

Who came first - larvae or adults? Origins of bilaterian metazoan larvae

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ABSTRACT There is a classic controversy in zoology over whether the common ancestor of living bilaterian phyla was a benthic animal with a bilaterian body plan, or was a pelagic larva-like animal similar to what we see today in the primary larvae of indirect-developing bilaterians. We examine the current larva-like adult hypothesis, and present an alternate model for the evolution of complex life histories by intercalation of larval features into the ontogeny of an ancestral direct-developing bilaterian. This gradual accumulation of larval features results in a developmental regulatory program that produces a larva distinct in body plan from the adult. The evolution of a rapid and complete metamorphosis is made possible by the convergent evolution of set aside cells in the final stages of the emergence of indirect developing larval forms. Although convergences abound either hypothesis for the evolution of developmental pathways and life histories, the bilaterian first hypothesis is consistent with all stages of evolution of a complex life history being selectively advantageous, with the rapid evolution of larval forms, and with the frequent co-option of genes from the adult phase of the life cycle prevalent in the evolution of embryos and larvae.

KEY WORDS: *larval body plan, evolution of development, life history evolution, bilaterian origins*

Life history modes and metazoan phylogeny

Most phylum level diversity exists in the sea and most marine phyla exhibit indirect development, developing by means of a complex life history in which a feeding larval form distinct from the adult is produced (Fig. 1). Larval development is followed by metamorphosis to the adult form. Larval body plans are diverse, but some larval forms are shared among phyla. For example trochophore larvae are present in mollusks, annelids, and several other phyla. These shared larval forms have long been used as phylogenetic tools to unite phyla whose adult body plans are different. The existence of distinct larval and adult body plans, as well as the uniting genetic concept of the zootype (Slack *et al.*, 1993) exacerbate an old problem in zoology: Was the ancestor of living bilaterian phyla a benthic animal with a bilaterian body plan, or was it a pelagic larva-like animal similar to what we see today in the primary larvae of indirect-developing bilaterians?

The controversy is important because of the differences in interpretation of major evolutionary events that are forced on us by the alternatives. At issue is the interpretation of the role of development in the Cambrian metazoan "explosion" (Conway Morris, 1998; Giribert, 2002), which encompasses the nature of PreCambrian metazoans, the lack of a PreCambrian metazoan

fossil record, evolution of developmental regulatory mechanisms, role of convergence in major evolutionary transitions in body plans, the origins of life histories, and the impacts of evolving life histories on Cambrian ecology and diversity.

Metazoans use two primary life history modes, direct and indirect development. Direct-developing animals produce a post-embryonic form, sometimes called a larva, that is built according to the same body plan as the adult. Indirect-developing forms have a complex life history, in which the post-embryonic stage is a distinct larval form that exploits a different niche than the adult, and undergoes some degree of metamorphosis. Larvae generally fall into two categories. The most generally familiar larval forms, those of insects and amphibians are the so-called "secondary" larvae. Despite their having some or extensive metamorphosis involving imaginal cells, these larval forms have the same body plan as the adult, and therefore are part of a direct developing life history. However, most phyla are marine invertebrates that have what are called "primary" larvae. These "maximal" indirect developers have an entirely different body plan than the adult, and the larvae are able to perform necessary functions, such as feeding and locomotion. Within these larvae, set-aside cells, a group of cells that do not contribute to the development or functioning of the larvae, give

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rise to a second, more complex wave of development. This next round of development produces the adult body plan.

Figure 2 shows the distribution of developmental mode among metazoan clades. The three major basal non-bilaterian groups, the sponges, cnidarians, and ctenophores, exhibit varying forms of direct development. While these groups all produce a larva-like stage during development, including a feeding larva-like stage in some cnidarians, none of them undergo an extensive metamorphoses, and the adult body plan is patterned from the "larva" (Fell, 1997; Martin, 1997; Martindale and Irvine, 1997). The bilaterian animals are divided into the protostome and deuterostome clades. The protostomes are further split into the ecdysozoans and the lophotrochozoans. The ecdysozoans comprise clades of molting animals that include arthropods, nematodes and a number of other phyla. These are all direct developers. This is not intuitive as it is well known that arthropods, such as *Drosophila*, go through several larval stages that superficially do not look like the adult. Furthermore, the imaginal discs are similar to the set aside cells described in maximal indirect developers. However, the adult body plan is patterned from the embryo and larval stages, and in some, imaginal discs. No larval stage distinct from the adult body plan is present. Lophotrochozoans, which include annelids and molluscs, include many clades that are indirect developers. The larval type produced by a number of clades of lophotrochozoans is called the trochophore. The deuterostomes also contain taxa with varying modes of development. The ancestral mode of development in the sister clades of echinoderms and hemichordates is indirect development by way of a dipleurula larva. Chordates, on the other hand, are all direct developers. It is thus a fundamental question; Are ecdysozoans and chordates primitively direct developers, or is this state derived from a maximally indirect-developing ancestor?

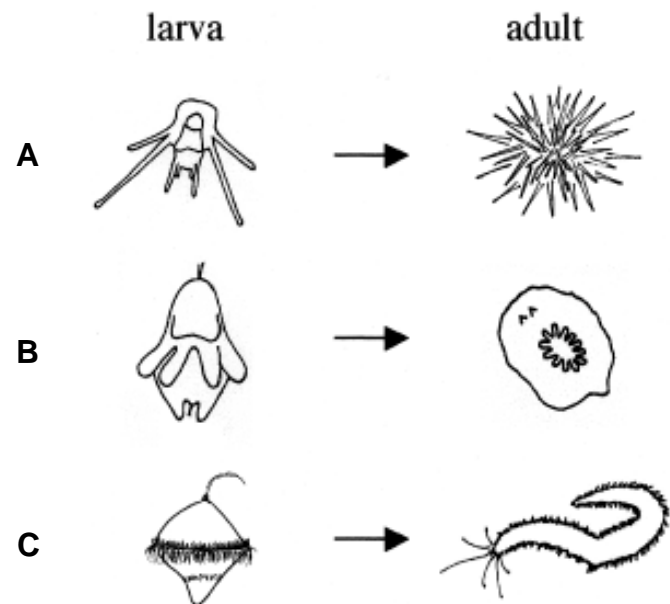


Fig. 1. Drawings of larva and adults from maximal indirect developers. (A) A pluteus larva and its sea urchin adult. (B) A Müller's larva and its polyclad flatworm adult. (C) A trochophore larva and its polychaete adult.

