Opinion

The evolution of early animal embryos: conservation or divergence?

Alex T. Kalinka and Pavel Tomancak

Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstr. 108, 01307 Dresden, Germany

There is a remarkable similarity in the appearance of groups of animal species during periods of their embryonic development. This classic observation has long been viewed as an emphatic realization of the principle of common descent. Despite the importance of embryonic conservation as a unifying concept, models seeking to predict and explain different patterns of conservation have remained in contention. Here, we focus on early embryonic development and discuss several lines of evidence, from recent molecular data, through developmental networks to life-history strategies, that indicate that early animal embryos are not highly conserved. Bringing this evidence together, we argue that the nature of early development often reflects adaptation to diverse ecological niches. Finally, we synthesize old and new ideas to propose a model that accounts for the evolutionary process by which embryos have come to be conserved.

New approaches to understanding a seminal observation

The 19th-century German embryologist Karl Ernst von Baer first noted that there was a striking similarity between animal species during periods of their embryonic development [1]. von Baer's observations, and in particular his third law (see Glossary), provided foundational evidence supporting Darwin's theory of common descent [2]. As a natural historian, Darwin was inclined to view embryonic conservation as reflecting a lack of adaptive opportunities available to the embryonic stages of the life cycle of an animal. In his view, the environmental variance to which adult stages are adapted is largely absent from the relatively protected embryonic stage. In 1922, Walter Garstang placed the embryo back at the heart of evolution by arguing that the origins of adult morphological novelties must often be traced back to changes in the embryo [3]. To Garstang, embryonic conservation was a consequence of the stepwise building of evolutionary novelties upon the foundations of older characters. In this stepping-stone model, older embryonic characters would be retained, perhaps as rudiments 'pruned' down to their essential elements, to ensure the correct development of the more recently evolved characters that they underpin [3,4].

The 20th century saw the advent of improved microscopy technologies, and the renewed observations that followed prompted researchers to propose that morphological conservation is greatest in the middle of embryogenesis, during the so-called 'phylotypic' period [5–7]. This proposal, now known as the hourglass model (Box 1), diverged from what is often viewed as the classic model, which states that embryos are most alike at the earliest stages and gradually accumulate differences as development proceeds (but see 'von Baer's third law' below). One question naturally ensued: why is the middle of embryogenesis highly conserved? In contrast to Darwin's view that environmental differences drive adult but not embryonic adaptation, Duboule and Raff, both developmental biologists, sought answers in the developmental system itself.

Duboule proposed that mid-embryogenesis is characterized by precise coordination between growth and patterning such that the sequence of temporal and spatial activation of genes is highly sensitive to perturbations [6]. Taking a more global view of conservation, Raff argued that the complexity of interactions between genes, cells and developmental processes reaches a maximum during mid-embryogenesis when the body plan of the organism is being established [7]. In both models, the deleterious nature of changes during mid-embryogenesis is contingent upon properties of the developmental system that are unique to this period. However, to what extent variation at mid-embryogenesis is limited purely by selective constraints, or by the interplay between selective and developmental constraints, is not specified in either model.

However, acceptance of the hourglass model and the concept of a conserved phylotypic period has not been universal. Comparative studies of vertebrate embryos, led by Michael Richardson, have shown that there is variation in the timing of appearance, relative shape and number of certain structures during the phylotypic period [8–10]. The authors argued that, although vertebrate embryos share many features of their body plan during the phylotypic period, heterochrony is so widespread that this period ought to be viewed instead as an archetype that, although useful as an organizing concept, is not observable as a clearly defined period of embryonic development. In addition, Richardson suggested that changes during the phylotypic period are more important evolutionarily precisely because they often have significant impacts upon adult morphology [9]; this is known as the adaptive penetrance model (Box 1). Other studies focusing on changes in the ranked order of developmental events have concluded that there is no evidence for any pattern of conservation during embryogenesis [11,12].

Now in the 21st century, comparative embryology is being tackled using the tools of the post-genomic era (Box 2). Several studies taking a molecular approach to

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Corresponding authors: Kalinka, A.T. (kalinka@mpi-cbg.de); Tomancak, P. (tomancak@mpi-cbg.de).

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Glossary

Cambrian period: a period 542–488 million years ago that marks a dramatic transition in the evolutionary history of life. Whereas life before the Cambrian period was relatively simple and mostly unicellular, the beginning of the period is characterized by a rapid emergence of fossils of complex multicellular life forms resembling the body plans of most currently existing animal phyla.

Cleavage: rapid cell division during early embryogenesis in most animal species. It occurs without significant growth and transforms the single-cell zygote into a multicellular mass of approximately the same size.

Developmental constraint: a limitation on the phenotypic variation that a developmental system can produce as a result of the properties of the system itself.

Early development: the period of embryonic development that starts with fertilization of the egg and encompasses the maternal–zygotic transition, cleavage and early nuclear divisions, and gastrulation, but does not include the phylotypic period during mid-embryogenesis.

Gastrulation: the formation of the three primary cell layers of an animal (ectoderm, endoderm and mesoderm) via cellular rearrangements of a single layer of cells.

Heterochrony: a change in the timing or rate of developmental events resulting in differences in the size or shape of different animal species.

