

Larval genome transfer: hybridogenesis in animal phylogeny

Donald Irving Williamson

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Abstract My larval transfer hypothesis asserts that mature adults became larvae in foreign animal lineages by genome acquisition. Larval genomes were acquired by hybridization when sperm of one animal fertilized eggs of another animal, often remotely related. There were no larvae in any phylum until the classes (and, in some cases, the orders) of that phylum had evolved. Since larvae were acquired by hybrid transfer, they are not directly related to the adults that metamorphose from them. The widely accepted classification that associates echinoderms and chordates as deuterostomes, and annelids and molluscs as trochophorates or lophotrochozoans, is flawed. Symbiogenesis, the generation of new life forms by symbiosis, accounts for the discontinuous evolution of eukaryotic cells from prokaryotes. Hybridogenesis, the generation of new life forms and life histories by hybridization in sexually reproducing animals, occurred at all taxonomic levels from species to superphyla. Not only were larvae acquired by transfer from foreign adults from the late Palaeozoic to the present, but also complex animals were generated from simpler ones by this process in the Cambrian explosion, and organ systems were transferred between remotely related animals. There are several types of evolution. Symbiogenesis and hybridogenesis are saltatory genome transfer processes that dramatically supplement the gradual accumulation of random mutations within separate lineages described by Darwin.

Keywords Saltatory evolution · Sequential and concurrent chimeras · Hybridization · Recapitulation · Planctospheres · Echinoderms · Hemichordates · Lophotrochozoans

1 Background and significance

Larvae are active immature animals that differ significantly from the adults that will succeed them in ontogeny. The larval transfer hypothesis claims that basic forms of all larvae originated as adults in other taxa, and they were transferred by sexual hybridization between species at all levels of relationship (Williamson 2003). The first larvae resulted when eggs of one species were fertilized by sperm from another species. The eggs hatched as larvae resembling one parent, then metamorphosed into juveniles (small adults) resembling the other. All descendants of this cross were animals with larvae, in which one animal form follows another: they were sequential chimeras (Williamson 1991). Corollaries of the larval transfer hypothesis are that larvae were later additions to the evolutionary histories of species with indirect development, and they do not represent evolutionary ancestors of such species. Metamorphosis represents a change of taxon during development.

Hybridogenesis is the generation of new life forms and life histories by hybridization in sexually reproducing animals. It accounts for the acquisition of larvae by many animals (larval transfer), and also for the production of complex animals from simpler ones in the Cambrian explosion, and for the transfer of organ systems between remotely related animals (component transfer). Symbiogenesis, the generation of new life forms by symbiosis, accounts for the origin of eukaryotes from prokaryotes. Hybridogenesis and symbiogenesis are both saltatory evolutionary processes that involve fusions of genomes of

D. I. Williamson
School of Biological Sciences, University of Liverpool,
Liverpool, UK

D. I. Williamson (✉)
14 Pairk Beg, Port Erin,
Isle of Man IM9 6NH, UK
e-mail: diwilliamson@manx.net

remotely related organisms. They dramatically supplement the gradual accumulation of random mutations within separate lineages described by Darwin.

Darwin (1859) insisted that evolution is gradual, and he assumed that the larva and adult of any species had evolved gradually from a common ancestor. He thought that larvae showed the true relationships of taxa, and he wrote, “Even the illustrious Cuvier did not perceive that a barnacle was, as it certainly is, a crustacean; but a glance at the larva shows this to be the case in an unmistakable manner.” (Darwin 1859: 420). Barnacles go through nauplius and cypris stages in their development, and both these larval forms had adult counterparts. Cambrian nauplii were non-crustacean adults, some descendants of which hybridized with a variety of crustaceans to give them nauplius larvae (Williamson and Rice 1996; Williamson 2006a, b). The cypris has its adult counterpart in the Cambrian crustacean *Canadaspis* (Briggs 1978). Also, barnacle larvae resemble rhizocephalan larvae. Adult rhizocephalans are parasites of crabs and hermit crabs, and are totally devoid of crustacean or arthropod characteristics (Williamson 2009). I agree that barnacles are crustaceans, but not because of their larvae.

Darwin regarded larvae as ‘active embryos’, and he said, “As the embryonic state of each species and group of species partially shows us the structure of their less modified ancient progenitors, we can clearly see why ancient and extinct forms of life should resemble the embryos of their descendants—our existing species” (Darwin 1859: 449). Haeckel (1866) made this the basis of his ‘biogenetic law’, also known as ‘the theory of recapitulation’. This postulates that larvae represent ancestral adults, ontogeny is a short and rapid recapitulation of phylogeny, and major evolutionary innovations are confined to adults. Applying his law to echinoderms, Haeckel reasoned that recent and fossil adults, all of which are primarily radial, had evolved from bilateral ancestors, similar to extant larvae.

Balfour (1880–1881) put forward a very different view on larvae. He distinguished between primary larvae, “which have continued uninterruptedly to develop as free larvae from the time when they constituted the adult form of the species”, and secondary larvae, “which have become introduced into the ontogeny of species, the young of which were originally hatched with all the characters of the adult.” He regarded all extant larvae, except the planula larvae of cnidarians, as secondary, but he made no suggestions on the sources of secondary larvae. He inferred from the structure of the nervous system of adult and larval echinoderms that “adult Echinodermata *have retained* (Balfour’s italics), and not, as is now usually held, secondarily acquired, their radial symmetry; and if this is admitted it follows that the obvious bilateral symmetry of Echinoderm larvae is a secondary character.” He noted that

“the auricularia [of sea cucumbers], the bipinnaria [of starfish] and the pluteus [of sea urchins and brittle stars], but not the transversely ringed [doliolaria] larvae of the Crinoidea [sea lilies], can be reduced to a common type.” He deduced that “the various existing types of [echinoderm] larvae must have been formed after the differentiation of the existing groups of the Echinodermata; otherwise it would be necessary to adopt the impossible position that the different groups of Echinodermata were severally descended from the different types of larvae.”