Although the ancestor of the bilaterian clade was likely a form lacking maximal indirect development (Fig. 2), phylogenetic arguments cannot be decisive.

The modern debate on body plan origins

Haeckel's 1874 theory of animal evolution, simply summarized as "ontogeny recapitulates phylogeny" had a profound influence on zoological thought. He postulated that the metazoan ancestor was an organism resembling the hollow ball of the blastula stage (Blasteae). Blasteae then evolved invagination to resemble a gastrula (Gastreae), a form that is common to the development of all present day animals (Haeckel, 1874; Jagersten, 1972). Although this line of reasoning fell out of popularity by the early twentieth century, it has re-emerged in modern discussions. Expanding on the Gastreae Theory, Nielsen suggested that the planktonic Gastreae animal later evolved into a Trocheae animal, which resembles extant marine invertebrate larval forms (Nielsen and Norrevang, 1985; Nielsen, 1995). He proposed that the protostome ancestor (Lophotrochozoa plus Ecdysozoa) resembled a trochophore larva and that the deuterostome ancestor (Deuterostomia) also resembled a feeding larval form (the tornaria). Both of these larva-like ancestors then evolved into benthic adults, which is recapitulated in the metamorphosis of extant marine invertebrates, by ceasing swimming, fusing their blastopore lips, connecting the apical organ and nervous center, and modifying the location of the ciliary band.

Modern discoveries regarding the genic controls of development have reinvigorated the investigation of this classical question. In a series of recent papers, Davidson and colleagues have proposed an intriguing and creative hypothesis that suggests that the ancestor of the bilaterians was an adult larva-like animal that had the developmental regulatory features characteristic of larvae of animals exhibiting maximal indirect development (Davidson *et al.*, 1995; Peterson *et al.* 1997; Peterson and Davidson, 2000; Peterson *et al.* 2000a; Erwin and Davidson, 2002). Their rationale was as follows. First, they argued that the similarities between primary larvae of different phyla, including those of both protostomes and deuterostomes, are too great to be due to convergent evolution. The alternative is that these larvae are homologous, and were present in the last common ancestor. Secondly, they suggested that the ancestral early patterning mechanism was most likely what Davidson (1991) has called type 1 embryogenesis, a developmental process in which cleavage is stereotypical and limited to about 10 rounds of cell division, and where the embryo is patterned by inductive interactions between individual blastomeres. As observed in a number of marine phyla, type 1 embryogenesis is sufficient to build a microscopic primary larva. These larvae contain many of the differentiated cell types that we see in modern bilaterians adults, including ectoderm, skeleton, muscle, ganglia, gut and eyes.

In this scheme, the ancestral bilaterian was much like these larvae. This animal would have possessed representatives from all of the major families of transcription factors and signaling molecules. However, it would not yet use these proteins for regional specialization that we see in modern bilaterian adults. The eventual evolution of set-aside cells, precursors to the developing bilaterian adult within the larva, would capitalize on these molecules to pattern the complex body plans we see today.

To avoid confusion, it should be noted that the larva-like ancestral metazoans would have been direct developers. Thus, the relatively simple developmental genetic controls of type I embryogenesis would have been deployed not in making larvae, but in building the larva-like adult. Complex life histories exploiting maximal indirect development to produce separate larval and adult developmental pathways would have arisen later, and would have been responsible for the origin of bilaterian body plans and large adults.

A number of authors have questioned the larva-like ancestor/set aside cell hypothesis (Knoll and Carroll, 1999; Wolpert, 1999; Jenner, 2000; Rouse, 2000; Bishop and Brandhorst, 2003). Of the issues raised, three kinds of objection carry the most weight. These are arguments on the distribution of developmental character states in bilaterian phylogeny, improbabilities in the selection of set aside cells before evolution of a bilaterian body plan, and on the improbabilities of required convergences.

Jenner (2000) pointed out that for the larva-like hypothesis to hold true, maximal indirect development has to lie at the base of the Bilateria. The more basal Cnidaria do not exhibit this mode of development. Davidson *et al.* (1995) and Peterson *et al.* (1997) recognized this point, and provided trees on which maximal indirect development was mapped. Jenner (2000) noted that neither of their cladograms allows an unequivocal determination of a primitive bilaterian developmental mode. By comparing maps of maximal indirect development on phylogenetic trees, including a consensus 18S rDNA tree, he made the interesting observation that the choice of taxa for such trees makes a big difference. Thus, "minor" phyla need to be included to give a fuller sampling of taxa basal to major phyla. In Fig. 2, minor taxa expand the tree beyond the taxa generally used. Large major clades are bundled. For example, lophotrochozoans, which include a number of large related phyla, constitute only a single clade in this representation. Figure 2 is modified from Jenner's mapping of maximal indirect development on the consensus 18S rDNA tree, notably in our use of Cnidaria, a clade lacking maximal indirect development (Martin, 1997), as the basal taxon and out group to the Bilateria. Mapping of maximal indirect development (Fig. 2) shows that this character is unlikely to have been a plesiomorphic feature of bilaterians, and is thus derived independently in several clades (as for example suggested for trochopore-like larvae by Rouse, 2000). Note that lophotrochozoans include a number of closely related major phyla, annelids, mollusks, brachiopods, bryozoa, nemertines, platyhelminthes, sipunculans and others. Thus, if each of these phyla are mapped separately, and without some of the "minor" phyla, one comes away with an impression of a vast preponderance of maximal indirect-developing taxa deep in bilaterian phylogeny. This phylogenetic argument (Fig. 2) results in set aside cells being secondary and convergent in these lineages (Valentine *et al.*, 1999). It also means that ecdysozoans and chordates did not lose maximal indirect development, but instead are primitively direct developers that possessed a bilaterian body plan rather than a larva-like form.

