

Protochordate body plan and the evolutionary role of larvae: old controversies resolved?¹

Thurston C. Lacalli

Abstract: Motile larvae figure prominently in a number of past scenarios for chordate and vertebrate origins, notably in the writings of Garstang, Berrill, and Romer. All three focus on the motile larva of a primitively sessile tunicate ancestor as a vertebrate progenitor; Garstang went further in deriving chordates themselves by neoteny from a yet more ancient larva of the dipleurula type. Yet the molecular evidence currently available shows convincingly that the part of the tunicate larva that persists to the adult expresses only a subset of the genes required to specify a complete bilaterian body axis, and essentially the same appears to be true of dipleurula larvae. Specifically, both are essentially heads without trunks. Hence, both are highly derived and as such are probably poor models for any real ancestor. A more convincing case can be made for a sequence of ancestral forms that throughout their evolution were active, motile organisms expressing a full complement of axial patterning genes. This implies a basal, ancestral form resembling modern enteropneusts, although a pelagic organism at a hemichordate level of complexity is also possible. A reassessment is thus required of the role played by adult and larval tunicates, and of larvae more generally, in chordate evolution. Tunicates need to be interpreted with caution, since the extreme degree of modification in the adult may have been accompanied by reductions to the larva. Dipleurula larvae may retain some ancestral features (e.g., of apical, oral, and anal organization), but are otherwise probably too specialized to be central players in chordate evolution. Garstang nevertheless remains a key figure in the history of evolutionary thought for his innovative ideas on the relation between ontogeny and phylogeny, and the way in which major innovations in morphology and body plan arise.

Résumé : Les larves mobiles jouent un rôle de premier plan dans plusieurs scénarios mis de l'avant dans le passé sur l'origine des chordés et des vertébrés, en particulier dans les travaux de Garstang, de Berrill et de Romer. Les trois auteurs s'intéressent à la larve mobile d'un ancêtre tunicier primitivement sessile comme un ancêtre des vertébrés. Garstang va encore plus loin en faisant dériver les chordés eux-mêmes par néoténie d'une larve encore plus ancienne de type dipleurula. Néanmoins, les données moléculaires actuellement disponibles montrent de façon convaincante que la partie de la larve de tunicier qui persiste jusqu'au stade adulte exprime seulement un sous-ensemble des gènes requis pour la spécification d'un axe corporel bilatéral complet et il semble en être de même pour la larve dipleurula. De façon spécifique, les deux sont essentiellement des têtes sans tronc. Ce sont donc des organismes fortement dérivés et, en tant que tels, ils sont donc probablement de mauvais modèles pour un quelconque ancêtre réel. On peut faire un argument plus convaincant en appui à une séquence de formes ancestrales qui, au cours de leur évolution, sont des organismes actifs et mobiles qui représentent l'expression de l'ensemble complet des gènes de structuration axiale. Cette approche implique une forme basale et ancestrale similaire aux entéropeustes modernes, bien qu'un organisme pélagique de niveau de complexité d'un hémichordé soit aussi envisageable. Il faut donc réévaluer les rôles joués par les adultes et les larves de tuniciers, et celui des larves en général, dans l'évolution des chordés. Il faut interpréter les tuniciers avec prudence, puisque le niveau extrême des réductions observées chez l'adulte peut s'être accompagné de réductions chez la larve. Les larves de type dipleurula peuvent avoir gardé certaines caractéristiques ancestrales (e.g., des organisations apicale, orale et anale), mais elles sont par ailleurs probablement trop spécialisées pour avoir joué un rôle central dans l'évolution des chordés. Garstang demeure néanmoins un auteur d'une importance capitale dans l'histoire de la théorie évolutive, à cause de ses idées innovatrices sur la relation entre l'ontogénie et la phylogénie et sur le mode d'apparition des innovations majeures dans la morphologie et le plan général du corps.

[Traduit par la Rédaction]

Received 30 January 2004. Accepted 17 November 2004. Published on the NRC Research Press Web site at <http://cjz.nrc.ca> on 19 April 2005.

T.C. Lacalli. Biology Department, University of Victoria, Victoria, BC V8W 3N5, Canada (e-mail: lacalli@uvic.ca).

¹This review is one of a series dealing with aspects of the biology of the Protochordata. This series is one of several virtual symposia on the biology of neglected groups that will be published in the Journal from time to time.

Introduction

With the techniques now available for sequencing genes, and indeed whole genomes, the phylogenetic position of a given group of animals can be determined with an increasing degree of precision. In some cases this has led to whole phyla being shifted from one major branch to another, or to the construction of entirely new groupings, as in the case of the Lophotrochozoa and Ecdysozoa (Adoutte et al. 1999). It is thus increasingly apparent that adult morphology is remarkably plastic over evolutionary time. This is a problem, because the construction of accurate phylogenetic trees is but the first step in a larger task of reconstructing the ancestral organisms themselves. One wants ultimately to know what these organisms were really like (e.g., were they pelagic or benthic, radially symmetric or bilateral, and so on). For basal metazoans, where there are as yet no reliable fossils and limited prospect of finding any, it is difficult to see whether any substantial progress towards this goal will be possible. When it comes to the origin of chordates, and subsequently vertebrates, there is a somewhat greater chance of finding fossils of key transitional forms. Indeed some may already have been found in deposits at Chengjiang, where soft parts are preserved (Holland and Chen 2001). Otherwise, however, we will have to rely on the comparative analysis of extant forms, which is likely to require a good deal of interpretive insight and ingenuity.