Living fossil: a living species that morphologically resembles fossil species over a long period of geological time; for example, velvet worms, dragonflies, scorpions, lungfish and several species of shark.

Maternal reproductive strategies: the provisioning of the resources of a mother into her offspring via differential strategies of investment into eggs and/or zygotes; for example, species that produce few large eggs versus species that produce many small eggs; or egg-laying species versus internal gestation species.

Maternal–zygotic transition: the transfer of developmental control from the mother to the zygote via depletion of maternal transcripts and transcriptional activation of the zygotic genome; this process occurs early during embryonic development.

Parent–offspring conflict: a conflict of interest between a mother and her offspring in terms of the optimal level of maternal resource investment; whereas a mother is equally related to all of her offspring, an individual offspring is more related to itself than to its siblings (more so in the case of multiple paternity) and, when possible, should attempt to extract more resources from the mother than it is optimal for her to give.

Phylotypic period: a period during mid-embryogenesis when animals belonging to the same phylum are said to exhibit the greatest level of morphological similarity (the term 'phylotypic stage' was introduced by Klaus Sander [5]; we replace 'stage' with 'period' because the morphological similarity is expressed during a succession of stages) [6,7]; the morphology during this period reflects the basic body plan associated with a particular phylum [10], corresponding to the tail-bud or 'pharyngula' stage in vertebrates [68], and to the segmented germ band stage in arthropods [5].

Phylum: a taxonomic rank grouping organisms that share the same general body plan in terms of morphology, internal structure and development; for example, arthropods and chordates.

Selective constraint: a limitation on the evolutionary trajectories that a species, process or molecular sequence can pursue owing to the removal of deleterious phenotypic variants by natural selection; we define a selective constraint as impeding (in a sense, greatly reducing the likelihood) of a particular evolutionary trajectory as opposed to forbidding it absolutely.

Stepping-stone model: a model proposed by Walter Garstang [3,4] to explain embryonic conservation by the sequential addition of adaptations onto the foundations of previous adaptations leading to a stabilization of the essential elements of the past upon which the body plan of a current species depends. A similar concept was advanced by Rupert Riedl [69] in which he described the old elements of a developmental system as 'burdened' by acting as the foundations for subsequent evolutionary adaptations.

Transcriptomics: techniques aimed at monitoring gene expression levels in a biological sample for all genes in the genome. DNA microarrays typically use single-stranded, sense DNA probes hybridized with labeled cDNA (the target) derived from RNA isolated from a biological tissue. The array is scanned and the amount of hybridized target proportional to the expression level for each gene is extracted by image analysis. More recently, massively parallel deep-sequencing technologies allow sequencing of short fragments of significant proportion of transcripts in a sample, which can then be mapped to a sequenced genome and used to count expression levels digitally.

von Baer's third law: animal embryos from different species most resemble one another at early stages of embryonic development and gradually accumulate differences as they reach later stages [1]. Although this is the usual interpretation of von Baer's third law, von Baer based his observations on post-gastrulation embryos, meaning that the similarity he described refers to the middle periods of embryogenesis.

the question of embryonic conservation have brought with them a more quantitative methodology to the traditionally qualitative discipline of comparative embryology [13–26]. Whereas several of these studies have provided support for a molecular signature of conservation during the phylotypic period [13–17,19,22,23,25,26], others have instead found support for conservation at the earliest stages of development [18,21]. Given the resurgence of interest in this field, we believe this is a propitious moment to revisit the classic ideas and more recent models put forward to explain embryonic conservation.

The role of early development in animal evolution

We focus our attention on early development and ask whether this period is conserved or whether it can tolerate change. We contend that, because conservation at the end of embryogenesis is not endorsed by any model (Box 1), building a picture of the forces acting on early developmental processes is crucial to furthering understanding of embryonic conservation.

In addition to clarifying the pattern of embryonic conservation, developing a better understanding of how evolutionary forces act on early development will help to shed light on the relation between development, ecology and broad patterns of animal biodiversity. For example, if early development provides an adaptable means by which animal species can exploit varied ecological niches through divergent reproductive strategies, then one might expect successful animal groups to evolve developmental systems that can accommodate adaptive changes during their early stages. An intriguing corollary of this proposition is that the observed paucity of animal body plans might, at least partly, be a consequence of the ability of a small number of animal groups to inhabit a wide range of ecological niches. Thus, we can ask: how much can be inferred about the ecological context of an animal species purely from studying its early embryo? In what follows, we answer this question by first reviewing the evidence that early development has a propensity to diverge across several biological levels and then, by bringing this evidence together,we evaluate to what extent it supports ecological adaptation of early development.

Is early development evolutionarily labile?

When reflecting on the early periods of embryonic development, we find that there are several events shared by most animals: the fertilization of the egg transforming it into a zygote; the establishment of the primary axes of the embryo (the anterior–posterior and dorsal–ventral axes); the rapid nuclear or cellular divisions transforming the single cell into a multicellular organism; the activation of the zygotic genome during the maternal–zygotic transition; and the rearrangement of a single layer of cells into the three primary cell layers of the animal via gastrulation. Does the near-universal presence of these events and processes suggest that early development is highly conserved? We argue that these conserved events are necessary steps to pass through to generate the raw materials needed for building a body plan, but that conservation of events does not imply conservation of the pathways that produce those events [27]. Indeed, we propose that early

Box 1. Models explaining patterns of embryonic conservation

Four main models have been proposed to both predict and explain different patterns of embryonic conservation in animal species (Figure I).