Garstang (1894, 1922, 1928) ignored Balfour’s works. He amended Haeckel’s theory of recapitulation by proposing that modern larvae represent ancestral larvae rather than ancestral adults, and that, contrary to Haeckel, ontogeny creates phylogeny rather than recapitulating it. Garstang’s innovation, however, was a modification rather than a rejection of the hypothesis that larvae represent ancestors. He drew attention to cases in which larvae of one taxon resemble adults in another, such as trochophore larvae and adult rotifers, and he proposed that such adults are descendants of ‘persistent larvae’: animals that had matured in the larval state. Trochophore larvae, also known as trochosphere larvae, resemble rotifers of the genus *Trochosphaera*, and occur in some members of at least eight phyla, including annelids and molluscs. According to Garstang (1922), the phylum Rotifera evolved from a form resembling *Trochosphaera*, which originated as a trochophore larva that had matured without metamorphosis. He maintained that annelids, molluscs and several other phyla evolved from a common ancestor which had trochophore larvae. No-one, however, has proposed a feasible phylogeny of rotifers based on their evolution from a form resembling *Trochosphaera*.

The affinities of the respective classes of adult echinoderms conflict with the affinities of their larvae. I, independently of Balfour (1880–1881), decided that this anomaly could be explained if early echinoderms had no larvae, and larval forms were ‘transferred’ after the establishment of the extant classes. In other phyla, such as annelids, molluscs and bryozoans, the presence or absence of larvae is again consistent with the acquisition of larvae after the establishment of the classes (or, in some cases, the orders) of the phyla. Many developmental anomalies are consistent with the hypothesis that genes prescribing larvae had been transferred, and the only known process that could transfer genes in sufficient quantity is hybridization, leading to mergers of genomes. In my early publications on the subject, I claimed that some larvae in eight phyla had been transferred from other taxa by hybridization (Williamson 1988a, b, 1991, 1992, 1996), and my publications from 1998 extended the larval transfer hypothesis to all larvae. Laboratory experiments on the fertilization of ascidian eggs with sea urchin sperm showed that interphyletic crosses are

possible and can produce either paternal or maternal larvae (Williamson 1992, 2003). The basic forms of all larvae were transferred by hybridization, and the simplicity or complexity of metamorphosis reflects the degree of relationship between the original animals that hybridized. I also came to disagree with Garstang on the interpretation of cases in which adults in one taxon resemble larvae in another. Garstang regarded such adults as descendants of persistent larvae, but I claim that they are surviving relatives of the adult sources of the larvae concerned. Garstang proposed that the phylum Rotifera evolved from a form resembling *Trochosphaera*, which originated as a trochophore larva that matured without metamorphosis. I regard *Trochosphaera* as too specialized to be close to the stem group of the Rotifera, which, I claim, evolved independently of other phyla. I theorize that rotifers resembling *Trochosphaera* hybridized with an annelid and a mollusc, and their respective descendants were annelids and molluscs with trochophore larvae. Haeckel (1874); Garstang (1928) and I (Williamson 1988a, 1992, 2003) expressed different views on the evolution of urochordates, which comprise tunicates, some of which have non-feeding tadpole larvae, and larvaceans, which are tadpoles throughout their lives. Haeckel proposed that larvaceans were the original urochordates, and some larvaceans later evolved tunicate bodies. Garstang suggested that larvaceans evolved from feeding tadpole larvae of tunicates, maturing in what was previously the larval state. I regard larvaceans as surviving relatives of the tadpole-like adult that hybridized with one or more tunicates to produce tunicates with tadpole larvae.

I conclude that the basic types of all larvae were transferred from animals in other taxa by sexual hybridization, and each larva originated as an adult, whether or not it has a living counterpart (Williamson 1998, 2001, 2002, 2003, 2006a, b, 2009; Williamson and Vickers 2007). New life histories generated by hybrids between animals at all levels of relationship is one form of hybridogenesis. Early simple sexually-reproducing animals in the Ediacaran and lower Cambrian frequently hybridized, and these hybrids were not animals with larvae (sequential chimeras) but more complex animals (concurrent chimeras). Hybridogenesis, therefore, played a vital part in the Cambrian explosion. Whole genomes fused, but not all genes in each genome were expressed. In some cases, this led to hybrid transmission of specific organs. For example, hybridization between an animal with a lophophore and distantly related non-lophophorate animals led to the acquisition of this organ by disparate taxa. This feeding organ with an array of hollow ciliated tentacles occurs in the four phyla Bryozoa, Phoronida, Brachiopoda and Entoprocta and the class Pterobranchia (phylum Hemichordata). No-one has proposed a phylogeny that derives these taxa from a common ancestor, and, even if consideration is restricted to the

Brachiopoda, Phoronida, and Bryozoa, LSU and SSU sequences of ribosomal RNA indicate that “the Lophophorata is not a monophyletic entity” (Passamanek and Halanych 2006). I suggest that the lophophore was one of several organs acquired by hybridogenesis, including the bivalve shell of brachiopods, some molluscs and some crustaceans (Williamson 2006a).

2 Evidence from echinoderms and hemichordates

The larval transfer hypothesis covers all larvae, and examples ascribed to larval transfer from all major and several minor animal phyla are discussed in Williamson (2003). Here, however, I confine myself to examples from echinoderms and hemichordates. I present evidence that (1) the ontogeny of echinoderms, and (2) the distribution of types of larvae in echinoderms and hemichordates, are consistent with the larval transfer hypothesis but not with the hypothesis that larvae and their corresponding adults evolved from common ancestors.

2.1 Ontogeny of echinoderms

Echinoderm larvae are bilateral, but adult echinoderms are primarily radial. “A superficial bilateral organization has evolved twice, in irregular echinoids and holothuroids, but is based on an underlying five-fold organization of skeleton and most organ systems, and is clearly secondary” (Wray 1999). Bilateral larval echinoderms, however, do not develop into radial adults. The bilateral and radial forms are distinct throughout their development.