The question of vertebrate origins remains a major unsolved problem and a source of continuing interest and controversy. Considerable progress has been made in the past hundred years, as can be seen from the nature of the arguments presented at the Darwin Jubilee Debate in 1909 (for an easily accessible commentary see Gee 1996). Then the perspective was a rather rigid one, based on assessing the ease of transforming one adult morphology to another, and various phyla, from worms to arthropods, were viable contenders to be the progenitors of vertebrates. By the middle of the 20th century, the nature of the argument had changed to one primarily concerned with whether the proximate ancestors of vertebrates were sessile, like ascidian tunicates, or motile, like enteropneusts and amphioxus (Gregory 1946). The chief proponent of the former view was Walter Garstang, who placed tunicates center stage (Garstang 1928a). His ideas were sufficiently influential that one can effectively break the whole subject in two parts, before and after Garstang, as indeed Gee does. Yet, although tunicates are clearly basal chordates (Zeng and Swalla 2005), there is now considerable doubt about the validity of Garstang's central premise that advanced chordates evolved from the motile larvae of an otherwise sessile ancestor.

Instead, as is increasingly clear from the expression patterns of genes involved in specifying the axial body plan, the sedentary habit in tunicates is almost certainly derived rather than ancestral. The intent of this review is to examine some implications of this realization, especially what it implies about the evolutionary role of motile larvae. Garstang considered larvae to be phylogenetically very important as a potential source of evolutionary novelty. But if they are secondarily derived, they become little more than a side issue in evolutionary phylogeny. Because Garstang is a key figure in the development of modern ideas about how evolu-

tionary change occurs, specifically how and where in the life cycle evolutionary innovations arise, one needs to consider the consequences if his specific proposals regarding chordate origins are proven wrong.

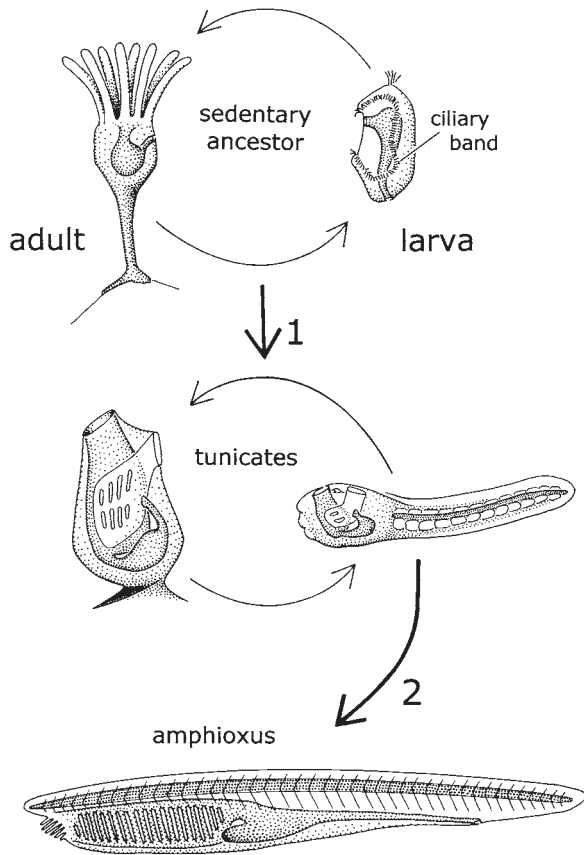
Scenario 1: sedentary ancestors with pelagic larvae

The importance of tunicates as models for the vertebrate ancestor was recognized by Kowalevsky, who first identified them as chordates, and they have played an important role in various evolutionary scenarios since then (Gee 1996). The most fully developed ideas are a legacy of the writings of three comparative zoologists of the past century: Walter Garstang, N.J. Berrill, and A.S. Romer. I will refer to the sum of their ideas, as they appear in most textbooks, as the GBR hypothesis (Fig. 1). Garstang and Berrill were tunicate specialists, among their other interests, and this is reflected in the weight of detail they bring to their arguments. However, the core idea is simple, that ancestral deuterostomes were sedentary, tentaculate animals like the modern pterobranchs, or crinoids without their armour. Chordates and eventually vertebrates, their actively motile descendants, then arose from the motile larval stage by paedomorphosis, a form of heterochrony roughly equivalent to neoteny, in which a young (i.e., larval) stage becomes sexually mature and replaces the adult. The ancestral life cycle is thereby truncated, and a new adult evolves that incorporates features originally evolved to serve the larval stage.

Garstang seems to have come to this idea early in his career through the realization that local concentrations of neural tissue in chordates could have evolved from the ciliary bands of dipleurula-type larvae like those of echinoderms and hemichordates (Garstang 1894). Thus, the neural tube could have formed by rolling up the mid-dorsal domain enclosed by the circumoral band (step 1 in Fig. 1). Ciliary locomotion would then be replaced by muscular activity with the evolution of axial musculature that eventually became segmented. This generates an advanced chordate with somites of at least an amphioxus level of complexity (step 2 in Fig. 1), with the tunicate larva being the transitional form (Garstang 1928a). These ideas form the basis for later treatments by Berrill (1955) and Romer (1967), who were, however, less enthusiastic about the role of the dipleurula in the first step of Garstang's proposal. Regardless of details, a central point in all such schemes is that the evolutionary action takes place in the pelagic realm, and does so independently of changes in adult morphology.