Early conservation

In this model, the earliest developmental stages are considered foundational and any apparent conservation in later stages is the delayed realization of the conservation of genes and proteins acting early [9,21]. Although von Baer's third law is often interpreted as proposing early conservation, von Baer in fact based his observations on post-gastrulation embryos [1].

Hourglass model

Conservation is considered greatest in mid-embryogenesis and is either the result of the need for coordination between growth and patterning when the body plan is being built [6] or the result of a global increase in the complexity of interactions between genes and processes during the phylotypic period [7]. Divergence in early development is considered the consequence of diverse reproductive strategies, and divergence in late development is the result of specialization for the diverse life histories of the larval or adult organisms of different species.

Adaptive penetrance

This model posits that the most important beneficial mutations are likely to occur during the phylotypic period precisely because this is when the body plan is laid down [9].

Ontogenetic adjacency

This model posits that small changes are most likely between events that are adjacent in the developmental sequence of events [11].

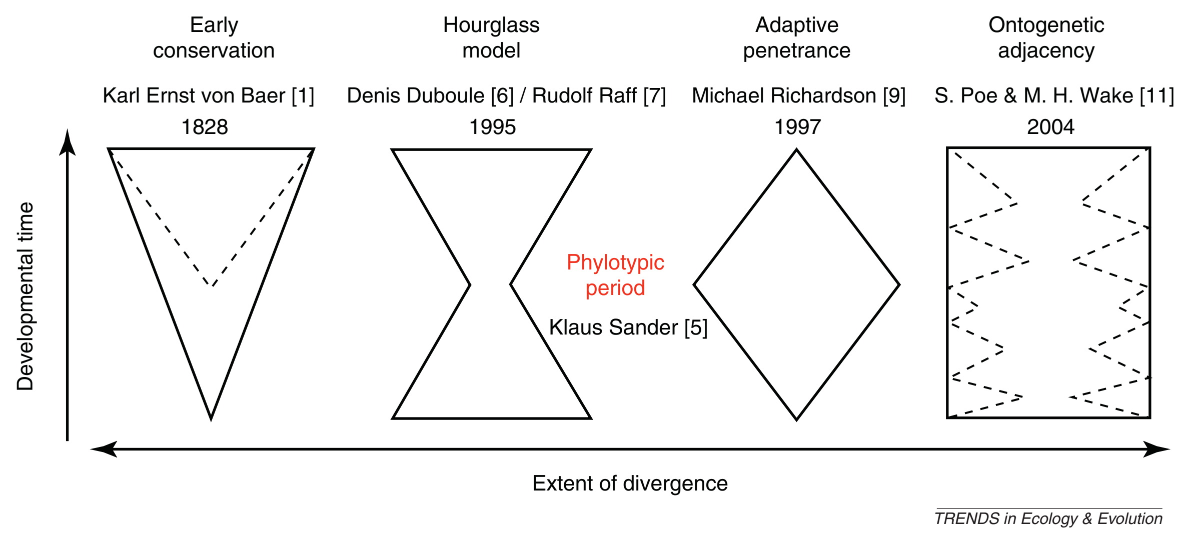

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Figure I. A schematic comparing four different models that posit different patterns of conservation during animal embryogenesis. In all of the models, development from egg to adult is shown on the y-axis, and evolutionary divergence is represented on the x-axis. Broken lines leading to mid-embryogenesis in the 'Early conservation' model indicate that von Baer based his observations on post-gastrulation embryos. Broken lines in the 'Ontogenetic adjacency' model represent an instance of a conservation pattern for a limited period of time, and the bold, unbroken lines indicate that this model does not predict any particular pattern of conservation over long periods of time; that is, all periods are equally likely to diverge.

development is characterized by conserved developmental events with divergent modes of development [28,29].

Developmental processes

In this section, we discuss the evidence that early developmental processes diverge between species belonging to particular animal phyla, but without consequences for the adult morphology. Two distinct modes of embryogenesis have been identified in insects: long- and short-germ development [5,30]. In long-germ developers, such as *Drosophila*, the specification of all of the body segments from anterior to posterior occurs almost simultaneously before gastrulation. This mode of early patterning is derived [30] and distinct from what is considered the ancestral mode of short-germ development, in which only the anterior segments are specified before gastrulation, with the remaining segments being generated progressively from a posterior growth zone. Whereas the patterning of long-germ developers occurs in a syncytium (a non-cellularized, multinuclear cytoplasm), short-germ patterning occurs in a cellularized environment (at least in the posterior region of the embryo), thereby requiring distinct mechanisms of patterning [31]. Despite the major differences between these two modes of development, there is no clear impact on the body plan of the larva. Rather, it is likely that the major effect of long-germ development is to accelerate embryonic development of the larva [5,32]. Long- and short-germ modes of development have also been described in velvet worms (Onychophora) [33] with the probable consequence of the long-germ mode being a shortening of the length of development [27]. The shortening of embryonic development is probably an adaptation to particular ecological circumstances, and this is a point we return to in the life-history section.

