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Phylogenetic analyses of mode of larval development

Michael Hart

Phylogenies based on morphological or molecular characters have been used to provide an evolutionary context for analysis of larval evolution. Studies of gastropods, bivalves, tunicates, sea stars, sea urchins, and polychaetes have revealed massive parallel evolution of similar larval forms. Some of these studies were designed to test, and have rejected, the species selection hypothesis for evolutionary trends in the frequency of derived larvae or life history traits. However, the lack of well supported models of larval character evolution leave some doubt about the quality of inferences of larval evolution from phylogenies of living taxa. Better models based on maximum likelihood methods and known prior probabilities of larval character state changes will improve our understanding of the history of larval evolution.

Key words: ancestral state reconstruction / maximum likelihood / parallelism / phylogeny / species selection

Introduction

The comparative analysis of marine invertebrate larval forms has a long and rich tradition.[1,2] The original approach involved species comparisons and correlations among trait values. We now recognize that, wherever possible, such comparisons should be made in the context of a phylogeny: comparisons should be made between clades, and correlations should be drawn between independent contrasts.[3] This need for phylogenetic knowledge, combined with the accessibility of molecular and analytical tools, has turned larval biologists into phylogeneticists. Has this professional metamorphosis been worthwhile? What do recent phylogenetic studies tell us about larval evolution that we did not already know? A short review reveals both successes and failures. Some studies have uncovered unexpected phylogenetic patterns or rejected previously suggested hypotheses. Multiple studies all point to similar patterns, such as massive parallel evolution of derived larval forms. However, the application of these phylogenies to the reconstruction of larval character evolution is sometimes suspect because models of larval character state changes are not well developed. In addition, few of these studies are large enough to permit statistically robust correlational analysis using independent contrasts.

From the Department of Biology, Dalhousie University, 1355 Oxford Street, Halifax, NS, B3H 4J1, Canada.
E-mail: michael.hart@dal.ca

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Case studies

Conus gastropods.

Conus is the most species-rich gastropod genus. Their phenotypic diversity extends to larval morphology and mode of larval development.[4] Many *Conus* and other species of caenogastropods develop via a planktonic veliger larval stage. Duda and Palumbi[5] used nucleotide characters from an intron to create a phylogeny for 70 *Conus* species, including eight species with nonplanktonic development. They found that seven of eight species with nonplanktonic larvae were reliably placed as the sister group to some other lineage with planktonic larvae (Figure 1). By forcing analytical programs to group together species with nonplanktonic development, Duda and Palumbi showed that this pattern was unlikely given their data, and that these eight species represented at least seven independent derivations of nonplanktonic development from an ancestor with planktonic dispersing larvae.

Lasaea clams.

Lasaea are small, cosmopolitan intertidal bivalves, most of which are polyploid, asexual brooders. ÓFoighil and Smith[6] used nucleotide characters from mitochondrial DNA (mtDNA) to build a phylogeny for various diploid, sexual and polyploid, asexual lineages. The one known diploid, sexual species (with a planktonic feeding veliger) was not the sister group to the asexual lineages [Figure 2(a)]. Instead, this species was a member of one clade of asexual polyploids. A second diploid lineage (in which the breeding system is not known) was also the sister group to some but not all polyploid, asexual lineages. ÓFoighil and Smith assumed that diploid sexuality is ancestral for these (and other) bivalves. If transformations to polyploid asexual reproduction are not reversible then this phylogenetic result implies that polyploidy and asexuality evolved on multiple occasions among *Lasaea*. Karyotype data support the suggestion that asexuality has been repeatedly derived from diploid sexuality by hybridization. The large genetic distances between diploid, sexual and polyploid, asexual lineages suggested that asexuality may have persisted in these clams for 10–20 million years, though asexual lineages of most animals are usually expected to be short-lived.

Molgulid tunicates.