To Garstang's contemporaries, his ideas were more than just an ingenious way to derive the neural tube. They also served as a compelling counterexample to the predictions of the biogenetic law, which had become something of an intellectual albatross by the second decade of the 20th century. According to the biogenetic law, largely an invention of Ernst Haeckel in its strongest form (Richardson and Keuck 2002), ontogeny was supposed to recapitulate phylogeny in a comparatively rigid way. Although it would have been apparent to proponents of recapitulation that eggs and embryos show various special adaptations, major evolutionary innovations were supposed to occur at the end of the developmental sequence. Ancestral features would then be displaced

Fig. 1. Origin of advanced chordates, represented here by amphioxus, from a series of sessile ancestors, which is essentially the GBR hypothesis as expounded variously by Garstang, Berrill, and Romer. The ancestral adults remain sessile, while their motile larvae evolve progressively in two steps: first to (1) a tadpole-like protochordate and then to (2) a more advanced and fully motile form.



progressively earlier in the life history and accumulate in the embryo or larva. The appearance of relic structures in embryos (e.g., gill slits in mammalian embryos) was then a natural consequence of this process. Garstang published a detailed critique of this idea in 1922, largely refuting it, but support for Haeckel's position has been steadily eroding already for some years. Lillie, for example, points out in the Introduction to his *Development of the chick* (Lillie 1908) that ontogenetic and phylogenetic histories are linked because the ontogenies are inherited, not because they are determined by the phylogeny. The idea that evolutionary novelties might be generated by the action of selection on early ontogenetic stages is discussed by both Veit (1920) and Van Name (1921), in the latter case in a proposal for chordate origins that closely parallels Garstang's. Nevertheless, Garstang's (1922) paper proved timely, formalizing the arguments against recapitulation in a comprehensive and useful way. The value of the paper to his contemporaries is evident in the assessment of Garstang's contribution by de Beer (1940) and in Alister Hardy's Introduction to a posthumous volume of his zoological poetry (Garstang 1951).

The specialized nature of larval adaptations is a persistent theme in Garstang's later work (e.g., Garstang 1928b). The

importance attributed to this change in perspective can be seen again in Hardy's Introduction, and in an almost exaggerated way in H.B. Fell's (1948) review of echinoderm larvae. To Fell, larval morphology was so variable and so frequently subject to evolutionary change, with reversion to lecithotrophy being the prime example, that it was unreliable as an evolutionary clue to anything. Fell's analysis begins a trend in larval studies, especially marked in Britain and North America, that is characterized by attention to specific larval adaptations (e.g., for feeding and locomotion) without reference to phylogenetic issues.

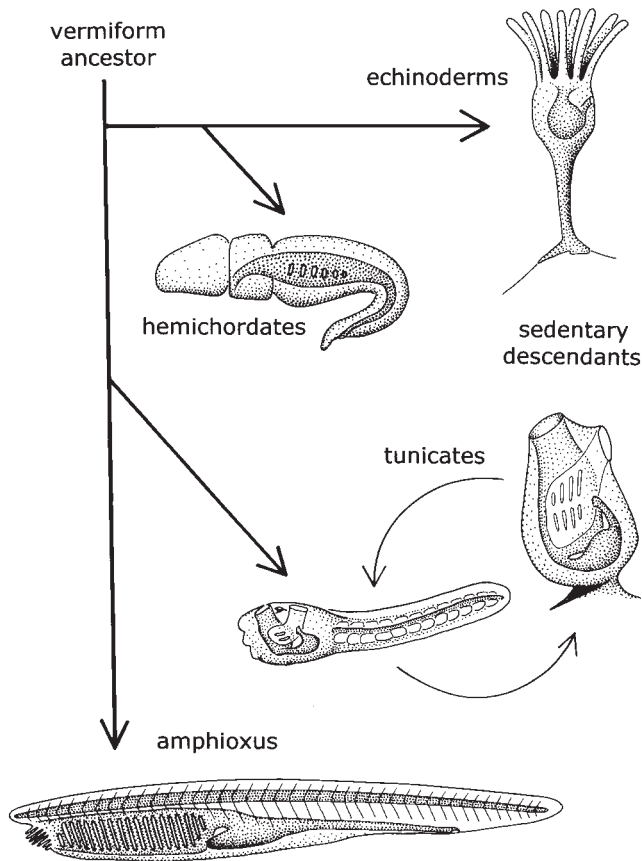
Larval biology in continental Europe developed somewhat differently, as there was a more continuous tradition of combining larval studies and phylogenetic analysis (e.g., Jagersten 1972; Nielsen 1987, 2001). The reasons for the different emphasis can be explained in various ways. In part, it seems to be due to a certain reluctance on the part of both British and American zoologists to engage in speculative exercises, especially concerning phylogeny. The experimental method dominated biology in both countries beginning in the early 20th century, and this produced a strong bias against questions that could not be answered directly by experiment. Biological research became the "art of the soluble" as Medawar (1967) puts it. In America, the trend away from speculative endeavors parallels the increasing scale of the scientific enterprise generally and an increased sense of professionalism among biologists (Allen 1969; Maienschein 1991). This led, perhaps for political as much as scientific reasons (Rasmussen 1991), to a rejection of the comparative methods and speculative theorizing of the previous generation. Thus, for example, T.H. Morgan would largely repudiate the scientific approach of his mentor, W.K. Brooks, who had been a major figure in late 19th century comparative morphology (Allen 1978).

