusca	*C. gigas* *H. discus hannai*	Phylum (Mollusca)	[26]
Echinodermata	*P. lividus* *S. purpuratus*	Order (Camarodonta)	[47]
Echinodermata	*A. japonicus* *L. variegatus* *A. japonica* *S. purpuratus*	Phylum (Echinodermata)	[32]
Annelida	*O. fusiformis* *C. teleta*	Class (Polychaeta)	[33]

monophyletic group of species sharing the same body plan [34]. Although further research is needed to determine the evolutionary scale to which the hourglass model applies, studies have demonstrated that the most conserved embryonic stage is maintained across phylogenetic groups from larger to smaller scales, and even among variations within a species. For example, the highly conserved, potential vertebrate phylotypic period has been documented as the most conserved phase in other several taxa, such as reptiles [40], *Xenopus* species [41] and even within intra-species lines in medaka (*Oryzias latipes*) [42], a phenomenon known as "persistent conservation" [43]. These findings suggest that the potential phylotypic period has been conserved through a variety of evolutionary scales while adapting to various niches, such as marine, freshwater, and terrestrial habitats, implies the existence of some non-environmental factors underlying the persistent conservation, such as developmental constraints [47]. On a different note, this persistent conservation from the intra-species level to broader phylogenetic scales could potentially be leveraged to resolve the circular definition of body plan and phylum. A phylum is often defined as a taxon sharing the same body plan while the body plan is defined as the set of morphological characters shared by species within the same phylum. To solve this issue, one could first identify the broadest monophyletic range of species exhibiting a persistently conserved mid-embryonic phase [31]. Then, animal phyla could be redefined based on this maximal group of species, and the body plan could subsequently be defined by the morphological features shared among the embryos at the most conserved developmental stages in each species.

Hourglass conservation: organ and tissue perspective

Given that the connection between the stage of highest transcriptomic conservation and body plan establishment is still enigmatic, the next question would be whether the observed hourglass-like conservation across the embryo reflects the cumulative outcome of hourglass-like conservation of individual organs and tissues. Recent studies seem to support this. For instance, a comparative analysis of mouse and zebrafish spinal cord development using scRNA-seq data and gene regulatory network data revealed an hourglass-like conservation pattern of cells with maximally similar cell composition at around the potential phylotypic period [48]. A similar tendency was also reported in the development of the mouse limb and bamboo shark fin, with cellular composition being maximally conserved at mid-embryonic stages, approximately E10.5 in mice [49], a period close to the phylotypic stage observed in whole-embryo comparisons (around E9.0). Complementing these tissue-focused results, a recent lineage-resolved single-cell comparison during embryogenesis in two nematodes (*C. elegans* and *C. briggsae*) found that transcriptomic divergence between the homologous cells of these species is not only reduced at mid-embryogenesis on average, consistent with an hourglass-like pattern, but also reveals cell–type–specific heterogeneity (e.g., intestine and muscle were found to be more conserved than many neuronal classes) [50]. These results support the notion that the conservation observed at the whole embryo level could be a reflection of the overall conservation of individual organs and cell types. Further organ-level comparative studies may help test this idea and reveal which organ primordia, if any, are more conserved than others.

Underlying mechanisms for the hourglass-like conservation

Currently, the hourglass model remains supported primarily as observed phenomena rather than as a universal evo-devo law with well-characterized mechanisms capable of explaining the causes of the hourglass-like conservation. Even if hourglass-like conservation observed at the whole-embryo level could be fully explained as a sum of organ-level phenomena, key questions remain regarding whether individual organs and tissues are governed by a common underlying rule or whether each independently exhibits hourglass-like diversity through distinct mechanisms.

One early mechanistic hypothesis was that the conservation of a mid-embryonic period might simply reflect a reduction in positive selection relative to other developmental stages. In this view, the more conserved mid-stage occurs between an early developmental period diversified due to adaptations in nutritional supply and reproductive strategy and later developmental periods diversified due to environmental adaptations [35, 51]. This, however, was found to be insufficient by the results of an experimental evolution study in *C. elegans* conducted by Zalts and Yanai [52]. Taking advantage of the self-fertilization capability of this species, the group randomly selected individuals from each generation, enabling the evolution of mutation accumulation (MA) lines in which the influence of positive selection was minimized by avoiding selection for specific phenotypes. By comparing temporal gene expression variations among MA lines, these authors found that the developmental stage exhibiting the least variability aligned with the evolutionarily most conserved period in nematodes [52]. Although these findings do not rule out potential contributions of positive selection to hourglass-like patterns, they indicate that the phylotypic period tends to remain conserved in intra-species evolution even under conditions where positive selection is largely absent. This supports the possibility that intrinsic factors in embryogenesis—namely, developmental bias,

defined as biases on phenotypic variability imposed by developmental systems [53]—play a crucial role during the mid-embryonic period.