Most tunicates develop as a swimming tadpole larva in which some of the distinctive chordate features appear and then are lost at metamorphosis. However, some species of Stolidobranchiata have reduced or lost the larval tail and do not swim. Hadfield *et al.*[7] used nucleotide characters from the nuclear ribosomal RNA genes to build a phylogeny for some tunicates in the families Styelidae and Molgulidae, including seven species with tailless or anural development. Instead of a clade of anural species, they found tailless development was widely distributed among clades [Figure 2(b)]. Later analyses based on sequences from muscle protein genes that are implicated in the loss of the tail confirm these patterns.[8] As many as six independent losses of the tadpole tail were inferred, and other anural species could have been included in the study so that this is a minimal estimate of the number of losses of the tadpole tail.

Asterinid sea stars.

Sea stars in the genera *Patiriella*, *Asterina*, and other Asterinidae include at least four distinctive modes of larval development: feeding planktonic bipinnaria larvae, nonfeeding planktonic larvae (schmoos), nonfeeding larvae in benthic egg masses, and internal viviparous brooding.[9] We used mtDNA nucleotide characters to build a phylogeny for 12 nominal *Patiriella* and *Asterina* species.[10] Similar larval forms did not occur as clades, and ordered transformations between the four modes of development could not be easily reconstructed [Figure 2(c)]. We concluded that many parallel changes in larval form, habitat, and dispersal potential had occurred. Small genetic distances between lineages with different modes of development suggested that some of these changes had been rapid or recent.

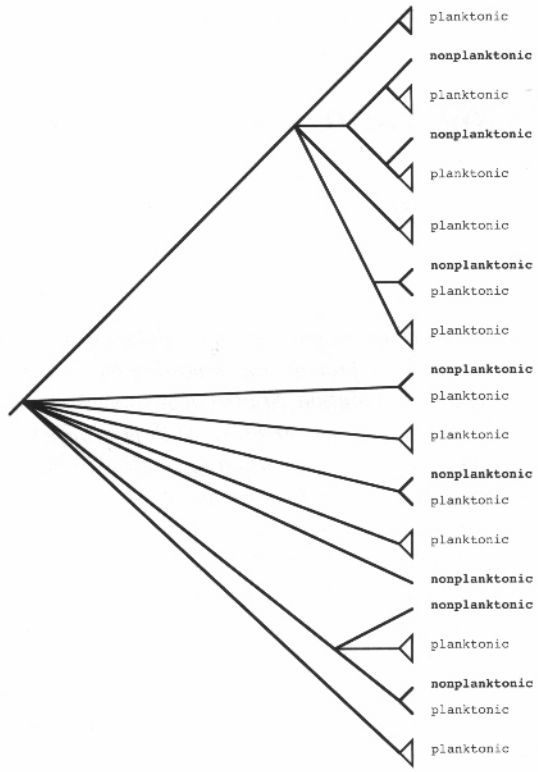

Figure 1. Phylogeny of *Conus* snails with planktonic and nonplanktonic larvae. Seven of eight species with nonplanktonic larvae are confidently resolved as the sister group to a lineage or species with planktonic larvae. Branch points in the original figure that were supported by low bootstrap values were collapsed to polytomies. Large triangles indicate groups of species or lineages that had the same larval form. The presumed derived form (nonplanktonic) is shown in bold typeface. After Duda and Palumbi[5] Figure 2.