Figure 1 shows postembryonic development in the starfish *Astropecten aurantiacus*. In common with other echinoderms with planktonic larvae, the deuterostome larva develops three pairs of coelomic pouches from the archenteron (Fig. 1a–c). These pouches grow and separate from the archenteron as sacs. The five lobes of the radial juvenile arise from undifferentiated cells lining the largest of these sacs. This is usually the left hydrocoel (mesocoel) sac (Fig. 1d, e), but occasionally it is the right hydrocoel sac, or twins may develop in both hydrocoel sacs. The cells from which the juvenile rudiment develops are stem cells, which have never been part of any larval tissue or organ. They are capable of developing into any shape or form, and the first shape or form that they develop into is that of a radial juvenile echinoderm.

As the radial juvenile and the bilateral larva grow and differentiate, the juvenile migrates to the outside of the swimming larva. Figure 2a shows such a case in the development of the starfish *Luidia sarsi*. There is a comparable stage in the development of all echinoderms with planktonic larvae, in which the juvenile can move its

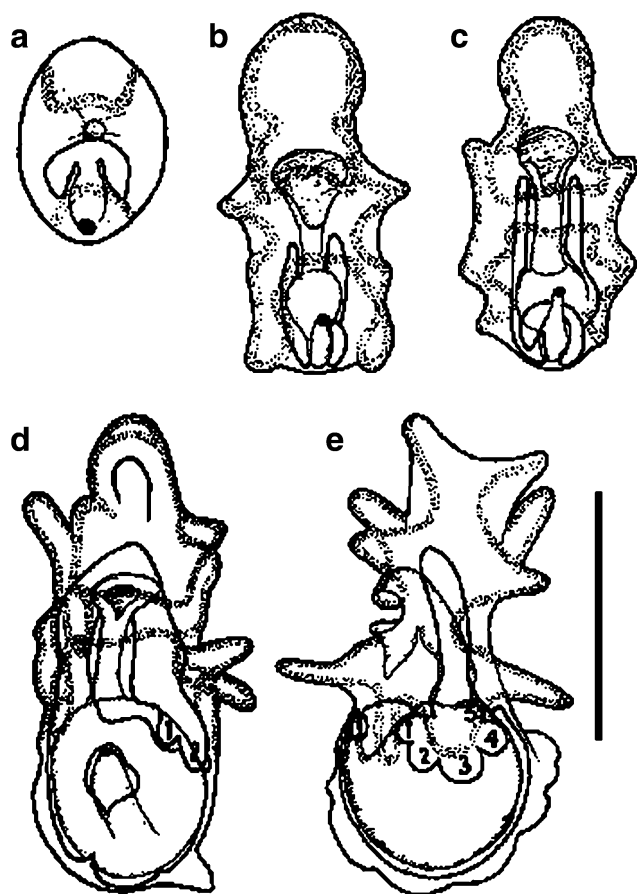


Fig. 1 Larvae of the starfish *Astropecten auranciacus*, **a** 3 days from hatching, **b** 10 days, **c** 14 days, **d**, **e** 70 days. **e** is lateral view of (**d**); 1–5 are lobes of developing juvenile. Scale = about 1 mm. (Redrawn and adapted from Hyman 1955)

arms (if present), spines and tube-feet independently of the swimming movements of the larva, indicating separate functioning nervous systems. Most echinoderms settle at this stage, and much of the larva is absorbed by the juvenile. In *L. sarsi*, however, the juvenile drops off the swimming larva (Fig. 2b), and the two products of the same egg can live independently for at least 3 months, when the larva eventually dies (Tattersall and Sheppard 1934). The radial juvenile of all echinoderms with planktonic larvae develops as a quasiparasite of the larva from its inception in the stem cells of (usually) the left hydrocoel sac. The bilateral larva does not ‘develop into’ the radial juvenile. The case of *L. sarsi* is striking because of the length of time that the larva and juvenile may live separately, but it is only one of many cases of ‘overlapping metamorphosis’, in which the larva and the juvenile co-exist for a time. Comparable cases occur (1) in polychaete worms with trochophore larvae, in which the wriggling segmented worm protrudes from the swimming larva, (2) in nemertean worms with pilidium larvae, and (3) in doliolid urochordates with tadpole larvae. In each case the juvenile may

break free while the larva continues swimming (Williamson 2003, 2006a). The remarkable independence of the juvenile and larva illustrated by these examples is consistent with the discrete genomes implied by larval transfer, but not with the single genome implied by common ancestry.

The ontogeny of direct-developing echinoderms is also explicable in terms of larval transfer but not in terms of common ancestry. Figure 3 shows stages in the development of Kirk’s brittle star, described by Fell (1941). The identity of this New Zealand species is still uncertain.

Eggs are laid at extreme low water on rocky shores, and the embryos develop pentaradial features before hatching. Bilateral echinoderm larvae develop as enterocoelous deuterostomes (*cf.* Fig. 1), but this species develops as a radial schizocoelous protostome. Ten primary podia develop round the blastopore, which becomes five-pointed, and the coelom develops from splits in the mesenchyme (schizocoely). There is no anus, as in all non-larval brittle stars. The New Zealand sea daisy *Xyloplax medusiformis* (Rowe et al. 1988) and the subantarctic brooding heart urchin *Abatus cordatus* (Schatt and Féral 1996) also develop directly, with no bilateral phase, comparable to the development of Kirk’s brittle star. The development of the other known species of *Xyloplax* and *Abatus* is undescribed. Fell (1941, 1948, 1963, 1968) repeatedly claimed that the development of Kirk’s brittle star represents the ancestral method for all echinoderms, that original echinoderms were schizocoelous protostomes, and that the phylogeny of echinoderms is unrelated to their ontogeny. I agree.