Wolpert (1999) has noted that the concept of a larva-like animal evolving a new adult form via evolution of set aside cells is unlikely to pass muster by natural selection. Wolpert based his argument on the principle that any evolutionary scenario has to account for the selective advantage of each new developmental stage. This is difficult for set aside cells if the adult has a larva-like morphology

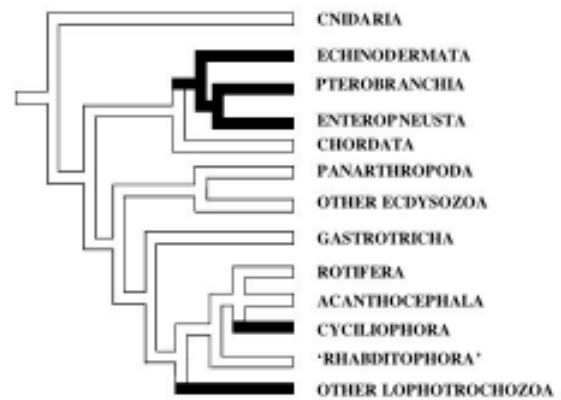


Fig. 2. 18S rDNA consensus cladogram with mapping of developmental modes. Maximal indirect development present: black branches. Absent: white branches. Cladogram from Jenner (2000), but modified by specific use of cnidarians as the out group to bilaterians instead of "diploblasts." Cnidarians do not possess maximal indirect development.

and system of developmental regulation. The advantage of set aside cells would be difficult to account for before the evolution of the new adult structures and the adult patterning mechanisms of bilaterians. In fact the origin of germ cells and gonads would also pose a problem for the hypothetical larva-like animals and their larva-like developmental programs. Finally, metamorphosis itself; How would it arise de novo to produce a new final body plan? Wolpert (1999) and Valentine and Collins (2000) have argued that larval stages must have arisen gradually in evolution by intercalation into early stages of an ancestral direct developing ontogeny. We will present below a hypothesis that allows for the origin of "primary" larvae, set aside cells, and metamorphosis that obviates the objections arising from natural selection.

Arguments from the improbabilities of convergence pose a different kind of problem because extensive convergences do occur frequently in evolution [and in larval evolution as well (Hart, 2000)]. Thus, it is not adequate to a priori simply prefer one convergence over another. In the case of the set aside hypothesis (Davidson *et al.* 1995, Peterson *et al.* 1997; Peterson *et al.*, 2000a), it is assumed that larval similarities and set aside cells are unlikely to have evolved convergently. But this is a prejudice without rigorous support. If the larva-like adult ancestor hypothesis is accepted, then the inescapable problem of convergence rears its head in another way; All the macroscopic bilaterian adult body plans that arose more or less simultaneously during the Cambrian radiation have to be convergent. There is no way to escape the necessity that a number of complex features evolved convergently whether maximal indirect development is primitive or evolved later by bilaterians. This convergence extends to the complex pattern forming mechanisms shared by these phyla. Thus, if the larva-like scenario is correct, the Hox gene anterior-posterior patterning system of the zootype (Slack *et al.*, 1993) would have been independently acquired from ancestors that contained some Hox genes, but did not use them in collinear A-P patterning. We will propose that the bilaterian adult body plan is plesiomorphic, and that the evolutionary interpolation of larval features, including gene regulatory systems, can be readily accounted for. In the process, we acknowledge our own prejudices as to probabilities

of convergences, and suggest that convergence is one of the truly important and interesting problems of macroevolution.

Larval origins from a direct-developing ancestral bilaterian

The prevalence of indirect development via a larva distinct from the adult body plan among marine invertebrates indicates a strong selective advantage to planktotrophic development (Olive, 1985; Jablonski, 1986; Strathmann, 2000). This is one mode of development among many, determined by a complex mix of phylogenetic and ecologic factors (Olive, 1985; Wray, 2000). Size has a strong influence on mode of development. Large animals have the luxury of being able to produce large numbers of small gametes. These gametes rapidly produce small feeding larvae that grow by planktonic feeding, and ultimately metamorphose into the different adult body form. However, animals with bilaterian body plans also can be very small. These animals cannot produce enough gametes to afford planktotrophic larvae with a distinct body plan. Thus, these organisms are direct developers with a bilaterian body plan and developmental genetic machinery. Proponents of the larva-like metazoan hypothesis suggest that the relationship of size to development in of ancestral metazoans lies in larva-like forms with a larva-like developmental regulatory system. The absence of large metazoan body fossils or traces in all but the very latest Precambrian fossil record until shortly before the Cambrian radiation suggests that Precambrian metazoans, particularly primitive bilaterians were likely quite small animals. Fortey *et al.* (1996), have argued in detail that major important evolutionary events may have taken place in small Precambrian

metazoans before the events of the early Cambrian that produced large fossilizable animals. These observations are in general agreement with a range of molecular clock estimates that place metazoan origins to at least 200 myr before the Cambrian (Ayala *et al.*, 1998; Valentine *et al.*, 1999; Wray *et al.*, 1996). However, this conclusion does not require that small Precambrian metazoans were either planktonic or larva-like.