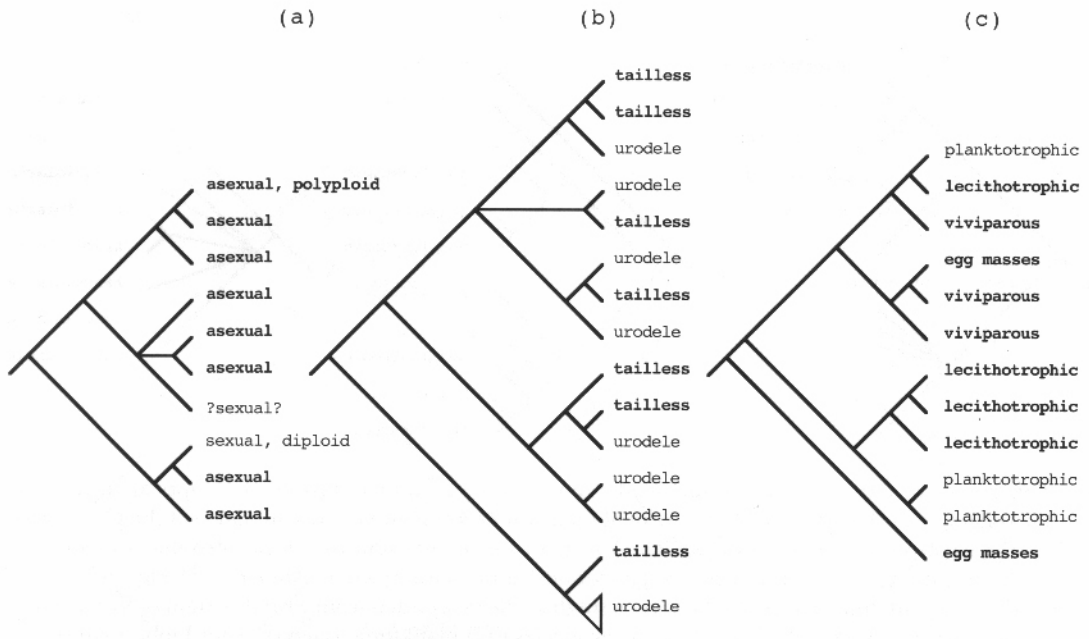

Figure 2. Phylogenies of larvae and breeding systems. (a) *Lasaea* clams. Both species that are known to be diploid and sexual (or diploid and probably sexual, shown by the question marks) are sister group to species or lineages that are asexual (all of these are also polyploid, indicated for the topmost terminal taxon only). After ÓFoighil and Smith[6] Figure 4. (b) Tunicates. Most species with tailless (or anural) larvae are close relatives of species with a normal (urodele) tadpole larva. After Hadfield *et al.*[7] Figure 2. (c) Asterinid seastars. Two clades include both feeding planktonic larvae and nonfeeding, benthic, or viviparous development. After Hart *et al.*[10] Figure 2. Triangles and derived forms shown as in Figure 1.

Littorinid gastropods.

Like asterinids, the family Littorinidae include species with several modes of development, including planktotrophic and nonplanktotrophic veliger larvae. Reid[11] used morphological characters to reconstruct relationships among species of *Lacuna*, *Tectarius*, *Littorina*, and other genera and species groups in this family. One of Reid's discoveries was a clade of six genera, the Lacuninae, that includes both larval forms [Figure 3(a)]. Three of these genera have planktotrophic larvae but their respective sister groups are nonplanktotrophic. Reid assumed that the feeding veliger is the ancestral larval form for all littorinids. However, the shells of feeding larvae of the Lacuninae resemble the shells of their nonfeeding sister groups, and not the shells of other feeding littorinids and other gastropods with feeding veligers. Reid concluded that this shell similarity (combined with the phylogenetic pattern) implied a recent reversal from nonfeeding to feeding veligers among the Lacuninae.

Turritellid gastropods.

In the first molecular analysis of larval evolution, Lieberman *et al.*[12] used mtDNA nucleotide characters to analyze relationships among 11 turritellids with planktonic or nonplanktonic veligers. Like Duda and Palumbi,[5] Lieberman *et al.* were specifically interested in whether all or most cases of derived nonplanktonic development were descended from one or a few such ancestors. They found, instead, that most instances of nonplanktonic larvae were independently derived (though the inference depends to some extent on the nature of development for species in which the larval form is not known).

Families of sea stars.

All Asteroidea with a planktotrophic larva develop initially as a bipinnaria larva. Some of these groups, as well as many nonplanktotrophic larvae, later develop a set of brachiolar arms at the anterior end as an attachment structure. These arms are absent in larvae of the Luidiidae and Astropectinidae. Wada *et al.*[13] used mtDNA nucleotide characters to determine relationships among some of these families. They found that luidiids (without a brachiolaria) are the