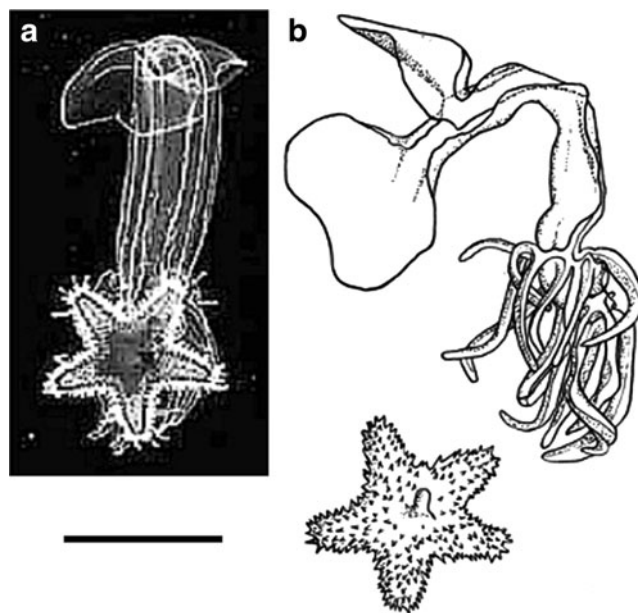


Fig. 2 Bilateral larva and radial juvenile of the starfish *Luidia sarsi*. **a** Juvenile still attached to swimming larva. **b** Shortly after separation. Scale = about 10 mm. (**a** from photograph by DP Wilson. **b** redrawn and adapted from Tattersall and Sheppard, 1934)

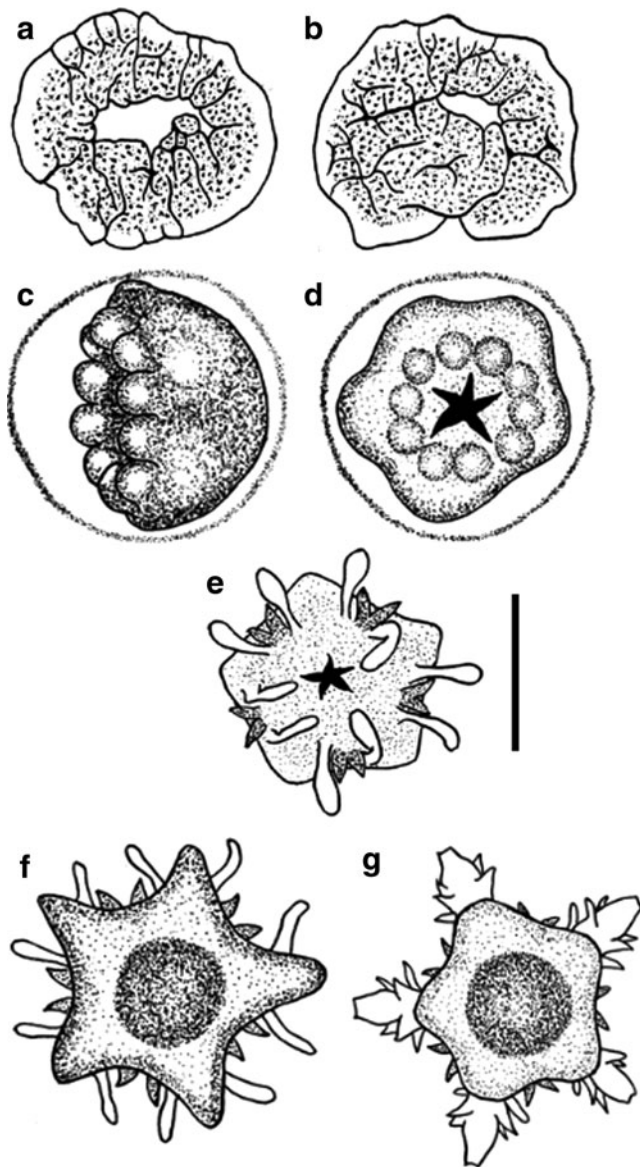


Fig. 3 Stages in the development of Kirk's brittle star. **a** Blastula. **b** Early gastrula. **c, d** Side and oral views of embryos with rudimentary podia. **e** Newly emerged juvenile. **f** 'Asterina' stage. **g** Juvenile with developing arms. Egg membrane omitted in (a) and (b). Scale = about 0.5 mm. (Redrawn from Fell 1941)

2.2 Echinoderms and hemichordates

Figure 4 shows examples of the extant classes of echinoderms and hemichordates and their larvae.

I use the ending '-omorpha' for the echinoderm classes. The more widely used ending '-oidea' properly applies to superfamilies (ICZN 1999). These classes are: Asteromorpha (starfish) with bipinnaria larvae (Fig. 4a), Ophiuromorpha (brittle stars) with ophiopluteus larvae (Fig. 4b), Echinomorpha (sea urchins) with echinopluteus larvae (Fig. 4c), Holothuromorpha (sea cucumbers) with auricularia larvae (Fig. 4d), and Crinomorpha (sea lilies)

with doliolaria larvae (Fig. 4e). A non-feeding doliolaria is the second larva of sea cucumbers, and a brachiolaria, with arms adapted for attachment, is the second larva of some starfish. The only known sea daisies (Concentricyclomorpha) (Fig. 4f) are three species of *Xyloplax*. They were originally described as a new class of echinoderms (Baker et al. 1986), but some now regard them as a subclass of starfish. They are recorded from decaying timber in deep water off New Zealand, the Bahamas, and the northeast Pacific. Adults lack alimentary systems. *X. medusiformis* has no bilateral stage in its development, and juveniles within the ovary have a vestigial gut (Rowe et al. 1988). The development of *X. turneri* and *X. janetae* is undescribed.