The existence of living small bilaterian direct-developing animals, such as nematodes and acoel flatworms, shows that tiny Precambrian bilaterian animals could well have existed before the evolution of any planktotrophic larval form. The discovery of phosphatized fossil embryos from the late Precambrian Doushantuo phosphorite of China, about 30 myr before the Cambrian, is suggestive in that these embryos fall into the size range of direct-developing eggs of living marine invertebrates (Xiao *et al.*, 1998). However, they represent only a small sample of potential embryo diversity, and as their adults are not known, they could be embryos of sponges, cnidarians, or other basal non-bilaterian taxa as well as of small bilaterians.

Hypothesis

How did planktotrophic development arise? We have to note here that whether one is proposing a larva-like ancestor or a bilaterian adult-like ancestor, these animals would have been small, and they would perforce have been direct developers in their ontogeny. Although both direct developers, their hypothetical ontogenies would have differed in crucially significant ways. The larva-like ancestor would have been limited to developmental processes and controls similar to that of living primary larvae of marine invertebrates. The bilaterian-like ancestor would have possessed the more complex developmental systems suggested for the zootype, that is the full range of patterning mechanisms characteristic of adult bilaterian development. Neither would have needed nor possessed set aside cells. We share the views of Wolpert (1999) and Valentine and Collins (2000) that larval stages arose through interpolation of larval features into early developmental stages of direct-developing animals of bilaterian body plan. Consideration of this evolutionary course shows that most problems that accrue to the larva-like ancestor hypothesis are avoided. Only convergence remains, and there is substantial evidence for convergent evolution among larvae.

Our hypothesis is diagrammed in Fig. 3. Each arrow represents an ontogeny, with blue showing the adult bilaterian developmental pathways, and red the evolving indirect-developing larval pathway. Figure 3A represents the ontogeny of the hypothetical ancestral bilaterian. It is a strict direct developer. Ontogeny B shows a descendant in which a facultative developmental sub pathway has evolved. A model for this kind of situation can be found in the dauer larva pathway of the nematode *Caenorhabditis elegans* (Gerisch *et al.*, 2001; Wang and Kim, 2003). *C. elegans* normally develops directly through a series of molts. In unfavorable conditions, a network of signaling pathways (TGF β , cGMP, IGF-I) couple the sensory inputs from cues such as crowding, lack of food, and warm temperatures to downstream genes that produce the dauer phenotype. Dauers revert to the growing and feeding larval molt series when conditions become favorable. About 2000 genes with a variety of functions have been found to show expression changes in dauers. Although *C. elegans* is terrestrial, significant parallels to

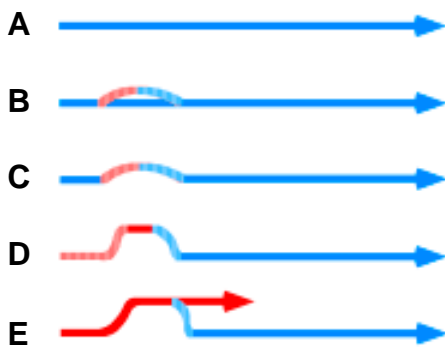


Fig. 3. Progressive evolutionary interpolation of larval development into an ancestral bilaterian ontogeny. (A) Direct development of an adult bilaterian. There is no larval stage. Development is by addition of adult features and growth of the body and its parts. Allometric growth may change proportions as development progresses. Developmental regulation uses genes and processes similar to those used in the adult body plan phase of development in living bilaterians. (B) Interpolation of a facultative novel feature into early development. Novel ontogenetic processes in light red; adult ontogeny in blue. (C) Integration of once facultative feature into a required "larval" stage. (D) Aggregation by co-option of additional features into an integrated larval entity in development (red). An integrated regulatory network distinct from that of adult ontogeny is arising. Most but not all adult features arise from remodeling of larval features. (E) Emergence of a fully developed biphasic ontogeny. Larval ontogeny is in red. Adult development arises primarily from set aside cells generated in the larva. Regulatory controls have diverged and dissociated into fully larval (red) and adult (blue) components of ontogeny.

the hypothetical ancestral bilaterian exist. The animals are small; environmental variations favor facultative variations in development; individual components of development can be co-opted into such facultative pathways individually and possess selective value. The dauer situation represents a well-established bundling of alternative features. These would appear to be dissociable, and were likely added one by one as the dauer evolved its full range of functions. These are the kinds of features that were co-opted to produce primary larvae and their selection.

In ontogeny C, the alternative developmental pathway has become constitutive, and a rudimentary larval stage has come into existence. No set aside cells exist, and there is no extensive metamorphosis. Most of the larval tissues (although not necessarily all) become incorporated into the adult.

In ontogeny D, a true larval stage (dark red) has appeared. In this ontogeny, some larval structures are discarded, and a more recognizable metamorphosis has begun to evolve. However, the metamorphosis is gradual, and no set aside cells exist. Situations analogous to this exist in living animals. For example, in hemimetabolous insects and amphibians, some larval structures are discarded, some are remodeled, and some strictly adult features unused in the larvae develop over a number of instars. In frogs the transition is gradual, with extensive changes in transcription and extensive growth of adult structures, as well as re-absorption or remodeling of larval features (Gilbert *et al.*, 1996). This process is highly distinct from the extremely rapid and extensive metamorphosis of marine invertebrates (Hadfield, 2000). The larval stage of this ontogeny (dark red) represents the beginning of a purely larval developmental pathway in which gene regulatory systems specific to tissues that will be discarded in later adult development become established.

Finally, ontogeny E diagrams an established fully biphasic life history. Two complete developmental genetic systems are present. Set aside cells are present. These allow an efficient transition to adult development in the pre-metamorphic larva, and rapid metamorphosis in response to appropriate environmental triggers.