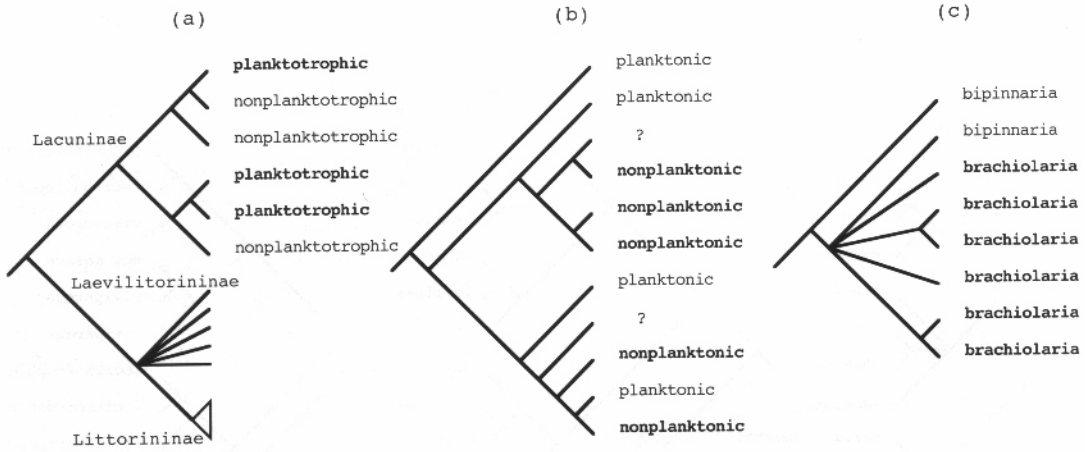

Figure 3. Phylogenies of larvae. (a) Littorinid snails. Among the Lacuninae, two lineages appear to have re-evolved a planktotrophic veliger larva (based on the larval form of their sister groups and the resemblance of the planktotrophic shells to the shells of the lecithotrophic sister groups). The Laevilitorininae are exclusively lecithotrophic, while the Littorininae are mixed planktotrophic (the ancestral form for gastropods) and lecithotrophic. After Reid[11] Figure 17. (b) Turritellid snails. Species with nonplanktonic larvae do not form a single clade descended from a single origin of this derived form, and instead are independently descended several times from species with planktonic veligers. After Lieberman *et al.*[12] Figure 2. (c) Families of sea stars. One of two sea star families lacking brachiolar arms in the larva is sister group to the other living families, implying that the bipinnaria larva without a brachiolaria stage is the ancestral form. After Wada *et al.*[13] Figure 2(a). Triangles, derived forms, and unknown larval forms as in Figure 2.

sister group to the other families, and concluded that the brachiolaria is the derived form and that lack of brachiolar arms in these two families is probably primitive.

Families of sea urchins.

Feeding and nonfeeding larvae are widespread among the crown group sea urchins. Wray[14] used the combined morphological and molecular analyses of previous authors (see his references) to reconstruct the evolution and loss of planktotrophic pluteus larvae [Figure 4(a)]. He noted that phylogenies based on larval morphological characters strongly contradicted phylogenies for the same species based on adult morphological characters and nucleotides. Nonplanktotrophic plutei evolved in parallel in several families, as did planktotrophic plutei that can develop without food (facultative feeding larvae). Facultative feeding larvae may be transitional forms but neither of these species was sister group to sea urchins with nonfeeding plutei.

Sabellid polychaetes.

The Sabellidae and related families have evolved a tremendous diversity of larval forms, breeding systems, and other life history traits. Rouse and Fitzhugh[15] used morphological characters of adults to build a phylogeny of sabellids and some related polychaetes [Figure 4(b)]. Earlier reviews[16] summarized some of the results of this work. Among many other patterns analyzed, Rouse and Fitzhugh examined the distribution of derived breeding systems in which individuals are first male and then female (protandric hermaphrodites) or male and female at the same time (simultaneous hermaphrodites). Such lineages did not form single large clades, but instead were distributed among gonochoristic sabellid lineages, which suggested multiple parallel origins of hermaphroditism.

Patterns

The studies summarized have all been important individually for framing questions about the evolution of larval forms in particular taxonomic groups. However, taken together these studies can also be used to explore the relative frequency of some general patterns of larval evolution. Several themes have been visited repeatedly in these studies. Most of these studies focused on one or more of the following questions about pattern.