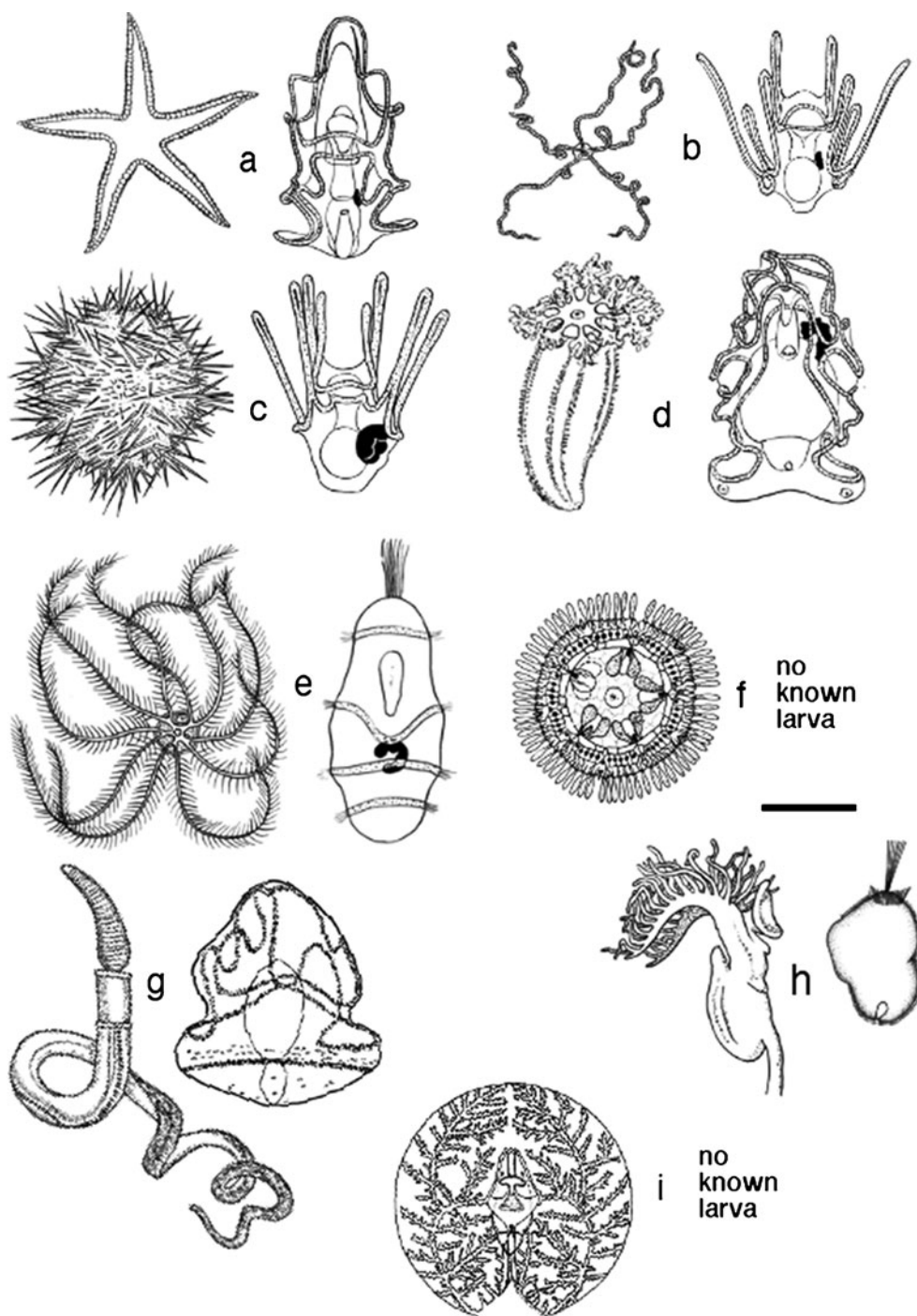
Hemichordates comprise the classes Enteropneusta (acorn worms) with tornaria larvae (Fig. 4g), Pterobranchia, some of which have yolk-filled trochophore larvae (Fig. 4h), and Planctosphaeromorpha with no known larvae (Fig. 4i). Adult enteropneusts and pterobranchs both have tripartite bodies. The anterior part in enteropneusts (Fig. 4g) is a proboscis, used in burrowing, while in pterobranchs (Fig. 4h) it is a lophophore, used in food collection. The middle part, the collar, contains a tubular outgrowth from the mouth cavity, previously thought to be a rudimentary notochord. The posterior part, the trunk, contains a perforated pharynx, with many gill slits in enteropneusts but only one pair on pterobranchs. The tornaria larva of enteropneusts (Fig. 4g), like all echinoderm larvae, is an enterocoelous deuterostome (the coelom forms from the archenteron, and the blastopore does not become the mouth). The only described larva of pterobranch hemichordates is a non-feeding trochophore (Fig. 4h) (Hyman 1959; Barnes et al. 1988). Feeding trochophores are schizocoelous protostomes, but the relevance of protostomy to the pterobranch larva is questionable. This larva, however, is clearly very different from a tornaria. The only known planctosphere (Fig. 4i) is *Planctosphaera pelagica*, and it is included in the hemichordates because of its resemblance to the tornaria larva of enteropneust hemichordates (Van der Horst 1936). It can grow to 25 mm with no sign of metamorphosis (Hart et al. 1994). I am convinced it is an adult and not a descendant of a 'persistent larva', as would be suggested by Garstang (Williamson 2003).

Sea urchins and brittle stars have similar pluteus larvae, with slender arms supported by calcareous rods, and the names echinopluteus and ophiopluteus link them to their respective adults. There are also obvious similarities between the auricularia larvae of sea cucumbers and the bipinnaria larvae of starfish (Fig. 4). As noted by Balfour (1880–1881), the doliolaria larvae of sea lilies are not closely related to other echinoderm larvae. Echinoderm larval affinities, however, are not matched by adult

Fig. 4 Echinoderms (a–f) and hemichordates (g–i): adults (*left*) and their larvae (*right*).

Developing echinoderm juveniles within larvae shown black. **a** Starfish and bipinnaria larva. **b** Brittle star and pluteus larva. **c** Sea urchin and pluteus larva. **d** Sea cucumber and auricularia larva. **e** Sea lily and doliolaria larva. **f** Sea daisy (no known larva). **g** Enteropneust and tornaria larva. **h** Pterobranch and trochophore larva. **i** Planctosphere (no known larva). Scale = about 10 cm for adults, about 1 mm for larvae.