Despite the appeal of the GBR hypothesis, both as an innovative scenario and a conceptual breakthrough, it contains an internal contradiction. On the one hand, it requires modern larvae to have preserved at least some recognizable ancestral features. On the other, the latter must have been sufficiently mutable as to give rise to entirely new body plans. If larvae were, in fact, more conservative than the corresponding adult in each instance, then they might well provide a better guide to the nature of the ancestral form. But Garstang's own work and his writings on larval adaptations emphasize the opposite, that larvae are molded by their adaptive response to the selective pressures of planktonic life. Given that this is clearly the case, the real question is whether any truly ancestral features survive in modern larvae, and if so, how can they be identified.

Scenario 2: progressive alteration of an actively motile adult

The main alternatives to the GBR hypothesis are scenarios that focus on the adult at each step (Fig. 2). These have received increasing attention (e.g., Bone 1960; Jagersten 1972; Jollie 1973; Nielsen 2001; Cameron 2005), in large part because of concerns that tunicates are secondarily specialized in various ways, not least in their sedentary habit, and so are probably poor guides to the ancestral condition. Instead, the sequence of ancestral forms is supposed to consist of motile,

Fig. 2. The main alternative to the GBR hypothesis. The ancestral adult is assumed to be a motile, roughly vermiform animal, either swimming or burrowing, throughout the evolutionary sequence. A selection of its descendants are shown, but the ancestral form is not, as its exact nature remains a matter of conjecture. The ancestral larvae, assuming there were any, can be disregarded in scenarios of this type, and all sedentary forms are then derived. In the case of the tunicates, the tadpole larva would be an evolutionary relic, probably much reduced from its original form, rather than a progenitor of segmented chordates.



bilaterally symmetric animals throughout, of at least moderate size, as opposed to being minute, like larvae. Motile forms such as enteropneusts and amphioxus are typically considered close to the main lineage (Presley et al. 1996; Salvini-Plawen 1998; Holland 2000), while tunicates are more distant. If the ancestral form was benthic, and a burrower, it could conceivably have been something like modern enteropneusts. However, a lineage of more active epibenthic or pelagic animals is also possible, in which case the enteropneusts may be rather poor models, since their nervous system and locomotory abilities would likely be secondarily reduced.

For the ancestor, therefore, one has to envisage something at an enteropneust grade of organization, but possibly pelagic and more active, with whatever that entails. Some putative fossil representatives have been suggested, among them the vetulicolians, although there is considerable disagreement as to the true nature of these organisms (Lacalli 2002). They are more likely pelagic than benthic, and the chief characteristic linking them with chordates is the presence of a series of doughnut-shaped lateral structures that resemble

pharyngeal (i.e., gill) slits. Pharyngeal slits occur in hemichordates, and pores that resemble them are known from carapoids, an atypical group of fossil echinoderms. Whether the latter are really protochordates or closely allied to them, the very fact that they are interpreted as such by some authors indicates the kind of features zoologists have come to expect of an ancestor, i.e., an elongate body, possibly subdivided in some way, perhaps segmented, with an expanded internal pharynx connected to the outside via slits or pores.

Thus, of the main chordate features, namely pharyngeal slits, a notochord, and dorsal nerve cord, it is the pharyngeal slits that are generally accepted as having evolved first. This leaves the origin of notochord and nerve cord to be explained. The homology between the chordate notochord and the hemichordate stomochords and pygostyles is now somewhat in doubt (Gerhart 2000), but their very existence illustrates the ease with which turgid outgrowths from the mesentoderm can be generated. The nerve cord, however, has no obvious counterpart in anything basal to chordates. Enteropneusts have an extended epithelial plexus for a nervous system, condensed into cords at certain points, but otherwise unlike the chordate CNS. This highlights one feature of Garstang's hypothesis: despite its other limitations, it provides a rationale for why a condensed nervous system might have evolved, since nerves in dipleurula larvae supply ciliary bands that are themselves condensed from the surrounding epithelium. If a condensed CNS evolved instead in the adult, some other explanation would be required.

Hypotheses that depend on adult organisms basically force one back to comparative morphology to re-examine the various adult forms and their development. Although many gaps remain in our knowledge of basal deuterostomes, especially hemichordates, differences between the various groups are so great that the true nature of the ancestral form is likely to remain for some time as elusive as it has been in the past.

Relevant molecular data

The results of gene sequencing and in situ analysis of expression patterns have provided enough new information that, in my view, they essentially resolve the above controversy in favor of a lineage of organisms that were active, motile forms rather than sedentary ones. The key question is how much of the ancestral body axis is represented in a given group. This can be monitored by examining the expression of genes involved in patterning the body along its anteroposterior axis. Among these, the *Hox* genes are best known; they are expressed in a series of domains at the hindbrain level in the head in vertebrates and through the trunk (Scott 1994; Finnerty and Martindale 1998). More anterior domains in the head are marked by sets of head genes, notably *Otx*, *Emx*, certain of the *Pax* genes, and *engrailed* (Hirth and Reichert 1999; Galliot and Miller 2000).