(1) What was the ancestral larval form? Often the ancestral form is asserted on the basis of morphological complexity or frequency among

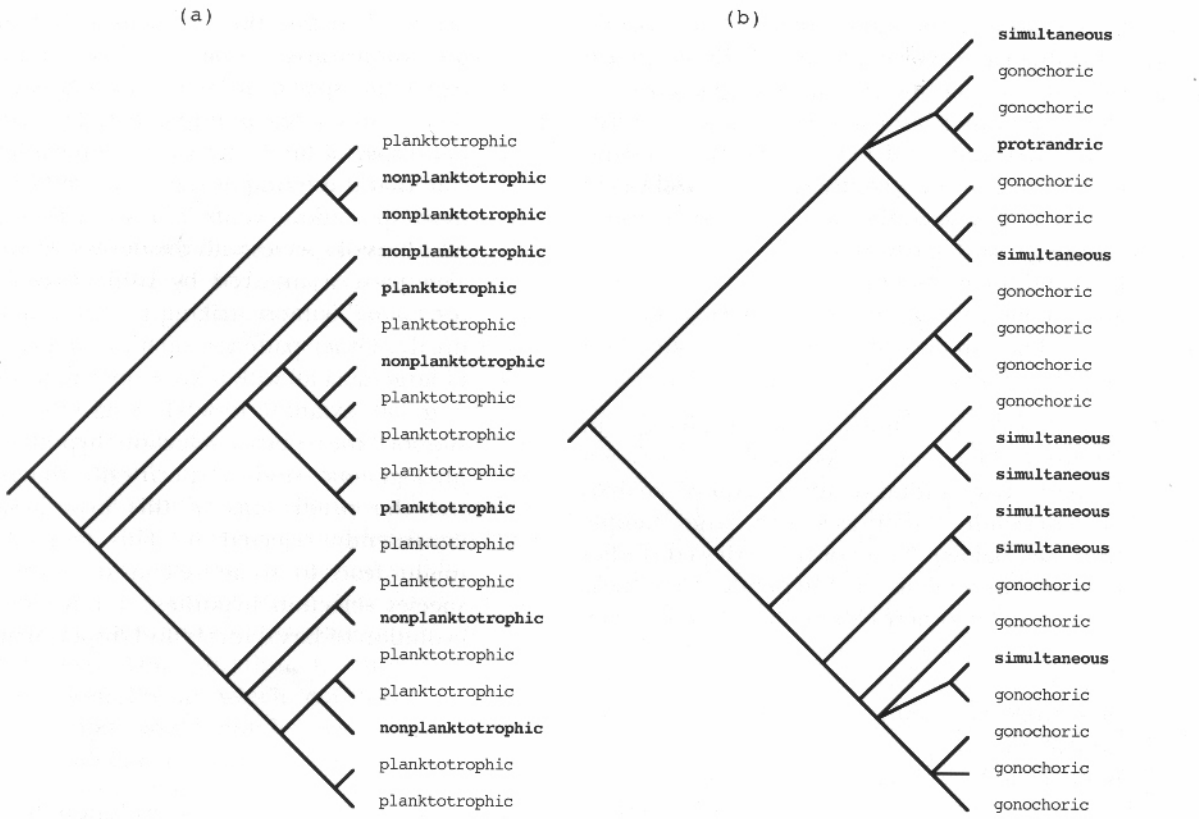

Figure 4. Phylogenies of larvae and breeding systems. (a) Families of sea urchins. Lecithotrophic larvae (bold face) evolved in parallel in several sea urchin groups. At least two planktotrophic species (bold face, italic) have large, yolk-rich eggs and a derived ability to develop without food (called facultative feeding). After Wray[14] Figure 2. (b) Sabellid polychaetes. Protandric and simultaneous hermaphrodites have evolved in parallel in numerous genera of higher sabellids. Gonochoric breeding systems (with separate male and female individuals) are presumed to be ancestral. After Rouse & Fitzhugh[15] Figure 45 (the higher sabellids only, including *Sabellastarte*, *Potamilla*, and *Schizobranchia*). Derived larval forms as in Figure 1.

members of the outgroup, but in other cases it is inferred by parsimony. In sea stars, the bipinnaria is inferred to be ancestral and the brachiolaria derived.[13] In polychaetes, gonochoristic breeding systems and brooding [not shown in Figure 4(b)] are inferred to be ancestral to hermaphroditism and broadcast spawning.[15]