(Adapted from Williamson 1992; Hyman 1959)



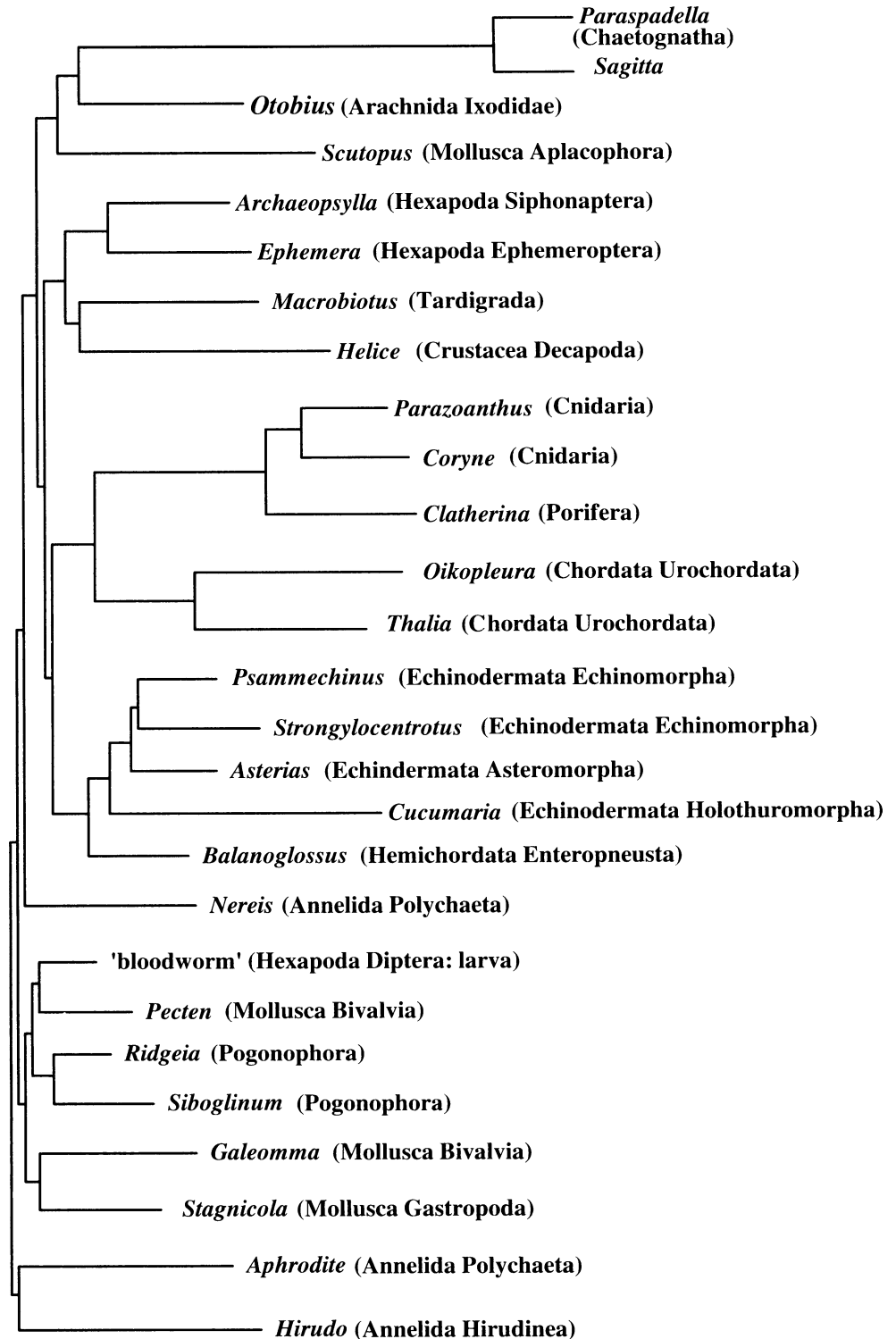
affinities. There is fossil evidence that sea lilies separated comparatively early from other echinoderms; brittle stars and starfish then evolved from one branch of the phylum, and, a little later, sea urchins and sea cucumbers evolved from another branch (Paul and Smith 1984). The lack of correlation between larval and adult affinities is consistent with the thesis that echinoderm larvae were not acquired until after the establishment of the classes of the phylum, as proposed independently by Balfour and myself.

Garstang (1894) pointed out the “remarkable similarity” between hemichordate tornaria larvae and echinoderm auricularias and bipinnarias (Fig.4). Several 20th century authors, including MacBride (1914), arranged tornaria, auricularia, bipinnaria, and pluteus larvae in sequence, implying that that was the order in which they had evolved. This sequence, however, bears no relation to the order in which the adults evolved. I claim that it is the order in which these larvae were transferred by hybridization, after

the classes of adult hemichordates and echinoderms were established, and that the adult source of the first of the series, the tornaria larva, was an ancestor of *Planctosphaera* (Williamson 1998, 2001, 2003). The same sequence, tornaria, auricularia, bipinnaria, pluteus, turned up unexpectedly in Michael Syvanen's phylogram of assorted

adult animals based on 18S ribosomal RNA (Fig. 5). This phylogram includes surprising associations, like those between a chaetognath and an arachnid and between a bivalve mollusc and an insect larva, and unexpected separations, like that between two polychaete worms. It shows, however, an enteropneust hemichordate and several

Fig. 5 Phylogram of some animals, based on 18S rRNA. (From Williamson 2002, after Michael Syvanen)



echinoderms, not in the order in which the adults evolved, but in order in which (I postulate) they acquired larvae. Of the animals investigated by Syvanen, that which showed the greatest affinity to *Balanoglossus* (enteropneust hemichordate) was *Cucumaria* (sea cucumber), followed, in order, by *Asterias* (starfish), and *Strongylocentrotus* and *Psammechinus* (sea urchins). No brittle stars or sea lilies were investigated. The 18S gene appears to have been transferred between taxa several times, not necessarily all by hybridization, and Syvanen's phylogram reinforces the conclusion of Abouheif et al. (1998) that "the 18S rRNA gene is an unsuitable candidate for reconstructing the evolutionary history of all metazoan phyla". In the case of enteropneusts and echinoderms, transfers of this ribosomal gene seem to be linked to transfers of nuclear genes that specify larval forms. This is consistent with mergers of whole genomes by hybridization.