In most insect species, the early rapid nuclear divisions and patterning of the embryo occur in a syncytium. However, the presence of a syncytium is not essential for the early patterning of the insect embryo as the early embryos of several endoparasitic wasp species have been found to develop almost entirely in a cellularized environment [34]. In some of these parasitic wasps, a more dramatic alteration of early development has evolved whereby several thousand embryos develop clonally from a single egg, known as polyembryony [35]. This novel and highly derived mode of development has also resulted in major changes in the axial

Box 2. Comparative embryology in the post-genomic era

The science of evo-devo is benefiting from a revolution in sequencing and transcriptomics technologies that together open the possibility for genomics analysis beyond traditional model organisms (Figure Ia). Genome sequences, gene annotations and sequence alignments can be used to design species-specific microarrays to measure and compare genome-wide gene expression in multiple related species (Figure Ib). Alternatively, one can exploit the sensitivity of RNA-seq and quantify transcripts from individual, precisely staged embryos, map the sequence reads to the genome and convert the coverage into gene expression levels for cross-species comparisons (Figure Ic). The RNA-seq analysis can be made cost-effective by bar-coding individual single embryo libraries and sequencing many of them in a single run.

Given that it is well established that morphological innovations in evolution arise typically from changes in gene regulation, by comparing species-specific gene expression across multiple stages of development, one can complement qualitative comparative morphology with quantitative genome-wide molecular data.

Recently, the microarray approach has been successfully applied to facilitate comparative analysis of early embryonic development in arthropods and vertebrates (Figure Id–g). Using species-specific custom-made microarrays, several groups collected new time course data sets on gene expression in the embryos of six *Drosophila* species [23] (Figure Id), four species of vertebrates (mouse, chicken, fish and frog) [25] (Figure Ie) and two related species of frogs [26] (Figure Ig). Analysis of the time course data revealed remarkably similar patterns of divergence that were all consistent with the morphological hourglass model. This analysis was complemented by a similar approach combining gene expression time course data in fish, flies and worms with a measure of gene emergence across the tree of life to show that genes expressed both early and late are younger than those expressed in mid-embryogenesis [22] (Figure If). All four studies point towards divergence in genome-wide expression programs early during development converging on a conserved phylotypic period and then diverging again, thus mirroring the morphological hourglass [23,25]. In the future, by making use of the power of RNA-seq, it will be possible to examine divergence in gene expression across several evolutionary scales. The macroevolutionary comparison across species can be complemented by microevolutionary studies of divergence within species using sequenced strains. In addition, allele-specific expression can be readily detected in RNA-seq data, which opens the possibility of measuring gene expression in interspecies hybrids. Quantitative genetic modeling using these rich data sets will reveal the modes of evolution in early development and beyond.