(2) How often did derived larval forms evolve? All of these studies indicate massive parallel evolution of derived larval forms, breeding systems, dispersal ability, and other life history traits. These include eight losses of planktonic dispersal in *Conus*[5] and four in turritellids,[12] four losses of sexuality in *Lasaea*,[6] six losses of the tadpole tail in tunicates,[7] four losses of a planktotrophic larva in asterinids[10] and many more among sea urchins,[14] and six origins of hermaphroditism among sabellids.[15] This appears to be a general trend confirmed for a wide variety of taxa and traits. Though the total number of evolutionary changes inferred in each of these studies is large, this discovery is perhaps not surprising. For each of these studies, most of the possible phylogenetic topologies would indicate multiple origins of derived life history traits. Only a small minority of the possible trees in each case would group all species with derived life histories together in a clade descended from an ancestor in which the derived form evolved just once. Moreover, larval phylogeneticists have been particularly drawn to taxonomic groups with especially diverse assortments of larval forms (in which convergence is more likely to be inferred). Thus, the null expectation in all of these studies should perhaps be parallelism and not homology among the derived modes of development.

(3) Can presumed ancestral forms such as a planktotrophic larva evolve by reversal? Reversals are difficult to identify on phylogenetic grounds alone, but other information (such as mixtures of derived traits and newly evolved 'ancestral' traits) can confirm hypotheses of reversal based on parsimony, as in the case of mixed ancestral (feeding) and derived (shell form) traits in littorinid snails.[11] In many other cases, reversals are assumed to be impossible or suggested to be less likely than parallel losses of the ancestral character state.[6,7,10,14]

(4) Are there correlations between change in different characters? Associations between changes in quantitative traits require analysis of independent contrasts with large sample sizes (see below). None of the reviewed studies includes an analysis of independent contrasts for testing hypotheses about evolution of larval forms. Associations between qualitative traits can often be inferred through mapping traits onto trees. For example, in sabellids the evolution of an elongate or cylindrical sperm head is associated with the evolution of brooding.[15] Because some brooders with the ancestral, spherical sperm head shape are the sister groups of other brooders with derived sperm forms, one might conclude that the evolution of brooding precedes and perhaps drives the evolution of changes in sperm morphology.

(5) Does larval evolution affect speciation and extinction? Larval traits that influence dispersal and population structure may predispose some lineages to different rates of speciation and extinction, resulting in long-term temporal trends in the frequency of different modes of larval development within taxa.[17] The main predicted trend is an increasing frequency of lineages with nondispersing larval forms. This hypothesis of species selection has been confirmed for some Mesozoic and Cenozoic molluscs (in which mode of larval development can be inferred from the fossils) through the direct enumeration of trends in species numbers. The hypothesis also predicts that phylogenies of living taxa should often reveal monophyletic groups of species with derived larval forms in which the rare evolution of a low-dispersal form leads to proliferation of lineages with that form and increased frequency of the derived life history. Two gastropod studies[5,12] were designed to test this prediction, but most of the studies are relevant to this question. All reject the species selection hypothesis. All of the studies infer multiple parallel origins of low-dispersal life histories, and only a few indicate that these origins were followed by one or more speciation events[7,10] leading to increased numbers of species with the derived larval form. The pattern inferred by Duda and Palumbi for *Conus*[5] is most striking in this respect (Figure 1). Other patterns, such as the occurrence of large taxa all with a low-dispersal larval form (e.g. all extant crinoids), might be used to support the species selection hypothesis, but phylogenetic studies specifically designed to test the predictions of this hypothesis have consistently rejected it. This general result might lead to some re-consideration of the species selection hypothesis for trends in the evolution of larval forms and dispersal ability.

Discussion

Phylogenetic studies of larval evolution have been most successful in identifying parallelism and in testing the species selection hypothesis. Both successes result from the same pattern: statistically well supported clades of mixed larval forms or life history traits, and rejection of the hypothesis that lineages with superficially similar derived larvae are close relatives. In the case of feeding larvae in groups like echinoderms, the parallelisms may arise because of the seeming ease of simplification and loss of feeding structures and functional constraints on the form of the feeding device.[18] In other cases the cause of such parallelism is not as clear. However, the discovery of widespread parallelism in larval evolution argues against the use of larval morphological or life history characters in phylogeny reconstruction. These characters appear to be more prone to homoplasy than many adult characters or nucleotides,[18] but a quantitative analysis of homoplasy levels in larval and nucleotide characters is required to confirm this suggestion.