The most striking feature of axial patterning from a phylogenetic perspective is the apparent conservation of the genes and expression domains across taxa as distant as fruit flies and vertebrates. Some workers have interpreted this to mean that the common ancestors of insects and vertebrates necessarily had both comparable genetic patterns and comparable structures, i.e., they were bilaterally symmetrical, segmented

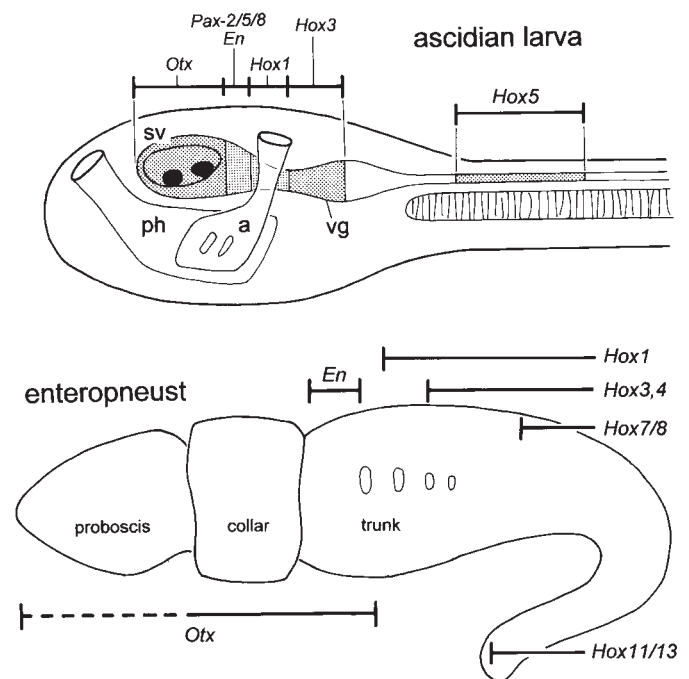
animals of some complexity (e.g., Kimmel 1996). A degree of caution is needed here, however, since the expression of a given gene in similar structures in otherwise distant organisms does not mean the common ancestor also possessed that structure. The textbook example is *Pax-6*, a key gene involved in controlling eye formation in both insects and vertebrates. Complex eyes are clearly derived and independently evolved in these two lineages, so what the two apparently share are conserved pathways involving *Pax-6* that are probably related to the specification of certain receptor cell types (Nilsson 1996; Hodin 2000). If the same logic is applied to axial patterning genes, one is led to question whether the ancestral bilaterian was anywhere near as complex an organism as its modern descendants. This leaves open the question of whether the ancestral form might have been, for example, sessile yet retained a complete set of axial patterning genes. Or, if the latter were suppressed in the adult, perhaps they were expressed in the larval stage, and so were available for later use as the larva evolved into something else. The test of such ideas is to examine the patterning genes of sedentary forms to see which genes are present, and where they are expressed in the larva and adult.

The tunicate body in relation to enteropneusts and vertebrates

The evidence from ascidian larvae is that the head genes like *Otx*, *Pax-2*, *Pax-5*, *Pax-8*, and *engrailed* (= *En*) are expressed in the larval CNS in the sensory vesicle to the level of the narrowed "neck" that connects it to the visceral ganglion (Fig. 3A). *Hox* genes are expressed in the neck region, visceral ganglion, and tail, all of which are lost at metamorphosis. A useful landmark here is the opening to the atrium. The atrium is a chamber derived from ectoderm into which the adult pharyngeal slits open. It arises as an invagination, paired in some species (Berrill 1950), at a level roughly equivalent to where *En* and *Hox1* are expressed (although at different stages), which is close to what will be the caudal end of the body once the tail is resorbed. Yet in vertebrates, both *En* and *Hox1* are expressed in regions that are unequivocally part of the head, corresponding roughly to the midbrain/hindbrain junction + anterior hindbrain. The gills and gill arches then form a series that extends caudally from that point. The situation in enteropneusts is similar (Fig. 3B), according to the recent study by Lowe et al. (2003) on a direct-developing species, *Saccoglossus kowalevskii* (Agassiz, 1873). From their data, the pharyngeal slits begin just forward of the zone of *Hox1* expression and extend from there through regions where more caudal members of the *Hox* cluster are expressed. This leads inescapably to two conclusions: (1) that essentially the whole of the adult body in ascidians, at least in terms of the ectoderm and its derivatives, is basically head-like in character, and anterior head at that, and (2) that the pharyngeal slits that initially perforate the atrium in ascidians (the protostigmata) are direct counterparts of at most the first pharyngeal slits of protochordates with an extended trunk (i.e., enteropneusts and amphioxus), instead of being an equivalent or homologous series simply squashed into a smaller space. In fact, the latter point accords with past views (cf. Berrill 1950) that the small number of protostigmata that develop initially in tunicates

reflects an origin from an ancestor with only one or, at most, a few pairs of pharyngeal slits.