Positive selection for larvae, set aside cells and metamorphosis

Why should direct-developing bilaterians invent larval stages? Selective pressures from a new ecological environment may have favored the advantages conferred by a larval life history phase, mainly locomotion, feeding, dispersal ability and site selection (McEdward, 2000).

Locomotion

Marine embryos are particularly vulnerable as they passively float and sink in the water column. An embryo that could swim would be competent to respond to the environment and would be subject to a whole new suite of selective pressures. There appears to be strong selective pressure for marine embryos to swim at an early stage, as this is seen in modern planktonic embryos. Evidence suggests that the combination of swimming and sinking may be important for predator avoidance and possibly feeding efficiency (Emler, 1983; Morgan, 1995; Young, 1995). Staver and Strathmann (2002) have shown that although most planktonic embryos swim at an early age, different taxa likely converged on early swimming rather than share an early swimming ancestor.

Feeding

Large bilaterians first appeared in the Cambrian radiation, and some became quite sizable. As only relatively large animals can produce large volumes of gametes, this increase in animal size also led to an increase in gamete number (Olive, 1985). With an increase in gamete number came the potential for an egg size versus egg number trade-off, which has been modeled and empirically supported within echinoderms (Vance, 1973a,b; Sewell and Young, 1997). Although Vance's specific model has been criticized, general resource versus number trade-offs are rampant throughout biology (Futuyma, 1995). With a finite number of resources, a female can either produce a large number of eggs, with relatively few resources or a small number of eggs, with relatively abundant resources. In certain environments, producing a larger number of less provisioned eggs may result in more offspring surviving to reproductive maturity compared to production of a smaller number of eggs with greater resources. However, with lower resource eggs, the larvae must feed to have enough resources to metamorphose. Therefore, the evolution of feeding larvae may have been selectively advantageous in some environments because it allows an increase in fecundity. The selective advantage of feeding may be indicated by the evolution of several types of feeding mechanisms, including filtering out particles via a sieve-like mechanism as in some brachiopods, directing particles along the food groove as demonstrated by veliger larvae or actively moving the particle into the feeding structure as in some echinoderm larvae (Strathman and McEdward, 1986; Emler, 1991). It is interesting to note that cilia play a role in all of these mechanisms.

Dispersal ability

For sessile benthic adults, the larval stage provides a unique opportunity for the organism to move and change environments from where it was fertilized. Dispersal allows for the colonization of new habitats and movement into areas of higher resources. Both could be selectively advantageous because there are unexploited resources such as food and space. Crowding has deleterious effects, including increased mortality and decreased fecundity because of resource competition. (Levitan, 1989; Hart and Strathman, 1995).

Site selection

Finally, larvae have been shown to exhibit settlement site preferences. They do not settle on a substrate until an appropriate spot is found, using such cues as water speed, chemical signals and bacteria (Morgan, 1995; Davidson and Swalla, 2002). Marine organisms seem to employ a variety of methods to sense environmental cues used in site selection such as the nervous system and perhaps innate immune system (Morgan, 1995; Hadfield *et al.*, 2000; Davidson and Swalla, 2002). They select for settlement in a favorable area, such as conspecifics or chemical cue from food items, both of which may indicate a high quality environment (Burke, 1986). Therefore, larva may play a role in selecting a high quality environment for settlement of the sessile adult. Although all of these selective advantages do not have to be present for the evolution of a larval stage, taken together, it is easy to understand potential pressures acting to favor the evolution indirect development in multiple phyla.

An important consequence of a biphasic developmental mode would be a dangerous vulnerability during the development of the

adult structures and during the “switch” from the functioning larva to the functioning adult. It is here that we can see the selection for set-aside cells. Strathman (2000) points out three important selective advantages of the adult body plan developing via set-aside cells. First of all, internalized or localized rudiments keep the developing adult body plan out of the way while the larva is still permitted to function. Secondly, the rudiment may be kept in a less differentiated state, which allows these cells to be nourished by the larva. Thirdly, by sequestering the adult rudiment until it is competent for metamorphoses, a rapid transition from larva to adult life takes place, decreasing the time in a nonfunctional state. Rapid metamorphosis is indeed observed in the majority of marine planktonic larvae, and there is evidence that delaying metamorphosis has negative effects on later fitness (Levitan, 1989). Hadfield’s (2000) “need for speed” hypothesis points out that larvae have adapted to selective pressures to decrease their helpless metamorphic stage not only by the formation of set-aside cells, but also by using cell to cell transmission of a metamorphic signal. This signal is usually external and brings about a rapid release of juvenile structures, accompanied by a loss of larval structures. While the exact signaling systems of metamorphosis are not known, the data that has been collected suggests that they are not the same across larva-producing phyla (Hadfield, 2000). Once again, we see how larvae have converged upon a solution for strong selective pressure.

Clearly larva and adults share many features though, such as cilia, gut, neurons and skeleton. According to our hypothesis, these features must all have been co-opted from the plesiomorphic direct developing adult form. Each of these has important fitness implications. Cilia play an important role in food acquisition and locomotion, necessary for survival, dispersal and predator avoidance (Strathmann and Mc Edward, 1986; Emler, 1991). The gut is of

evolutionary import for feeding larvae as they need to metabolize food for growth and energy. Neurons are important for sensing the environment and settlement (Hadfield *et al.*, 2000). The skeleton is important for overall structure, defense, and possibly feeding (Hart and Strathman, 1995).

On many levels, from stages of larval development, to specific structures, it is likely that there is a selective advantage to individual elements of a biphasic life history. This addresses the question of why direct-developing bilaterians would invent larvae: in certain environments, progressive acquisition of larval features increased fitness.

