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The Origins of Larvae

Mismatches between the forms of adult animals and their larvae may reflect fused genomes, expressed in sequence in complex life histories

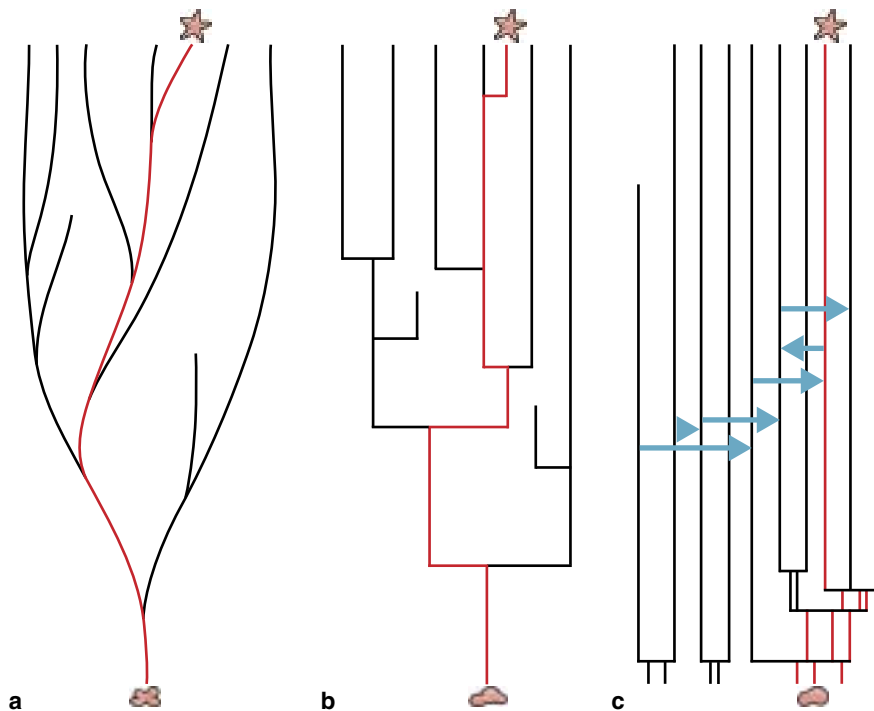Donald I. Williamson
and Sonya E. Vickers

Since the earliest days of the field—when a young Charles Darwin worked on his beloved barnacles, shelled shrimplike creatures, cemented to rocks, which lie on their backs and kick food into their mouths—evolutionary biologists have been fascinated by life's myriad odd forms. The rigorous naturalist confronted with unexplained peculiarities of form, life history or behavior is compelled to search doggedly for a scientific explanation. Just such a search some 150 years ago gave the world *The Origin of Species*, which remains the foundational scientific explanation of how plants and animals have changed through time. More animals are hatched or born than possibly survive to have offspring in their environment, Darwin wrote. Thus pressured to adapt, populations change gradually through time; they "descend with modification." Darwin emphasized the gradual nature of change in living form that today we call evolution: the accumulation of changes, or mutations, through heredity.

Not all modern theorists have accepted that change is gradual. Indeed, nature presents forms that can only be explained by *sudden* change. One of us (Williamson) has focused on such a case: larvae, the distinctive young forms of many animals. These forms can differ so markedly from the adults into which they develop that an observer is tempted to classify them as different species. And such an observer, we argue, might be right in a way. Williamson's "larval transfer" hypothesis proposes that larvae, and the genes that specify them, have been transferred from one hereditary animal lineage to another by cross-species, cross-genera and even cross-phyla fertilizations. We feel compelled to ask a question that is obvious to those

D. P. Wilson / FLPA

Figure 1. *Luidia sarsi*, **a starfish found in the North Sea and along the southern and western shores of the British Isles, develops from a fertilized egg into a larva with bilateral symmetry, inside which grows a juvenile form with the radial symmetry of the starfish. In this unusual life history, the juvenile migrates to the outside and drops off the swimming larva (the translucent shape in this photograph); both continue to live independently for up to three months. Author Williamson's larval-transfer hypothesis explains this oddity as the expression of genomes fused when two ancestral marine animals hybridized, one becoming the larva of the other. This argument is not only a radical proposal for solving open questions in the evolution of development but also one of several proposals that suggests a bushier shape to the "tree of life" that Charles Darwin proposed.**