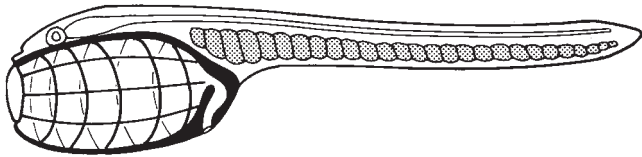
Fig. 3. How the body of an ascidian larva (top) maps to that of a more basal, direct-developing enteropneust (bottom), according to gene expression data. The figure shows the extent of the expression domains for an assortment of axial patterning genes, notably the *Otx*, *En*, *Pax*, and *Hox* genes. In the ascidian larva, these are expressed in the CNS, which consists of an anterior sensory vesicle (sv), separated by a narrow neck region from the visceral ganglion (vg), which then tapers to a slender nerve cord that extends into the tail. The expression data (from Locascio et al. 1999; Jiang and Smith 2002; for a review see Satoh 2003) are from larvae that have not as yet differentiated the pharynx (ph) and atrium (a), so the diagram is a composite of several stages. The position of the atrial opening relative to the expression domains, an issue discussed in the text, must be inferred, and is hence approximate. Enteropneusts lack an internalized nerve cord, and comparable genes are expressed in circumferential bands in the embryonic ectoderm (data from Lowe et al. 2003). In vertebrates, *Otx* expression extends to the caudal limit of the midbrain, which is marked also by a band of *En* expression. The comparable zone in ascidians would extend to about the level of the visceral ganglion, which means everything forward of this point has forebrain + midbrain character (i.e., anterior head). This corresponds, in the enteropneust, with all of the proboscis plus collar to a level just caudal to the first pharyngeal slit.



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This leads to something of a paradox. The body axis of adult ascidians as expressed in the ectoderm and its derivatives is truncated, literally, so that trunk components are lost, yet internally there are structures and organs that would originally have extended into the trunk. This illustrates a point that is evident also in the larvae of both protostomes and deuterostomes (see below): that external events are decoupled from internal ones so that, for example, a perfectly good intestine can transiently occupy the head during early

Fig. 4. The somatovisceral organism of Romer (1972), a cartoon emphasising the essential separation of the branchial/pharyngeal structures (heavy lines) and the locomotory axial complex containing the nerve cord, notochord, and somites. Although this continues to have heuristic value, as a way of understanding the basic layout of the vertebrate body, current evidence no longer supports it as a model for a direct tunicate-level antecedent of vertebrates, as Romer originally supposed. This could change if the fossil vetulicolians, with their bipartite body, prove to be bone fide basal chordates, but the nature of these organisms is currently a matter of some controversy.



development or, as in ascidians, it can be permanently anchored there by evolution.

What then can one say about the ascidian larva, which in the GBR hypothesis is the direct progenitor of vertebrates? In modern ascidians, the larvae are small and swim only briefly before settlement. They have limited sensory capabilities, and those they do have are specialized for larval functions. If ancestral tunicates were actively motile, rather than sessile, then the modern larvae might well reflect this heritage by retaining at least some of the ancestral locomotory equipment. They are, however, clearly scaled down versions of any such ancestor, so the relevant question to ask is how much have they lost.

It is useful in this context to consider Romer's concept of the somatovisceral animal (Fig. 4), which is basically a conceptual device for thinking about the anatomical separation of vertebrate locomotory structures from branchial and visceral ones. The way the body is drawn also, however, reflects Romer's conviction that the ascidian larva was a plausible starting point for vertebrate evolution. Ignoring the eyes and expanded brain, it bears more than a passing resemblance to a modern tunicate with a persistent, segmented tail (i.e., rather large ascidian larva). However, given that the extreme forward segregation of the internal organs in tunicates is probably a derived feature, the somatovisceral animal may be a less satisfactory ancestor than something with a more uniformly extended body like amphioxus.

This also illustrates a general problem of using modern organisms as models for otherwise unknown ancestors that may be inescapable where major morphological change has been a necessary correlate of survival. Ascidians are a good example because they likely avoided extinction in large part by adopting a mode of life that minimized direct competition with more advanced chordate lineages. Among modern tunicates, there are a few forms that compete directly with vertebrates in the pelagic realm, notably salps, which are major competitors of fish larvae for food. But it is not clear whether this reflects the primitive condition in any way. The various pelagic tunicates may all be as derived as the sessile ones. If so, this is unfortunate, because it is not at all clear where else one should look for clues to the nature of basal chordates.

Dipleurula-type larvae

Turning to the larvae of basal deuterostomes (the auricularia, tornaria, and other dipleurula types), the logic of the exercise is basically the same: one wants to know which axial patterning genes are expressed in the larval phase and where. If Garstang were correct, one would expect an anteroposterior series of expression zones coinciding with those in vertebrates. The available data on echinoderm larvae are puzzling, however, and somewhat ambiguous. Echinoderm genes are typically divergent (Long and Byrne 2001), and expression patterns show surprising differences between the larvae of different echinoderm classes (Lowe and Wray 1997). In particular, *Hox* genes are not expressed in obvious ectodermal domains in larvae. They do show up in the coelomic compartments (Arenas-Mena et al. 2000), but it is not clear that these are ancestral larval structures. One gene that is expressed in larvae is the *Otx* homolog, usually in association with ciliary bands. Expression patterns are quite variable, however, so that *Otx*-positive cells may occur in the bands or along their margins, and either along the whole band or in a restricted zone, for example, in the oral region (Harada et al. 2000; Shoguchi et al. 2000; Lowe et al. 2002). Exactly what this means is not clear, but it does show that, in some species at least, *Otx* can be expressed across much of the larval body, while well-defined *Hox* domains are not observed. This is consistent with the idea that the larval body, or at least its ectoderm, is largely head-like in character, but it is hardly compelling evidence. The better case comes, again, from the *S. kowalevskii* data of Lowe et al. (2003). Here the first pharyngeal slit coincides roughly with the caudal limit of *Otx* expression and the beginning of the *Hox* region. Classical studies of the tornaria larva of enteropneusts that have them show that this region arises from a growth zone located just anterior to the telotroch (Nielsen 2001). Thus, the body of the larva forward of this point, i.e., most of it, is head-like in character, and so finds its vertebrate counterpart in the region forward of the mid-hindbrain junction. Modern deuterostome larvae can thus be considered in essence to be swimming heads packed with a compressed set of visceral organs. Their value as models for an ancestral form with a complete anteroposterior body axis is consequently somewhat limited.