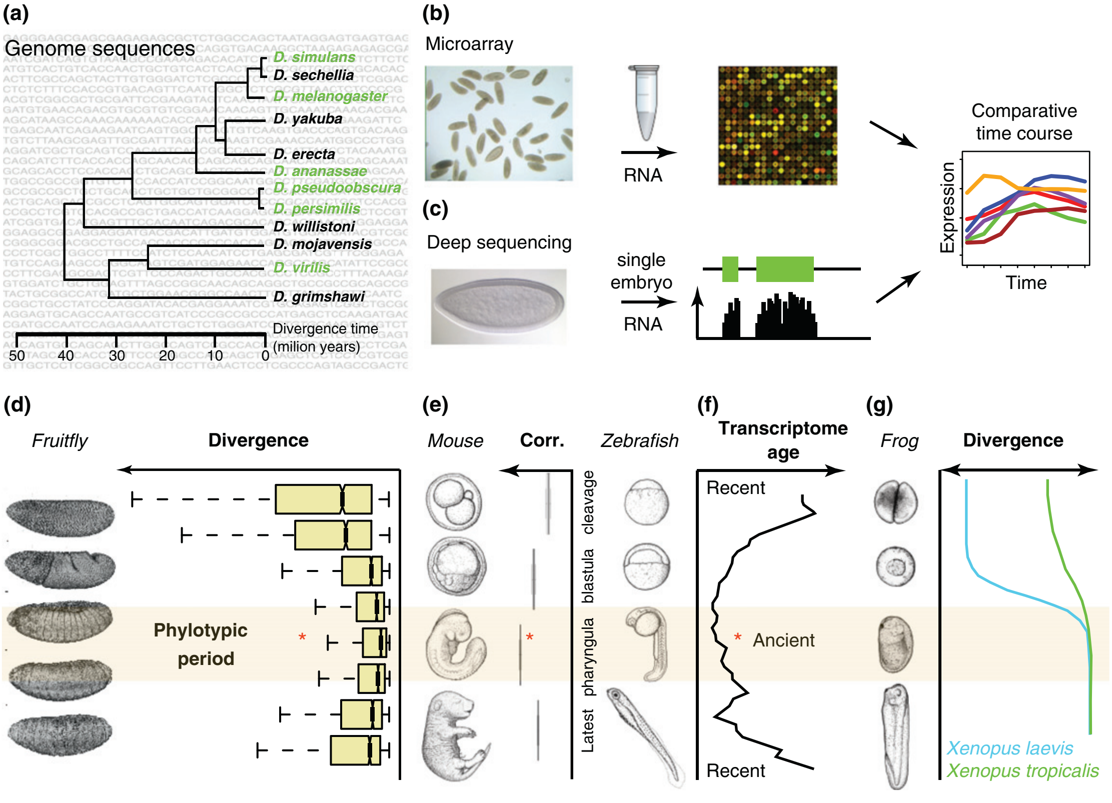

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Figure I. Transcriptomics technologies for studying the evolution of animal development. **(a)** Complete genome sequences are available for many species related to commonly used model organisms, for example *Drosophila melanogaster.* **(b,c)** Embryonic gene expression time courses of different species can be compared by isolating RNA from populations of staged (*Drosophila*) embryos followed by microarray analysis or by deep sequencing of RNA amplified from single embryos. **(d)** Divergence of gene expression, measured as the variance of all pair-wise species comparisons for each gene, at individual time points (0–16-h-old embryos collected in 2-h periods) during embryogenesis across six species highlighted green in (a) [23]. Divergence shows a minimum during germ band retraction (asterisk), which is considered the insect phylotypic period. Embryo images are three-dimensional renderings of time-lapse embryonic development of *Drosophila melanogaster* using selective plane illumination microscopy (SPIM). **(e)** Correlation of gene expression levels, measured as the average Spearman correlation coefficient for orthologous gene expression between equivalent developmental stages for pairs of species (Corr.), for mouse, fish, frog and chicken embryos. The asterisk indicates peak correlation at pharyngula stages [25]. Mouse, fish and frog embryo drawings adapted from [25]. **(f)** Transcriptome age index, combining the relative age of genes with their expression levels, plotted as function of embryonic time, shows that ancient genes common to all multicellular organisms are most active at the phylotypic (pharyngula; asterisk) period of zebrafish development [22]. (g) Schematic representation of gene expression divergence across time for an orthologous gene from two frog species shows that gene expression differences concentrate in the earliest stages of development [26]. The phylotypic period of arthropods (d) and vertebrates (e,f) is highlighted across the panels.

patterning of the early embryo [35]. Even more surprisingly, two distinct larval castes are produced by the polyembryo: a soldier caste, which defends the larvae; and a reproductive caste, and these castes are determined by cellular asymmetries that appear at the four-cell stage [35]. Highly modified early development, including polyembryony, has also evolved in insect species that give birth to live young (e.g. some species of earwigs, cockroaches and aphids) [36]. Such changes in early development are the adaptive consequences of the particular life-history strategies used by different species [34].

The rearrangement of cells that occurs during gastrulation is an essential process resulting in the three primary cell layers that give rise to the structural topology and organ system of the animal. Despite the importance of this event, the modes of gastrulation in animal species are highly varied [37]. For example, sea urchins that develop into nonfeeding larvae (so-called 'direct' developers) have evolved divergent modes of gastrulation, axis formation and cell lineage-patterning mechanisms relative to species that develop a feeding larval stage ('indirect' developers) [38,39]. One of the clearest effects of these changes is in the length of development, which lasts just three to four days in direct developers versus several weeks in indirect developers. Again, we see that early development evolves to accommodate a shortening of development time, a change that has significant ecological and evolutionary consequences that we revisit later. In vertebrates, hylid frogs, which have especially large eggs for amphibians, have evolved a mode of gastrulation that is more akin to avian gastrulation than to the gastrulation of other frog species [40,41]. In nematodes, peculiar patterns of cleavage and gastrulation have been described in a freshwater species, distinct from all other nematodes studied so far [42]. A more comprehensive comparison of 41 nematode species across 12 different clades found extensive divergence in several early developmental events, including cleavage and the specification of cell lineages, but again with no effect on the phenotype of the adults [43]. Cnidarian species (i.e. corals, sea anemones, jelly fish and hydroids) exhibit a remarkable diversity in their modes of gastrulation and this appears to be best explained by selection acting directly on life-history strategies as opposed to adult morphologies [44]. Cephalopods (i.e. octopuses, squid and cuttlefish) use a mode of cleavage that is distinct from the cleavage of all other mollusk groups; because cephalopod eggs are yolk-rich and this limits the region of the egg in which cleavage can occur, it seems probable that this distinct cleavage mode is a result of the evolution of a maternal investment strategy in cephalopods, which is linked to their loss of a larval stage [45]. Finally, in two similar species belonging to a phylum of microscopic, water-dwelling animals (Tardigrada), three-dimensional time-lapse observations of embryonic development have uncovered differences in both gastrulation and early cell fate specification processes, despite a broad similarity in the morphology of the adults [46].

Further examples of divergence in early developmental processes, particularly in vertebrates, are discussed by Hall [27]. In all of the cases covered, there is no change in the body plan resulting from major changes in the mode of early development, and these changes are often adaptations to the particular ecological circumstances of individual species.