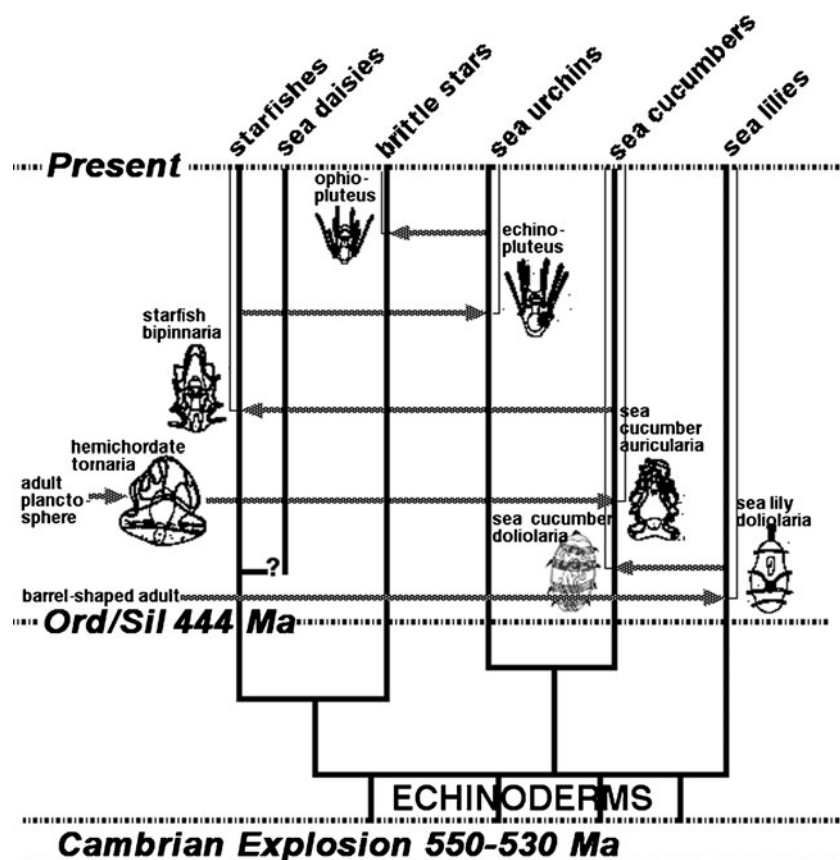
Nineteen classes of echinoderms were established by the end of the Ordovician. Most of these have not survived, but no new classes have evolved since (Paul 1979). I claim that the larval series: tornaria (of enteropneust hemichordates), auricularia (of sea cucumbers), bipinnaria (of starfish), pluteus (of sea urchins and brittle stars), is consistent with the acquisition of larvae after the evolution of the classes of hemichordates and echinoderms, but not with the assumption

that larvae and adults arose from common ancestors. The doliolaria larva of sea lilies, which is also the second larva of sea cucumbers, is not part of this series, and I suggest it was acquired when a former sea lily hybridized with a barrel-shaped adult, which is now extinct (Williamson 2006a). The sequence of evolution of the extant classes of echinoderms and acquisition of their respective larvae by hybrid transfer is shown in Fig. 6.

3 Discussion and conclusions

Symbiogenesis (Kozo-Polyansky 1924; Margulis 1970, 1993), the generation of new life forms by symbiosis, and hybridogenesis (Williamson 2003, 2006a), the generation of new life forms and new life histories by hybridization, both involve mergers of genomes of organisms at all levels of relationship. Both are saltational forms of evolution, independent of the gradual evolution by "descent with modification" described by Darwin (1859) or evolution by specific increments, as described by Eldredge and Gould (1972). All organisms, however they evolved, may be subject to natural selection. Symbiogenesis was responsible for the creation of eukaryotic cells (of protocists, plants, fungi and animals) from prokaryotes (bacteria, etc.).

Fig. 6 Sequence of events in evolution of extant classes of adult echinoderms (*thick lines*) in Cambrian and Ordovician, and subsequent acquisition of larvae (*thin lines*) by hybrid transfer (*speckled horizontal arrows*). Time (*vertical axis*) not to scale. Ord/Sil = Ordovician/Silurian boundary



Hybridogenesis was responsible for the evolution of complex animals from simple animals, for the acquisition of organs such as lophophores, and for the acquisition of larvae by many animals. A corollary of symbiogenesis is that “the functions now performed by cell organelles are thought to have evolved long before eukaryotic cells existed” (Margulis 1993). A corollary of larval transfer by hybridogenesis is that “the basic features of larvae are thought to have evolved long before animals with larvae existed” (Williamson 2003). Darwin (1859) persuaded biologists that organisms have evolved, and he proposed one method of evolution. I am among those who try to convince biologists that organisms have evolved in more than one way.

Although Haeckel’s recapitulation theory is largely discredited, Garstang’s modification, which claims that modern larvae represent ancestral larvae, is widely accepted by modern biologists. This leads to phylogenetic trees, such as Fig. 7, which group together (1) annelids and molluscs, and (2) echinoderms and chordates. Annelids and molluscs are widely regarded as trochophorates or lophotrochozoans. The vast so-called clade, Lophotrochozoa, was set up by Halanych et al. (1995) to include lophophorates and trochophorates, on evidence from 18S ribosomal RNA. As mentioned earlier, Passamanek and Halanych (2006) concluded that “the Lophophorata is not a monophyletic entity” on evidence from LSU and SSU ribosomal RNA, so invalidating the clade Lophotrochozoa. A clade is, by definition, monophyletic, but the fact that one of the founders of the Lophotrochozoa later showed it to be polyphyletic has had little effect on its widespread acceptance. Some annelids and some molluscs have trochophore larvae, but other annelids, including oligo-

chaetes (earth worms) and hirudineans (leeches), and other molluscs, including cephalopods (squids etc.), have no larvae or any indication in their embryonic development that they ever had larvae. I claim that larvae were later additions to the life histories of all indirect developers, transferred by hybridization. It is no coincidence that direct developing earthworms, leeches and squids mate to breed, and eggs are fertilized within the female’s body. The possibility of heterosperm fertilization in such animals is remote. On the other hand, polychaete worms and bivalve and gastropod molluscs with indirect development release their gametes into the sea, where fertilization takes place, and occasional heterosperm fertilizations are not unlikely. I maintain that the similar trochophore larvae of some annelids and some molluscs were acquired after the classes of these phyla were established, and they are not evidence that annelids and molluscs evolved from a common ancestor (Williamson 1992, 2003). No annelids or molluscs have lophophores.