What then is the phylogenetic significance, if any, of larvae?

One could argue that Garstang's ideas about larval paedomorphosis and evolutionary innovation are sound in principle, but simply don't apply to modern larvae in which a significant fraction of the ancestral body has been suppressed. Ancestral larvae were presumably less modified, and if so, they might be more plausible candidates to be progenitors of chordates. But we are then left with no way of reconstructing what such larvae might have looked like based on modern forms. A thoughtful review by Strathmann (1993) addresses some of the interpretive problems. Among these is the very real possibility (see below) that feeding larvae of the dipleurula type evolved after the divergence of the chordate lineage from other basal deuterostomes, in which case there would never have been a larva of this type, with

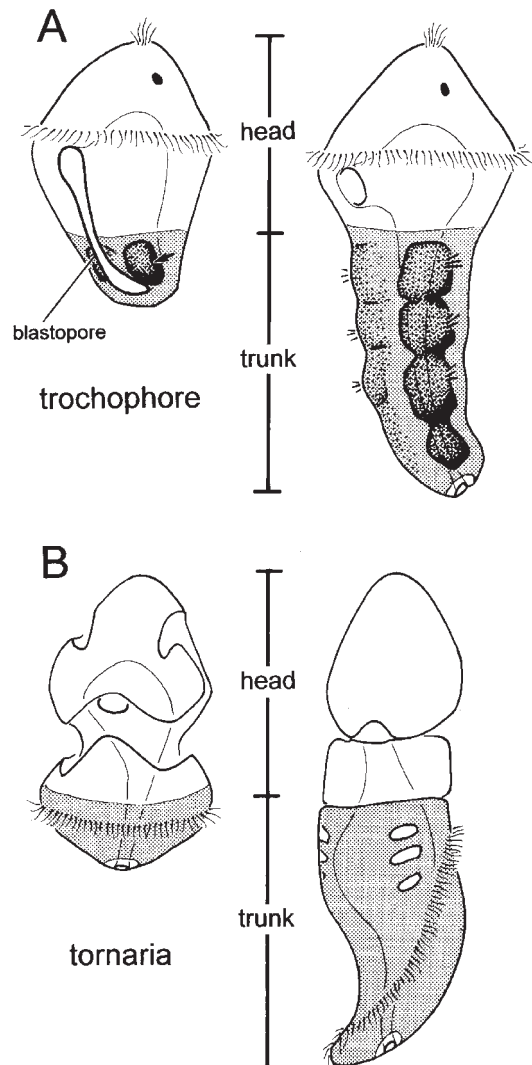
its characteristic arrangement of ciliary bands, in chordate ancestry.

A significant part of my own research has been an attempt to find morphological evidence in support of the initial step in the GBR hypothesis, the conversion of an auricularia-type larva to something with a nerve cord. If this occurred, it should be possible to find similarities in neuronal cell types and organizational patterns linking the two structures. The results to date have been disappointing, providing at best ambiguous evidence for Garstang's hypothesis (e.g., Lacalli 1996a; Lacalli and Kelly 2002). What does this mean in terms of the evolutionary significance of larvae? If, as above, they are secondarily reduced to swimming heads, not only do Garstang's ideas need to be reassessed, but those of Jagersten as well. Jagersten (1972) argued that planktotrophic larvae extend in an unbroken lineage back to the earliest metazoans as part of an ancestral pelagobenthic life cycle. Body axes, apical sensory organs, mechanisms for organizing ciliary bands, and positioning main body orifices would then all be shared, homologous features. Critics of his ideas (e.g., Haszprunar et al. 1995) consider direct development primitive, so that planktotrophic larvae would have had to arise repeatedly during evolution. In this view, it is not a useful exercise to seek homologies among larval structures like ciliary bands.

My own view is that the current evidence supports a somewhat intermediate position. There are common features shared by larvae of otherwise divergent groups that are indicative of a common heritage, yet there are also major differences, most notably between the downstream larvae of protostomes and the upstream ones of basal deuterostomes (Nielsen 1987, 1998). The terminology here refers to whether food particles are captured behind (downstream) or forward (upstream) of the principal ciliary band. In developmental terms, this translates also into a difference in the mode of formation of the mouth and anus. In protostomes, the blastopore is a slit that produces mouth at one end, anus at the other, and transects the site of the developing ciliary band ventrally (Fig. 5A). In deuterostomes (Fig. 5B), the formation of the mouth is a separate event, resulting from the fusion of the archenteron to the body wall well forward of the future site of the principal ciliary band. One way to explain this is to suppose that a tubular gut supplied with a separate mouth and anus evolved independently in protostomes and deuterostomes (Lacalli 1996b). In doing so, the mouth in each case could have been formed and positioned in a different way. This would account for constraints on how food was captured, whether by upstream or downstream mechanisms.