Co-option of genes in larval evolution

In our scenario, planktonic larvae have evolved secondarily from ontogenies that directly produced bilaterian adults. We predict that the favored path for this process was through co-option of genes and perhaps even gene modules from the adult to function in the larva. If this holds true, we would expect to see a subset of genes that function in adult ontogeny to play roles in the development of the larva (Fig. 4). Hox genes comprise a group of transcription factors that have been thoroughly studied in model systems, and now characterized in a number of other phyla as well. Because of the roles of Hox genes in establishing anterior-posterior domains in the developing adult, these genes have shed more light on body plan specification and relatedness than any other group of genes.

Hox gene expression has been examined to a lesser extent in the larvae of maximal indirect developers, but have been studied in detail in the dipleurula larva of the sea urchin *Strongylocentrotus purpuratus* (Arenas-Mena *et al.*, 1998, 2000) and in the modified trochophore larva of the polychaete *Chaetopterus* sp. (Irvine and Martindale, 2000; Peterson *et al.*, 2000b). The mRNA content per cell of eight of the ten echinoderm Hox genes, *SpHox2*, *SpHox3*, *SpHox4/5*, *SpHox7*, *SpHox8*, *SpHox9/10*, *SpHox11/13a*, and *SpHox11/13b*, was examined in the developing sea urchin. Only two of the genes, *SpHox7* and *SpHox11/13b*, were significantly expressed in the developing embryo and larval tissue (Arenas-

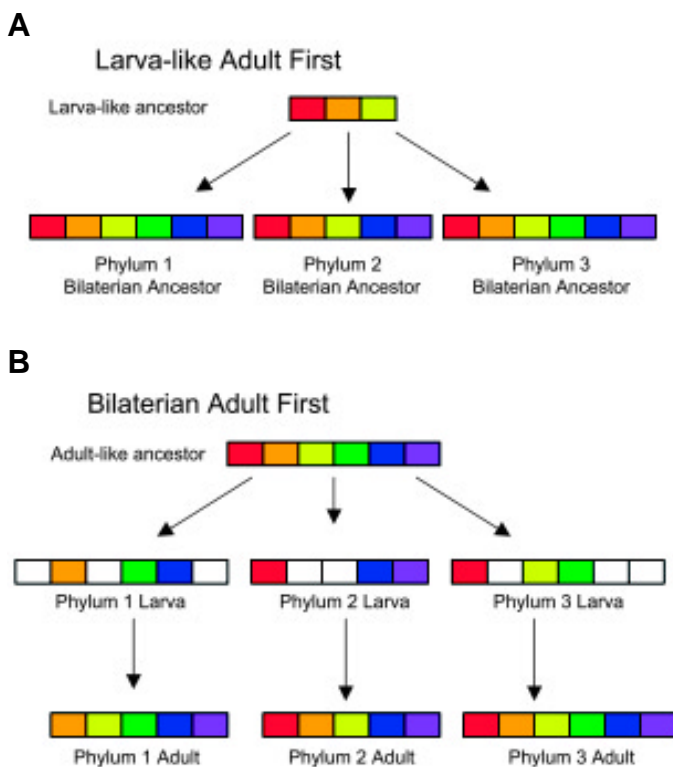


Fig. 4. Disparate genetic consequences of evolution of biphasic bilaterians from larva-like adult ancestors vs. evolution from ancestors possessing an adult bilaterian body plan. (A) Evolution of developmental genetic systems from a larva-like ancestor. The larva-like ancestor possess a simple developmental regulatory system, schematically diagrammed as three colored elements. The bilaterian phyla proposed to descend by acquisition of set aside cells and adult developmental processes are represented as possessing more complex developmental regulatory systems, schematic represented as additional colored elements. These are similar but not identical, representing divergence of developmental pathways in diverging phyla. **(B)** Evolution of developmental genetic systems from a bilaterian adult-like ancestor. The bilaterian ancestor already possess a complex developmental regulatory system. This ancestor is a direct developer, but as it evolves maximal indirect-developing larvae, genes are co-opted to form simpler, but novel larval phase developmental regulatory systems. The larvae of the descendant phyla have in many cases evolved independently, and although convergent, their gene regulatory systems are not identical. Note that the diagram shows the larvae as containing both expressed genes (in color) and non-expressed genes (in white). These non-expressed genes are expressed in development of their respective adults. In ontogeny, these larvae produce adults possessing complex adult developmental regulatory systems that arise in the prospective adult “set aside” cells and are involved in adult patterning.

Mena *et al.*, 1998). However, all of the genes were expressed in the adult rudiment (Arenas-Mena *et al.*, 2000). In *Chaetopterus*, a similar study found expression of 5 Hox genes, *CH-Hox1*, *CH-Hox2*, *CH-Hox3*, *CH-Hox4*, and *CH-Hox5* not to be initiated until the adult body plan had started to develop (Peterson *et al.*, 2000b). *In situ* hybridization revealed that the expression of these Hox genes was limited to the growth zone of the developing larva, the region that is thought to contribute to the segmental tissues of both the larva and adult body plans (Irvine and Martindale, 2000). Peterson *et al.* (2000a) conclude that the lack of Hox gene expression in the larva, and therefore the lack of regional specification, supports their argument that the bilaterian ancestor developed by type I embryogenesis only. However, if Hox genes have few, if any, functions in the bilaterian ancestry there would be no selective advantage for their conservation. Furthermore, their logic implies that the use of Hox genes in regional specification in virtually all metazoan phyla studied would have resulted from convergent evolution, as discussed above.