Figure 7 also links echinoderms and hemichordates as deuterostomes. In the previous section I point out that some direct-developing echinoderms are protostomes, which would place them on the other main branch of the tree, and also that pterobranch hemichordates are ‘lophotrochozoans’, which would also place them on the other main branch. I argue that the similar larvae of echinoderms and enteropneust hemichordates were later additions to the phylogenies of these animals, and they are not evidence of descent of adult echinoderms and hemichordates from a common ancestor. This absurd classification is a logical deduction from the hypothesis that larvae and adults gradually evolved from common ancestors. If a hypothesis leads to absurdity, it should be abandoned, and hybridogenesis is a reasonable alternative hypothesis. I claim that, during the Cambrian explosion, many early simple animals hybridized. (Modern rotifers may be descendants of one such group of early simple animals.) The products of these crosses were not animals with larvae (sequential chimeras) but more complex animals (concurrent chimeras), and most modern animal phyla evolved from survivors of this process. The majority of animal phyla are thus of multiple origin, and their histories and relationships cannot be depicted in a simple tree-like diagram. Animals in some classes of some of these phyla later acquired larvae by hybrid transfer (Williamson 2006a).

I hope that geneticists will test my hypothesis by comparing the genomes of related animals with and without larvae. The simplest tests will compare numbers of base-pairs of functional genes, with due allowance for polyploidy, polyteny, and other repetitions of gene sequences. Recent articles by Hart and Grosberg (2009) and Minelli (2009) claim that genome sizes undermine my thesis that caterpillars evolved from onychophorans by hybridogenesis

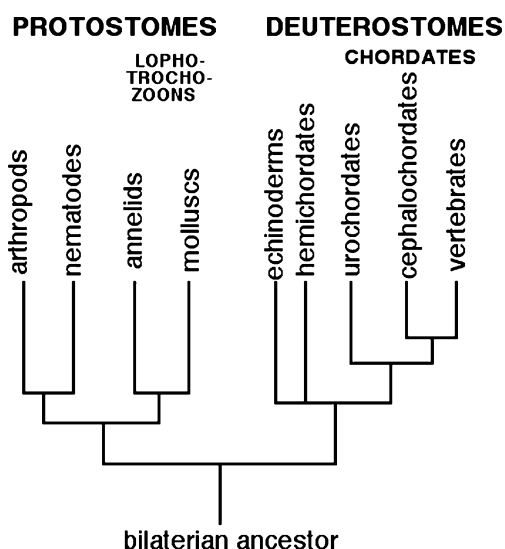


Fig. 7 Common misconception of relationships between animal phyla. (Redrawn and adapted from Dehal et al. 2002)

(Williamson 2009). Both of these critical articles, however, are based on C-values of DNA (weights in picograms) (Gregory 2009), but these weights lump together coding and non-coding DNA and include repetitive sequences. Most of the DNA of eukaryotes is non-coding: about 95% of the human genome consists of non-coding DNA, and the percentage in many animals is even greater. The unicellular *Polychaos dubium* contains more than 200 times as much DNA per nucleus as a human cell. Different species of *Drosophila* should have very similar evolutionary histories, but their C-values range from 0.12–0.41 pg, the largest being more than three times the smallest. Unmodified C-values show no obvious correlation with numbers of functional genes. In some cases, however, many of an animal's functional genes may be of bacterial origin and not related to the production of proteins that give the animal shape and form. Nikoh and Nakabachi (2009) present evidence that aphids have acquired many genes from bacteria by horizontal transfer. These genes are used to maintain symbiotic bacteria, not to produce and maintain animal protein. A proper study must be based on the protein-sequence level, where functional enzyme active sites, insertions and deletions, and other clues to relevant genotype-phenotype developmental data in a biological context are compared.

I urge geneticists to obtain draft genomes of the following pairs of animals and to make comparisons between them: (1) Kirk's brittle star and a brittle star with larvae, (2) a direct-developing heart urchin, such as *Abata cordatus*, and a heart urchin with larvae, (3) a direct-developing sea daisy and a starfish with larvae, (4) a cephalopod and a gastropod mollusc with larvae, (5) an earthworm and a polychaete worm with larvae, and (6) an enteropneust hemichordate and a pterobranch hemichordate. Due account must be taken of repetitive sequences and other factors outlined in the previous paragraph. If, however, any of these comparisons of genomes showed no significant difference, it would rock my hypothesis that links larvae to foreign genomes. If there were no significant difference in all cases, it would sink the hypothesis. I am confident that my hypothesis will survive such tests, but, if it is rejected, a new hypothesis will be needed to justify many unexplained developmental anomalies. These include 'overlapping metamorphosis' (as in the starfish *Luidia sarsi* (Fig. 5)), 'start-again metamorphosis' (as in bryozoans), multiple larvae (as in penaeid shrimps), incongruous larvae (as in dromiid crabs and hermit crabs), and recently evolved larvae (as in the hydroid genus *Hebella*), all discussed in Williamson (2006a).

I also exhort biologists to produce more experimental hybrids. Unpublished work by Nicander Boerboom and myself indicates that pre-treatment of sea urchin eggs for 40 s with acetic acid seawater (pH 5) (Raff et al. 1999)

before exposure to dilute ascidian sperm can lead to a high percentage hatch of healthy hybrid larvae. I call for more laboratory hybrids between members of these taxa to compare with my results (Williamson 1992, 2003), for crosses involving other phyla, including species with and without lophophores, and for investigations of the chromosomes and genes of experimental hybrids.

Comparatively recently, several authors, including Jenner (2000); Sly et al. (2003); Peterson (2005), and Page (2009), without reference to Balfour or myself, have discussed the possibility that some larvae might have been 'secondarily acquired' and could have been 'intercalated' into life histories. I claim that all larvae were secondarily acquired and were intercalated into life histories, and acceptance of this could resolve most of the developmental incongruities discussed in the *Biological Bulletin* Virtual Symposium: Biology of Marine Invertebrate Larvae (Emlet et al. 2009). All contributors to this symposium also ignore Balfour and myself.

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