The notion that the phylotypic period is under developmental bias has also been explored from a mechanistic perspective, with influential hypotheses emerging in the 1990s. Duboule proposed that Hox gene expression and the resulting establishment of a shared body plan impose limitations on phenotypic and genetic diversification during the mid-embryonic period [13]. Similarly, Raff argued that alterations during mid-embryogenesis could disrupt numerous global tissue interactions, increasing the likelihood of developmental failure [14]. Although the mechanisms proposed by Duboule and Raff differ, they share essentially the same perspective that the phylotypic period is subject to strong negative selection, which limits phenotypic diversification of this stage. If such mechanisms indeed contribute to the conservation of the phylotypic period, one would expect that phenotypic changes arising at this stage are more likely to be eliminated by negative selection, often through embryonic lethality, than are changes that occur at earlier or later stages. A pioneering meta-analysis study tested this prediction by assessing rodent developmental stages that are prone to lethal phenotype by exposure to teratogens, and reported that the potential phylotypic period appeared to experience the highest rate of embryonic lethality [18]. However, the explanatory value of these findings may be limited by the fact that, by definition, teratogens tend to target the organogenesis stage. Indeed, the highly conserved organogenesis phase has been shown to have less lethal phenotypes than earlier stages to mutational and various perturbations [54].

An alternative but complementary possibility is that the phylotypic period may inherently exhibit reduced phenotypic variation, or less evolvability, as has been suggested by limited intraspecific phenotypic variation observed in nematodes [52] and medaka [42]. As Darwin, and later Fisher [55], emphasized, the rate of phenotypic evolution depends critically on the amount of phenotypic variation within a population. Then, do phylotypic periods conversely show reduced variability? A study utilizing single-embryo transcriptomics in an inbred *Drosophila* population found that gene expression variability among individuals was lower during the phylotypic period than in earlier and later developmental stages [56]. Similarly, Uchida et al. [42, 57] performed single-embryo transcriptome analyses on inbred medaka sister embryos raised under identical environmental conditions, minimizing genetic and environmental differences, and found reduced stochastic variability in gene expression patterns specifically during the phylotypic period, a pattern also observed at the individual gene expression level. Although this does not necessarily mean that reduced potential for phenotypic variations, or lower evolvability, leads to long-term evolutionary conservation, when considered together with the persistent conservation observed during the phylotypic period in medaka, this possibility cannot be ruled out [42, 57].

What factors, then, underlie this reduced intraspecific variation observed during the phylotypic period? One possible factor is that the conserved period is phenotypically robust against mutational perturbations, as implied by less lethality of the mid-embryonic period than other embryonic stages following exposure to ultraviolet light [54]. Another possible factor would be that reduced phenotypic flexibility against non-mutational perturbations, or "developmental stability" underlies the reduction in evolvability, as outlined below.

A growing body of empirical and theoretical evidence indicates that low developmental variation is coupled to evolutionary phenotypic conservation. Developmental stability, states in which non-genetic phenotypic variations are less likely to arise [58], is often quantified by assessing fluctuating asymmetry [59]. Across diverse taxa and traits, patterns of developmental stability correlate with phenotypic conservation at multiple levels (e.g., individual, population, and species). Hallgrimsson et al. showed that morphological variations between left and right limb structures within individual mice correlate with morphological variations across individuals [58]. Similarly, studies of mouse molar tooth evolution have revealed that morphological variation within populations aligns with evolutionary divergence observed between populations and species—a pattern described as evolution along the "line of least resistance" [60]. Such evolutionary patterns have been attributed to developmental instability in molar morphogenesis, wherein stochastic phenotypic variations are directionally consistent with variations driven by genetic differences [61]. Research on *Drosophila* wing morphology also demonstrated congruence between fluctuating asymmetry, individual variation, and interspecific morphological diversity [62, 63]. Furthermore, theoretical studies using mathematical and cellular models of evolutionary simulations have demonstrated that phenotypic variation resulting from non-genetic, stochastic noise [64, 65] tends to be larger than that from mutational origins. Consistently, one theoretical study employing gene regulatory network (GRN) evolutionary simulations reported that phenotypic resistance to genetic variation can evolve simply through a requirement for developmental stability (defined here as the GRN's capacity to produce stable expression patterns) without explicit stabilizing selection for specific phenotypes [66]. Taken together, these findings support the hypothesis that phenotypes exhibiting high developmental stability are more likely to display long-term evolutionary conservation.

What makes the phylotypic period developmentally stable?