In the primitively direct-developing chordates and arthropods, essentially all of the Hox family members play a role in the specification of the body plan in the early stages development, including insect larvae and frog tadpoles (Godsave *et al.*, 1994; Burke *et al.*, 1995; Pownall *et al.*, 1998; Lombardo and Slack, 2001; Hughes and Kaufman, 2002). The expression of only a handful of Hox genes in maximal indirect-developing larva supports the hypothesis that these genes were co-opted from the genetic toolbox of the adult to perform a secondary function in the larva (Valentine and Collins, 2000). It will be instructive to see how other maximal indirect-developing taxa have co-opted subsets of the Hox genes into larval development. A glimpse of the flexibility of Hox genes in co-option for development of novel developmental features is provided by the work of Lee *et al.* (2003), who have shown in cephalopod development the recruitment of Hox genes into evolutionary novelties, although not in the expected collinear way.

Immunolocalization studies of the homeodomain-containing genes *engrailed*, *distal-less*, and *orthodenticle* (Lowe and Wray, 1997; Lowe *et al.*, 2002) in echinoderms have revealed an enormous amount of diversification of gene expression among echinoderm clades. These results provide strong evidence that regulatory genes have been co-opted repeatedly to function in echinoderm larva.

Shifts in gene expression are often associated with shifts in morphology and life history strategies, suggesting an ongoing flexibility in gene usage during larval evolution. We agree with Lowe and Wray's (1997) prediction that the recruitment of genes to function in the larvae of complex life histories is likely common among other phyla, including those of the lophotrochozoans. Studies to examine such gene expression must take in to account the importance of deep phylogenetic sampling, as a single species is often not necessarily representative of an entire phylum (Lowe *et al.*, 2002). In many cases, similarities may record deep shared genetic homologies, or convergent co-options. For example, Arendt *et al.* (2001) compared the expression patterns of *Otx* and *Brachury* in a protostome larva, *Platynereis dumerilii*, to those in the hemichordate larvae, *Ptychodera flava* (Tagawa *et al.*, 1998; Peterson *et al.*, 1999; Harada *et al.*, 2000). Similarities prompted the authors to make the reasonable conclusion of foregut homology between protostome and deuterostome larvae (Arendt *et al.*,

2001). More comparisons may be necessary before a final conclusion can be made. In other cases, phylogenetic distributions of characters leaves little doubt that numerous convergences of regulatory gene use have taken place. A good example is provided in the convergent use of distal-less in the development of independently evolved projections from the bilaterian body axis (Panganiban *et al.*, 1997).

Sea urchins have provided some of the most clear-cut examples of gene co-option. The enzyme arylsulfatase is only present at low levels in lysosomal vesicles of adult sea urchins (Mitsunaga-Nakatsubo *et al.*, 1998). However, it is found at very high levels in the extracellular matrix of the larva of both indirect and direct developing species (Akasaka *et al.*, 1990; Yang *et al.*, 1993; Haag and Raff, 1998). The physiological conditions in the extracellular matrix yield arylsulfatase nonfunctional as an enzyme, suggesting that the protein must have a secondary role, possibly in cell morphogenesis (Mitsunaga-Nakatsubo *et al.*, 1998). This is case of an enzyme being co-opted for a non-enzymatic role in the extracellular matrix of the larva. Haag and Raff (1998) have identified two novel genes that have been co-opted in the evolution of the non-feeding larva *Heliocidaris erythrogramma*. HeEL-1, a lectin-like molecule, is expressed at high levels in the early larval ectoderm of *H. erythrogramma*, but not until late stages of development of a pluteus forming sister species, *H. tuberculata*. HeET-1 has a similar expression pattern in *H. erythrogramma*, but is not detectable during the development of *H. tuberculata*. A homolog of this gene is present in the *H. tuberculata* genome (Haag *et al.*, 1999). It is likely that these genes have a role in the late larval or adult stages of *H. tuberculata*, but have been co-opted for a roles associated with life history strategy in *H. erythrogramma*.

Regulatory genes that control major features of larval development have also been co-opted into novel expression patterns and roles in the transition from indirect to direct development (Raff *et al.*, 2003). Genes such as *gooseoid* and *Msx* have played major roles in the transition to direct development in *H. erythrogramma* (Wilson and Raff, in preparation). Such examples demonstrate the frequency and speed of co-option in larva, as *H. erythrogramma* and *H. tuberculata* diverged approximately 4 million years ago (Zigler *et al.*, 2003).

The prevalence and rapidity of larval evolution

One of the major assumptions of the set-aside theory is that maximal indirect-developing larva of both protostome and deuterostome clades are too similar to have arisen by convergent evolution. However, there are several well documented cases demonstrating the prevalence and speed at which new larval forms evolve, and these forms often converge upon on another. Hart *et al.* (1997) studied mtDNA nucleotide characters to show that four different developmental modes have evolved in parallel in asterinid sea stars, and over short spans of evolutionary time. The evolvability of larvae has also been demonstrated in cone snails (Duda and Palumbi, 1999) gastropods (Reid, 1989) and sea urchins (Wray, 1996). In an effort to address the prediction that the ancestral bilaterian was a downstream feeding trochophore-like form (Nielsen, 1995; Davidson *et al.*, 1995; Peterson *et al.*, 1997, 2000a), Rouse (2000) used cladistic parsimony methods to examine downstream feeding in trochophore larvae of lophotrochozoans. The results of this study show that