Developmental networks

The embryonic development of animals is governed by a complex set of regulatory interactions between genes. These interactions produce a cascade of gene activation, from the initial maternal inputs right up to the zygotic genes responsible for building up the body plan of the organism. Together, the ensemble of interactions forms a hierarchical developmental network within which several subnetworks control the development of particular body parts and regions [47]. The topology of these subnetworks is crucial for determining the nature of the selective constraints acting on particular genes at particular times in development.

At the beginning of embryogenesis, the maternal factors and early-expressed zygotic genes that establish the major axes of the embryo are key elements that must be deployed or development will fail. At this stage, the developmental network is composed of a relatively small number of genes that determine broad domains in the embryo within which the more complex regulatory networks expressed by the zygote can function. This portion of the network is small yet crucial for the functioning of the downstream portions of the network, and thus can be viewed as a bottleneck through which the developmental cascade must pass [48]. Therefore, these early-acting genes are more likely to be essential genes, without which the embryo cannot develop. Studies of gene knockouts in vertebrates [18] and comparative studies of gene expression in nematode species [49] corroborate the notion that genes acting at the earliest periods of development are often indispensable. However, the essentiality of these early-acting genes does not imply that their expression, function, or sequence cannot be altered so long as their function is not lost. An example of such an alteration can be found in the early axis-patterning pathways of insects; the regulatory interactions of the early-patterning gene *orthodenticle* have evolved rapidly across different insect orders despite broad conservation of its role in axis formation [50]. In addition, several lines of evidence, in both vertebrates and invertebrates, suggest that the earliest periods of development are the most divergent both in terms of gene expression and protein sequence evolution [13–17,19,22,23,25,26] (Box 2). These measures of gene divergence are well suited for assessing the ease with which early development can be altered and, hence, the constraints active in early development, because changes in expression or sequence do not necessarily completely ablate the function of individual genes. Thus, we argue that gene knockout studies provide only partial measures of selective constraints acting on genes [18], and that the topology of regulatory networks will provide a more complete picture [47,48,51].

The wiring of early developmental networks varies substantially among insect species [52]. The gene networks underlying the differences in long- and short-germ developing insects have probably resulted from the recruitment of a set of genes into the early segmentation pathway in long-germ developers resulting in a shift in the timing of segment determination to occur before gastrulation [32,52]. The rapid mode of early developmental patterning that is typical of long-germ developers has also affected the dorsal–ventral patterning network. In short-germ developers, two distinct phases of dorsal–ventral patterning are required, whereas in long-germ developers, this axis is maternally established and globally stable [53]. These examples illustrate that selection for shortening of the length of embryonic development has probably driven a shift in the expression of early patterning networks.

Novel additions to early developmental networks have also evolved. The maternal patterning gene *oskar* is responsible for the assembly of germ cells in *Drosophila* and several other insect species. Recently, it was shown that *oskar* first evolved in the ancestor of all holometabolous insects (i.e. species with a complete metamorphosis) and that its appearance coincides with a shift in the determination of germ cells from the zygote to the mother [54]. Another important maternal patterning gene in *Drosophila*, *bicoid*, evolved from a gene duplication of the *Hox3* gene in the ancestor of the higher dipterans (i.e. true flies) [55]. Since then, the gene has evolved novel, and now crucial, functions in the early patterning of the embryo, and the most plausible explanation for these changes is that *bicoid* has facilitated faster development in long-germ developing flies [55]. In both of these cases, the recruitment of genes into early developmental networks has accompanied a shortening in the length of development that is probably driven by adaptation to particular ecological circumstances. Therefore, changes in expression, temporal coordination, or addition of novel components to early gene regulatory networks are common, and this suggests that there is significant plasticity in the networks active in early development.

Life-history strategies

Many of the changes in early development described above result in an increase in the tempo of embryogenesis, a shift from late to early-patterning processes, and an increase in maternal contributions to development. Changes such as these are the product of life-history strategies, which are an adaptive response to different environmental pressures [56]. For example, in an ephemeral environment where a food resource disappears quickly, there will be strong selection for rapid embryonic development so that individuals can exploit the resource while it is still available and while competition is not too intense. Such a scenario seems a plausible explanation for the modifications observed in the early development of *Drosophila* allowing segmentation specification to occur before gastrulation. Thus, one can view changes such as these as embryonic adaptations where the trait modified by selection is not expressed in the larval or adult stages.

Maternal reproductive strategies will also strongly influence early embryonic development. Changes in the size of eggs place various pressures on the early-patterning processes of embryogenesis. For example, as eggs increase in size, gastrulation methods must evolve to accommodate the increased size of the egg [40,41], and the early patterning processes of *Drosophila* eggs have been shown to change in response to artificial selection for increased and decreased egg sizes [57]. Maternal reproductive strategies have been linked to specific environmental variation in both invertebrates and vertebrates (e.g. [58–60]), supporting the notion that reproductive strategies are highly adaptable. In addition, the embryonic development of an egg-laying lizard species, in terms of developmental rates and yolk metabolism, is influenced strongly by the environment in which the egg develops, whereas the phenotypes of the hatchlings are minimally affected [61]. This suggests that the mode of development, and not the adult morphology, is often under strong pressure to adapt to varying environments.