A less radical alternative is to suppose the ancestor was a motile vermiform organism already possessing a tubular gut as an adult, with a separate mouth and anus, and a motile but nonfeeding ciliated larva. Thus, in the primitive state, differentiation of the gut, mouth, and anus would be delayed until after metamorphosis. Then, at some point along each lineage, with increased selective pressure to reduce egg size and take advantage of plankton as a food source, larvae in each lineage became planktotrophic. To do this most efficiently (i.e., with least yolk), unnecessary trunk structures could have been suppressed in favor of what was essential (i.e., head, mouth and associated oral structures, gut, and

Fig. 5. Head and trunk in invertebrate larvae: the basic head-like character of the early larva is evident in spiralian protostomes (the trochophore, A) because trunk precursors are sequestered in the mesoblast (arrow) and an overlying zone of prospective trunk ectoderm (shaded). These later expand in concert to form the segmented trunk. In deuterostomes (e.g., the tornaria, B), the trunk develops by proliferation from a posterior growth zone, probably circumferential. The spatial extent of the growth zone in relation to the blastopore is significantly different in protostomes and deuterostomes, which is a possible indication that the specialization of the larva as a precociously differentiated planktotrophic "head" occurred independently in the two lineages (see text for discussion).



anus). Assuming the anus is represented already in the posterior blastopore, this means superimposing a more anterior mouth on a previously existing ciliary field. Judging from the result, there are two ways to do this. In the protostomes, the blastopore has expanded forward as a slit, which temporarily divides the ciliary band and trunk rudiment into two parts (in fact, the sequestering of the trunk as a symmetrical pair of laterally positioned stem cells may be what allowed this change). In contrast, in deuterostomes the evidence to date suggests that the trunk arises from a complete cir-

cumferential growth zone, and this could in part be contingent on the formation of the mouth well forward of this zone where it will not interfere with subsequent growth.

Why these differences first arose is not clear. It may be due to the deuterostome mouth having evolved in a quite different fashion from the protostome mouth before either had feeding larvae, with the differences then being carried through as mouth formation is accelerated. Regardless of details, the overall implication is that the existence of two separate types of organization in feeding larvae, upstream and downstream, implies independent evolution of the two types from an ancestral form with either a nonfeeding larva or no larva.

Even if feeding larvae are secondarily derived rather than primitive, they may still possess primitive features worthy of serious study. Structures like the apical sense organs and mechanisms for ordering the ciliary fields will all pre-date the imposition of feeding, and may thus reflect the primitive condition (e.g., see Tagawa et al. 2000, 2001). Furthermore, the structures and specification mechanisms associated with mouth and anus formation may still be primitive ones, although carried forwards in terms of their developmental timing. The oral and perianal domains are thus potentially of special interest. There is clearly a need for caution when interpreting larvae, however. As with tunicates and their larvae, some features will be primitive, while others are derived. The problem is to determine which is which.

Conclusion

The molecular data provide increasingly strong evidence that both adult tunicates and dipleurula-type larvae lack a full complement of body regions, and so, are poor candidates to be transitional forms in any evolutionary scenario leading to advanced chordates. Instead, it seems clear that adult ascidians are secondarily reduced and highly modified. Although they may still be fascinating organisms in their own right and preserve ancestral characters worthy of detailed study, they are at best misleading guides to the ancestral mode of life and overall body plan. Hence, for example, there is still much to be learned about the nervous system and basic physiology of growth control, nutrition, and reproduction in tunicates for the clues they may provide regarding the primitive chordate condition. There are also persistent puzzles, the hypocord (Lacalli 1999) and neurenteric canal (Salvini-Plawen 1999) being examples, whose very existence show how incomplete our understanding of chordate origins still is. With regard to dipleurula-type larvae, the mechanisms by which apical structures, oral and anal domains, and ciliary bands are specified and organized may incorporate primitive features, but such larvae are probably otherwise of limited value as guides to the nature of the ancestral forms, their habits, and body plan.

To a considerable degree, the more we know in detail about living forms, the more aware we become of how derived they truly are, and the more elusive the ancestral forms become. An unfortunate consequence of rejecting Garstang's ideas is that we lose an otherwise appealing explanation for the evolution of a condensed nervous system from a diffuse one, and in more general terms, for the origin of new body plans by paedomorphosis. These events remain to be ex-

plained, perhaps in a less dramatic way through a sequence of gradual changes. Yet, in seeking evidence for these, there are no obvious points of reference among either living forms or fossils. The molecular data has so far been effective in terms of reducing the number of viable hypotheses, but less so in generating new ideas about the true nature of the ancestral forms. The essential problem has not changed. It is one of recovering, by inference or otherwise, some understanding of now extinct groups of organisms, and it is not at all clear how to proceed.

Acknowledgments

Financial support was provided by the Natural Sciences and Engineering Research Council of Canada. I thank Ed Ruppert and Ian Meinertzhagen for comments on the manuscript, and especially Nick Holland for sharing his insights into the historical development of the ideas discussed here.

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