Phylogenetic studies of larval evolution have been less successful in two other respects. First, few of these studies include a sufficiently large number of taxa to permit statistically robust analysis of quantitative traits by the method of independent contrasts. In some studies, the number of analyzable events is small.[6]

In others, too few of the extant species have been sampled, in some instances because the taxonomy of the study organisms provided misleading clues to which species should be included. The asterinid study included almost all of the putative species of *Patiriella* and was intended to focus on those lineages, but it revealed instead that *Patiriella* and *Asterina* are not mutually monophyletic and thus all of the several dozen *Asterina* species should have been included (rather than a select few as a putative sister group to *Patiriella*).[10] In other cases where taxon sampling is excellent, many nodes are not well-resolved and more phylogenetic characters (usually more DNA sequence data) are required. In the case of *Conus* gastropods,[5] this constraint does not affect the inference of parallel larval evolution but analysis of other characters using the same phylogeny might be limited by the resolution of internal nodes.

Second, the reconstruction of these evolutionary changes in larval form requires some model of larval character evolution. Strathmann and Eernisse[19] reviewed examples in which controversies about larval evolution would not be resolved even with perfect knowledge of past phylogenetic events. In these cases, several plausible alternative models of larval character evolution (for example, reversible versus irreversible loss of planktotrophic larvae) give different inferences about the ancestral larval form and the pattern of descent from that ancestor even when the phylogeny is known.

The simplest model is the unweighted, reversible model of maximum parsimony used in most of the studies reviewed here. These maximum parsimony reconstructions of larval evolution may fail because they do not take into account other kinds of information (other than the distribution of larval characters on a given phylogeny) that larval biologists have about the probable ease or difficulty of evolving particular character state changes. Instead of inferring the pattern of change in larval characters from the phylogeny alone, we could instead apply knowledge of embryology, functional biology, physiology, and other aspects of larval life developed from observations or manipulative experiments.

Methods for applying such information are not yet sophisticated. One rule of thumb concerning the likelihood of convergence uses the degree of metamorphosis from larva to juvenile. In taxa with a dramatic metamorphosis (such as bryozoans or echinoderms), the convergent appearance of identical larval forms (such as the cyphonautes or pluteus) by reversal in independent lineages seems unlikely if many of the convergently evolved structures are unique to the larval stage.[19] In taxa with many larval structures carried over through metamorphosis into the juvenile (such as molluscs or decapod crustaceans), reversals to an ancestral form might be more likely if such reversals require relatively few changes.

In some groups, such information on the likelihood of particular directions of larval evolution extends to the genetic and developmental basis of alterations in larval form.[8] We could use such information to replace *ad hoc* assertions about the improbability of certain events (such as reversals from brooding to planktonic development, or from nonfeeding to feeding larvae) with models in which these improbabilities are replaced by likelihoods. The preferred reconstruction of larval evolution would then be the one that maximizes the likelihood of all inferred changes given a phylogeny and a specified model of character state changes.

Specific methods for inferring ancestral character states by maximum likelihood are still in development.[20,21] The simplest involve estimating the rates or probabilities of particular character state changes in a maximum likelihood statistical framework for a given phylogenetic topology and branch lengths.[21] More sophisticated methods will include prior estimates of these probabilities from other kinds of studies.[19,20]

Cunningham[22] has made an initial attempt to apply maximum likelihood methods to two of the phylogenies reviewed here: sea urchins[14] [Figure 4(a)] and asterinid sea stars[10] [Figure 2(c)]. In both cases, he found that maximum likelihood estimates of ancestral larval forms (either feeding or nonfeeding) were highly uncertain, in part because both phylogenies included a large number of changes in larval form distributed among relatively few nodes. Prior probabilities of changes (in these cases, the loss or gain of a feeding larva) estimated from studies of larvae themselves are necessary to improve the likelihood estimates of ancestral larval character states.[16,19] Cunningham[22] pointed out that these prior probabilities are equivalent to statements about homology of similar larval forms in different lineages.

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