A recent study of maternally expressed genes across both vertebrate and invertebrate species sheds some light on how differences in reproductive mode can impact early development [62]. The authors found that the regulation of maternal genes involved in early development is more complex in species that give birth to live young relative to egg-laying species. Here, a shift is seen in the responsibility for early patterning from the zygote to the mother, which correlates with an evolutionary shift in reproductive mode. A particularly interesting suggestion is that conflicts of interest between the mother and the embryo (known as parent–offspring conflict) are responsible for driving divergence in the expression of early zygotic genes [63]. Overall, we argue that there are many potential avenues leading to adaptive change during the early periods of development, and as such we are persuaded to re-evaluate Darwin's view of embryonic evolution in a modern context.

A pluralistic perspective on embryonic conservation

We believe that a common objection to the concept of early embryonic divergence arises from the intuitive notion that the first foundations laid down when building any structure must be the most essential and, therefore, most conserved elements in the overall construction. We propose two answers to this objection. The first is rooted in a body of empirical observations, much of which we have discussed above, showing that conserved developmental events can emerge from divergent modes of development [28,29]; or there are, as Schierenberg puts it, 'many roads to Rome' [42]. One explanation for the apparent flexibility of early events is that the morphological development of the embryo, although established early in terms of the broad axes and domains of the animal, is not physically realized, via cellular movements and interactions [64], until the embryo reaches a crucial size or cell number and, hence, changes in early stages might be buffered before the construction of the body plan [64]. The second answer urges a fundamental shift in how the life cycles of animals are perceived (Box 3). If one views the egg and the early stages of embryonic development as an extension of the mother [64–66] and so subject to varying environments and the adaptive pressures that they elicit, then this period of the life cycle will no longer be viewed as the foundation of the animal body plan, but as an adaptable means by which the body plan can be reconstructed from widely different starting points [64].

With these considerations in mind, we believe that the notion of conservation of the early periods of embryonic development is no longer tenable and, as such, models that do not accommodate early divergence are not supported

Box 3. The animal egg and the evolution of the phylotypic period

The models advanced to explain the persistence of the phylotypic period do not explain how this conserved period evolved (Box 1). Tautz and Schmid [64] argued that development should be viewed as a continuous cycle within which complexity and diversity can increase through evolutionary time; in this view, there is no beginning or end to development. Newman [64] argued that the origin of the animal phylotypic period must have its roots in the origin of multicellularity in animals and suggested that the animal egg is an evolutionary innovation that, as a specialized enlarged cell, enabled faithful reconstruction of adult morphologies. Here, we synthesize these ideas with Garstang's stepping-stone model to introduce a model that can account for both how the phylotypic period evolved and why it is conserved.

In keeping with Newman's approach, we seek the origins of the phylotypic period in the origin of animal multicellularity. The first multicellular animals arose in the pre-Cambrian either through the cooperation of cells derived by division of a single cell founder or by aggregation of genetically distinct cells into a cooperating, primitively patterned colony. We contend that the evolution of a specialized egg cell in this primitive, morphologically indistinct animal would have provided a means to survive adverse environmental conditions (e.g. lack of nutrients or high population density), while enabling reconstruction of the simple multicellular structure when conditions improved. In addition, the single cell bottleneck created by the egg would have produced a genetically uniform population of cells in the multicellular animal, which in turn would have eliminated competition between the cells [70]. By removing competition within the organism, the egg would have laid the foundations for the evolution of increased complexity in the body plans of its descendant lineages. As adaptations accrued over time, they would be layered upon the foundations of earlier adaptations, thereby stabilizing an echo of the historical past of the organism. Key to this model is the notion that the evolution of increased complexity along certain lineages places greater pressure on maintaining the rudiments of past developmental trajectories (Figure I). Thus, this model predicts that animal phyla with simpler body plans should retain a weaker signal of their historical past, and hence display less embryonic conservation.

Adopting such a model, we can also shed some light on the old question of which came first, the chicken or the egg; viewing the early multicellular animal as a metaphor for the chicken, we see that the egg must always be considered an evolutionary novelty, emerging as it does from a functional adult organism.