The next question, then, is how the phylotypic period exhibits such high stability at the whole-embryonic level. Conceptually, at least two non-exclusive possibilities can be considered. One possible mechanism involves stable inter-tissue interactions; Raff highlighted potential dense, cross-module interactions during mid-embryogenesis that could limit variation [14]. While influential, this idea still awaits systematic experimental validation. A second possibility is that the observed transcriptomic stability arises primarily from reduced stochasticity at the intracellular level. Indeed, previous work has suggested that whole-embryonic gene expression variability largely reflects cell-intrinsic fluctuations rather than differences in tissue composition [67]. Investigating the regulatory mechanisms that suppress stochastic expression noise at the cellular level, such as properties of underlying gene regulatory networks, alongside studies of tissue-level interactions, may help explain how the phylotypic period shows both high developmental stability and low evolutionary potential.

Several regulatory features could underlie such cell-intrinsic stability. Pleiotropy may provide one clue; pleiotropically expressed genes are enriched in developmental stages conserved among vertebrates developmental stages in vertebrates [31]. A study in a medaka inbred line also showed that genes with lower expression variability in the conserved stages tend to be expressed across multiple developmental stages and tissues, indicative of pleiotropy [42]. In this perspective, gene regulatory architecture is another candidate, since longer enhancer regions have been associated with stable transcriptional outputs and with promoting pleiotropic gene usage [68–70].At the chromatin level, Liu et al. analyzed embryonic transcriptome data and ChIP-seq data in *Drosophila*, reporting that reduced inter-individual expression variability during the phylotypic period coincided with denser and broader histone modifications at developmental gene loci, suggesting that robust histone marking may contribute to transcriptomic stability at this stage [56]. Demethylation at developmental gene enhancers during the phylotypic period has also been documented in vertebrates [71]; the authors of that study assert that the regulation of Tet proteins, which mediate DNA demethylation, may help to elucidate mechanisms underlying the hourglass model of conserved gene expression patterns [71] lending additional support to a role for epigenetic control. Together, these observations suggest a future direction; investigating how these regulatory features evolve and become concentrated at specific developmental stages may provide insights into understanding the transcriptomic stability and lower evolutionary potential of the phylotypic period.

In addition to these features, the architecture of GRNs can itself restrict how perturbations propagate across fields. von Dassow et al. [72], through their simulations, showed that GRN controlling segment polarity structure may contribute to correct segmental patterns despite substantial perturbations in individual gene expression levels. Rather than the architecture of GRN, Kohsokabe et al. [73] showed the potential contribution of "slowly expressed" regulatory genes toward the hourglass-like conservation through their simulations. In their model, genes whose expression changes only gradually over developmental time acted as upstream controllers of large-scale transcriptional switches during the conserved mid-stage, and these slow regulators attenuated fluctuations in gene expression arising in early development, thereby stabilizing downstream developmental processes. Identifying similar network structures or dynamics in developmental systems related to body plan formation, and experimentally evaluating their ability to limit phenotypic variation, could provide valuable insights into mechanisms that limit developmental variability and reduce evolutionary potential during the phylotypic period.

Potential approaches to the identification of mechanisms underlying the conservation of phylotypic periods

Having surveyed potential intracellular sources of mid-embryonic stability, a different but classic idea would be inter-tissue coordination as a candidate mechanism for the conservation of the phylotypic period [14]. Rather than presupposing a single "responsible" tissue, Raff posited that dense cross-module interactions among organ primordia during mid-embryogenesis may limit the phenotypic variability. While past comprehensive tests were hindered by the effective approach of evaluating signaling interactions, recent advances—such as spatial transcriptomics and predictions of cell–cell and tissue–tissue signaling—now enable the identification of conserved interaction architectures. For example, recent advances in spatial transcriptomics, including multi-slice pseudo-3D approaches [74], and tools such as CellChat [75], COMMOT [76], and LIANA [77] enable us to objectively define organ modules within and across developmental stages, and further to estimate potential signaling interactions among the organ primordia. That said, because these strategies can only provide static snapshots of tissue interactions during development, further perturbation studies, such as gene knockouts, are needed to assess the extent of phenotypic variation is actually produced and to determine whether these interactions truly underlie evolutionary conservation.