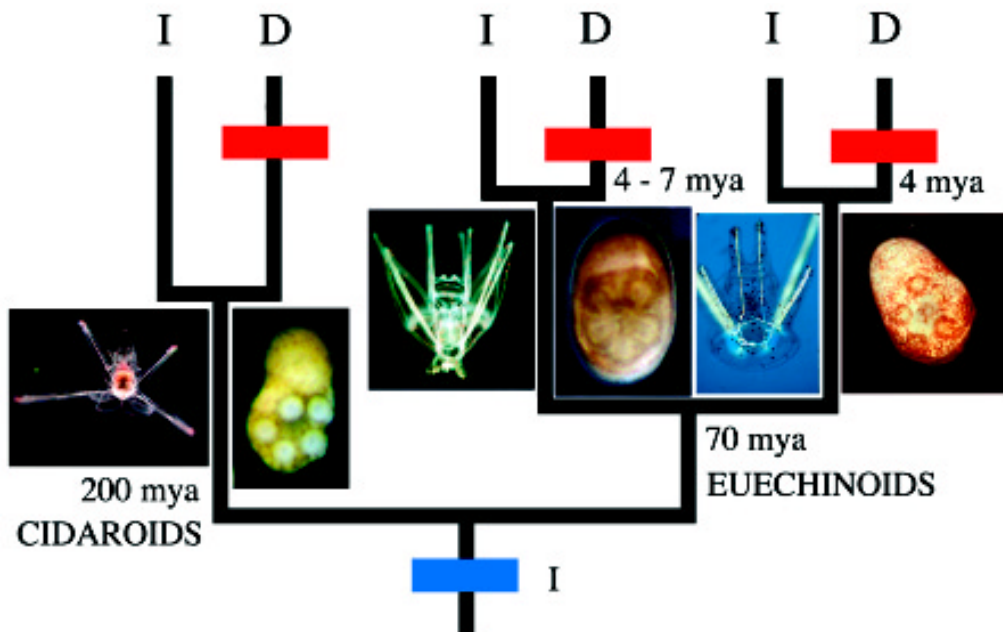


Fig. 5. Rapid convergent evolution of larval forms in three sea urchin lineages. Three clades are shown in a simplified phylogeny. Cidaroids are basal among living echinoids. The indirect-developing pluteus larva is plesiomorphic for echinoids. These larvae are bilaterally symmetric, and have ciliary bands and skeletonized arms for swimming and capture of plankton. These are thus all maximal indirect-developing larvae (I). Highly derived direct-developing larvae have arisen independently in these three lineages (and others not shown here), (D). These events are marked by red bars on the cladogram. Note that in two lineages, that of the echinometrid *Heliocidaris erythrogramma* (right branch), and the temnopleuroid *Holopneustes purpureescens* (middle branch), convergent direct-developing larvae have evolved within a few million years. Photographs: Cidaroid pluteus courtesy of Chisato Kitazawa, temnopleuroid pluteus courtesy of Richard Emlet, and other photographs by the R. Raff laboratory.

downstream feeding has been gained and lost several times among this group and suggests that the plesiomorphic ancestor of this group had a lecithotrophic larva whose ciliary bands were used for locomotion rather than feeding.

An example of the frequent convergence of larval forms is presented in Fig. 5, which shows the convergent evolution of non-feeding direct-developing larvae in a simplified sea urchin phylogeny of three clades in which the transition from indirect development to direct development has been studied (Parks *et al.*, 1989; Jeffery *et al.*, 2003; Raff *et al.*, 2003). Wray (1996) has shown from phylogenetic considerations that this event has occurred independently in a number of sea urchin groups. Molecular clock calibrations make it possible to show that in at least two of these cases, the evolution of *Heliocidaris erythrogramma* and *Holopneustes purpureescens* from the feeding pluteus larva, dramatic changes in larval development have occurred rapidly. We now have evidence that in these independent lineages convergent evolution of regulatory systems has taken place (Raff *et al.*, 2003).

Bilaterian life histories and the Cambrian radiation

The earliest bilaterians were small benthic animals, perhaps resembling flatworms, and having many of the characteristic bilaterian tissues. They possessed essentially all the regulatory genes involved in the patterning of bilaterian body plans, including gene regulatory systems involved in the development of tissues deeply shared among metazoan phyla; *tinman* (heart), *pax 6* (eye), *otx* (anterior part of the brain), *-dachshund* (eyes, etc), *caudal* (gut)

(Erwin and Davidson, 2002). In addition, anterior-posterior axis regulation by the Hox gene family (Slack *et al.*, 1993). Erwin and Davidson have summarized the genetic “toolkit” that the common ancestor would have possessed, including representatives of the suite of signaling systems and transcription factors that are used in the development of living metazoans (Erwin and Davidson, 2002). As small animals, the basal bilaterians were of necessity direct developers, and their ontogenies used the basic bilaterian genetic “toolkit.” Metamorphosis was gradual and partial, with retention and remodeling of features as development proceeded.

We propose that the rapid increase in size of bilaterians in the Cambrian radiation was not driven by the invention of set aside cells and addition of a bilaterian body plan to pre-existing planktonic larvae. Instead, the invention of planktonic larvae by new evolved large bilaterians opened the possibilities of planktonic development through selection for larval planktonic feeding, motility, rapid development, rapid metamorphosis, settling site selection, and dispersal. The evolution of set aside cells in evolving biphasic life histories would have increased the speed and efficiency of metamorphosis (Bishop and Brandhorst, 2000). The evolution of planktonic primary larvae would not have affected adult body plan evolution directly. However, the evolution of planktonic larvae did have an impact on diversity of metazoans by allowing more diversity in developmental modes, exploitation of new resources, and increasing dispersal into new environments (Bishop and Brandhorst, 2003). As a consequence, the introduction of complex life histories would have accelerated the Cambrian radiation and helped produce more complex marine ecosystems.

Acknowledgements

This work was supported by a grant from the US National Science Foundation to R.A.R., and from NSF-IGERT training grant awards in Evolution, Development and Genomics to B.J.S. and M.S.S., as well as from a NIH training grant and a McCormick Fellowship to B.J.S. We thank the School of Biological Sciences, University of Sydney for generously providing laboratory facilities, and Richard Emlet, University of Oregon, and Chisato Kitazawa, Duke University, for their gifts of photographs used in Fig. 5, and Alan Love for critically reading the manuscript.

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