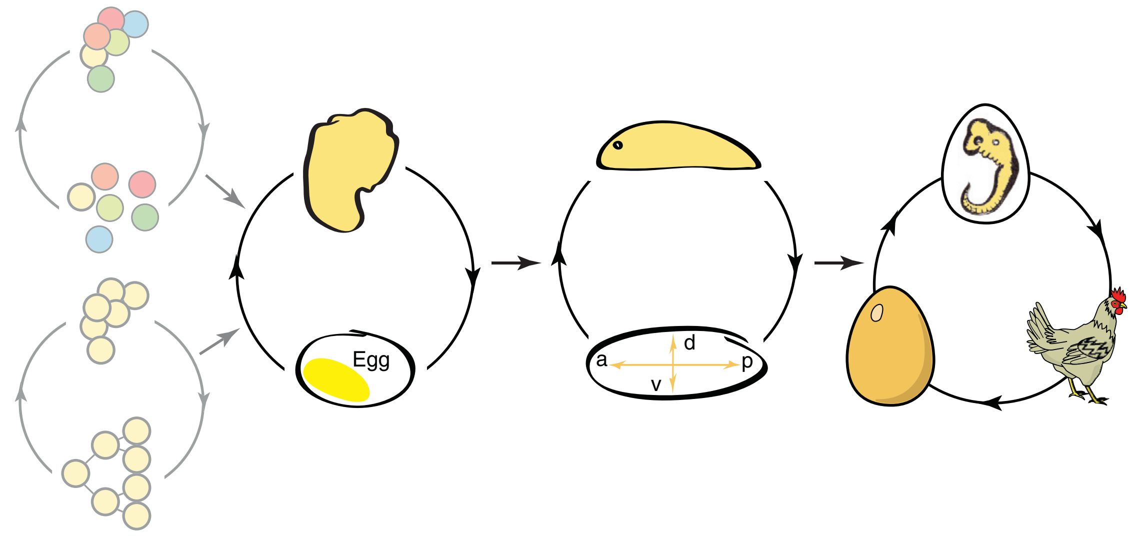

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Figure I. A model for the evolution of the phylotypic period. The left-most cycles show two alternative origins for multicellular animals: at the top, aggregation of genetically distinct, cooperating cells (depicted as colored circles) into a simple multicellular animal, and at the bottom, a founder cell divides asexually to produce a multicellular animal. In the next cycle, a specialized egg cell evolves, into which energy-rich yolk is deposited (depicted as a yellow region inside the egg). The egg allows the animal to survive adverse conditions and produces a genetically uniform animal, which we depict as a simple multicellular animal with variable morphology. Genetic uniformity prevents competition between the cells allowing complexity in the body plans of its descendants to increase, which is depicted in the next cycle as a simple animal with defined axes that are delimited within the egg [(a) anterior; (p) posterior; (d) dorsal; and (v) ventral]. Along certain lineages, the complexity of the body plan of the animal will continue to increase and each incremental step is built upon the body plan of previous generations, thereby stabilizing the historical past of the current animal species (i.e. the 'stepping-stone model'). This process is depicted in the last cycle on the right, where the complex animal is a chicken, the phylotypic period is depicted as the tail-bud stage [68], and its position in the life cycle indicates its historical correspondence to the body plans of simpler ancestors; the increased distance between the egg and the adult highlights the increased length of the developmental trajectory that must be taken to reconstruct the adult form in this complex animal.

(models 1 and 3 in Box 1; model 4 cannot be entirely rejected in this view). However, we note that we have described data from species belonging to eight different animal phyla (Arthropoda, Chordata, Cnidaria, Echinodermata, Mollusca, Nematoda, Onychophora and Tardigrada), whereas there are 35 animal phyla currently described. It is possible that early development is more conserved in some or all of these less-explored phyla as a result of phylum-specific developmental constraints [67]. Indeed, divergence in early development could be a signature of the evolutionary success of a particular animal phylum, and such success could in turn bias the sampling of species towards finding a pattern of early divergence. Conversely, it is possible that animal phyla with simpler body plans exhibit less embryonic conservation than their more complex counterparts (Box 3).

In attempting to understand the forces responsible for embryonic conservation, we favor an approach that views the development of an organism from egg to adult as part of a continuous cycle that has increased in complexity through evolutionary time [65] (Box 3). Attempts to discern the mechanistic underpinnings of embryonic conservation by focusing on the phylotypic period independently of the rest of the life cycle can provide only partial solutions to the problem [6,7]. By contrast, we believe that the evolution of the phylotypic period can be understood only as part of the larger process by which the life cycle has evolved. Such an approach appreciates the deep historical contingency that is embodied in the embryo and necessitates an understanding of the interplay between ecological forces and developmental constraints in their mutual shaping of the animal life cycle.

Concluding remarks

In conclusion, we believe that both empirical evidence and theory support the notion that early embryonic development has a propensity to diverge. Although all animal species must pass through certain conserved events necessary for constructing their body plan, there appears to be less conservation in how these events occur. Thus, we offer an extended answer to the question posed earlier: given the early embryo of an animal species, we believe that it would be possible to infer several aspects of the ecological niche of the organism, but comparatively little about its evolutionary trajectory; conversely, given an embryo from the phylotypic period, we would have less confidence to speak about its ecology, but would have remarkable insight into its phylogenetic history.

Based on our reflections above, we propose four avenues for future research: (i) comparisons of patterns of change in development across different animal phyla coupled with a broad sampling of ecological niches will assess the generality of these patterns beyond the major developmental models; (ii) experimental dissection of how variable modes of development can produce the same output will yield important insights into the relation between developmental mechanisms, their robustness and how these aspects influence their evolutionary potential; (iii) measuring selective constraints acting on genes or proteins while accounting for the topology of the regulatory networks to which they belong and, in addition, measuring the distribution of fitness effects resulting from mutations in regulatory or protein sequences, will provide greater insight into how different periods of development are evolving; and (iv) comparative studies using highly derived species known to have experienced strong selection on their morphology, such as endoparasites, will yield insights into how much change different periods of development can tolerate, and whether the body plan can be remodeled or simply pruned to its rudiments; in addition, the obverse strategy of studying embryonic development in so-called ‘living fossils’ will provide clues as to what extent different periods of development can change without affecting adult morphologies. Approaches such as these will help to reveal the forces responsible for shaping both animal morphology and the tree of life itself.

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