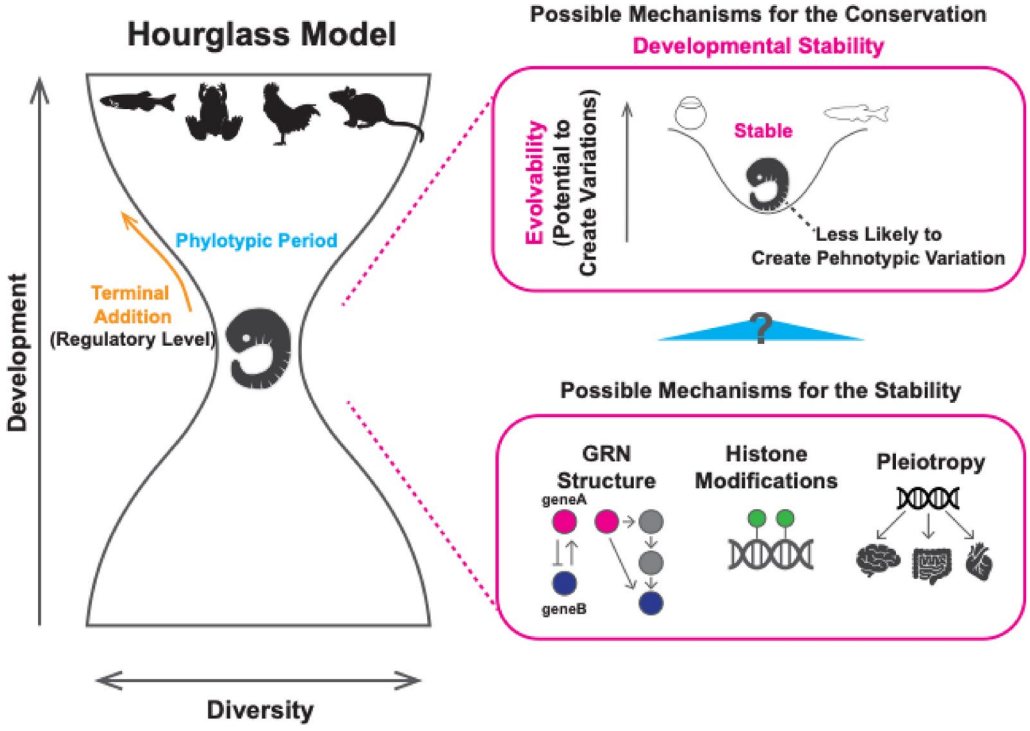

Fig. 1 Conceptual summary of the developmental hourglass model and possible mechanisms underlying the conservation of the phylotypic period

Haeckel's ideas in the hourglass framework

Finally, though we have so far focused primarily on the developmental hourglass model as the general law linking ontogeny and phylogeny, it is worth revisiting how this view relates to classical ideas proposed by Haeckel [7] (Fig. 1). First of all, although the funnel model is derived, at least in part, from the recapitulation theory, support for the hourglass model and rejection of the funnel model should not be taken as a direct denial of the recapitulation theory itself or all related concepts proposed by Haeckel. This is because recapitulation theory assumed that earlier developmental stages reflect more distant ancestral states, rather than a higher degree of conservation in earlier stages within a given phylogenetic group. For example, if a nested hourglass model—where the most conserved embryonic stages of larger phylogenetic clades shift to earlier developmental phases than those of smaller phylogenetic clades—had been supported, it could have potentially coexisted with both recapitulation theory and von Baer's law. However, as mentioned in previous sections, the discovery of persistent conservation during the potential phylotypic period finally refuted this possibility. That said, this only means that development is not a quick replay of evolution, or a recapitulation of evolutionary ancestry; concepts such as terminal addition remain open to further investigation. For instance, ATAC-seq analyses performed to evaluate the activities of potential regulatory elements during development suggested that evolutionarily newer regulatory regions tend to be enriched in later, post-phylotypic embryonic stages [78]. Further studies are still needed to reveal why such trends have been observed after the bottleneck stage of the hourglass model. Additionally, it is possible that evolutionarily older regulatory sequences were simply selected against in later development through evolution, rather than that new regulatory regions were added during later development. In any case, it seems that some of Haeckel's ideas remain to be thoroughly tested.

The hourglass model highlights the phylotypic period as the most conserved stage of embryogenesis, contrasting with the diversity of early and late stages. Potential mechanisms contributing to the developmental stability of the mid-embryonic period include robust gene regulatory networks, dense chromatin modifications, and pleiotropy. While these processes may buffer variation and maintain conservation, concepts such as terminal addition may still operate after the bottleneck stage, shaping evolutionary diversification.

Conclusion

In this review, we have explored the relationship between ontogeny and phylogeny within evolutionary developmental biology, and provided an overview of both historical perspectives and recent advances. As we have detailed, the concept of phylotypic periods, a central feature of the hourglass model, has been robustly supported as an evolutionarily conserved developmental stage in numerous cross-species comparative studies.

The precise mechanisms underlying this conservation, however, remain the subject of active investigation. Recent advancements in single-cell analyses and spatial transcriptomics technologies now offer unprecedented opportunities for the comprehensive investigation of tissue-level interactions and developmental network architectures. These emerging methodologies promise to significantly advance our understanding of the mechanisms driving phenotypic stability and evolutionary constraints associated with the phylotypic period.

Abbreviations

TAI	Transcriptome age
TDI	Transcriptome divergence index
MA	Mutation accumulation
GRN	Gene regulatory network

Author contributions

Both YU and NI were major contributors in writing the manuscript. All authors read and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

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Consent for publication

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