Figure 2. From Darwin forward, classical theory has viewed life's evolutionary history as taking place through continuing adaptation and mutation. Darwin's tree of life may be seen as a smoothly branching tree in which each bifurcation indicates common ancestry, and truncated branches indicate extinctions (a). In 1972, Niles Eldredge and Stephen Jay Gould, noting spurts of activity in the fossil record, introduced the concept of "punctuated equilibrium," pointing out that species appear fully formed (b). Williamson's larval-transfer theory introduces another wrinkle: the notion that one animal can become the larva of another, even distantly related animal by hybridization, thus establishing a connection between two branches of the tree of life (c). The diagrams trace the ancestry of a hypothetical starfish (red), one of several species that may have acquired larvae through hybridization (blue arrows). (Adapted from a diagram by Sonya E. Vickers.)

not trapped in conventional evolutionary thinking: Could animals with larval forms be hybrids, the products of successful fusions of genomes that are expressed in sequence during the animal's life history?

Larvae, familiar as immature stages in many animal life histories, are especially common in marine plankton. The caterpillar larva that spins the chrysalis from which an adult butterfly emerges lives on land, but most dramatic larval-adult transformation takes place in the sea. Clams, starfish and sea urchins cast their eggs and sperm into the sea, where they merge in fertilization. The larval-transfer hypothesis proposes that all larvae transferred into their present-day lineages from other distantly related animal groups by cross-fertilization.

In 21st-century scientific language, we would say that a portion of one animal's complement of genetic material, or genome, was acquired by another, creating a chimeric organism.

Following Darwin, most biologists today assume that a larva and its adult began as a single individual and that over time, the young gradually became more and more different from their adult forms. This can be labeled the "same stock" or "direct filiation" assumption. Until recently, there was no alternative theory and no need to defend the presumption. There were unexplained anomalies, but these were regarded as mere curiosities. Conventional thinking offers convergent evolution as the dominant explanation for the similarities of many larvae, speculating that many

organisms came to the same solution to problems such as dispersal and feeding.

In this article we will tell a different version of the origin of larvae, distinct from the "same stock" concept but consistent with Darwin's theory of natural selection:

Larva and adult began as different animals, each developing from its own type of egg. At a point in their evolutionary past, their ancestors interbred and produced offspring. Most hybrid offspring did not survive. Genomes so unlike each other had many difficulties in expression—the translation of genes into proteins. The few hybrids that did survive solved the dilemma by expressing their combined genomes sequentially rather than concurrently, first the larval genes and then the adult. The new combined animal survived and went on to reproduce specialized forms, usually highly advantageous in the procurement of habitat and food, then spread their genes as adults. The legacy left by the patching together of dissimilar ancestral lines is the perilous transition we call metamorphosis, the stage at which the larva transforms into the adult. The outcome is frequently not transformation but death.

The Tangled Tree of Life

Darwin's *Origin* gave science a powerful metaphor: the tree of life. The Darwinian view of life's history is a tree whose central trunk is rooted in common ancestry. The tree's branches—main limbs from which further branches diverge, each bifurcation indicating common ancestry—represent the diversity of life that arose over time. Most branches fail to reach the top (the present), as more than 99 percent of all past life on Earth is estimated to belong to extinct species.

A new concept, "punctuated equilibrium," was introduced by Niles Eldredge and Stephen Jay Gould in 1972 to replace Darwin's gradually branching tree. Eldredge and Gould pointed out that the fossil record contradicts the "numerous, successive, slight modifications" described by Darwin. "A species does not arise gradually by the steady transformation of its ancestors," they argued; rather, "it appears all at once and fully formed." The concept of punctuated equilibrium explains the fossil record by spurts of activity followed by stasis. The Eldredge-Gould replacement for Darwin's tree looks like a candelabra.

Until he retired in 1997, Donald I. Williamson was Reader in Marine Biology at the University of Liverpool in the United Kingdom. He specialized in marine plankton, particularly marine larvae. Most of his publications are on crustacean larvae. He is the author of Larvae and Evolution: Toward a New Zoology *(Chapman and Hall, 1992) and* The Origins of Larvae *(Kluwer, 2003). Sonya E. Vickers is a teacher and naturalist. She has traveled extensively within the U.S. and around the world to sites of natural interest. Address for Williamson: 14 Pairk Beg, Port Erin, Isle of Man IM9 6NH, UK. Internet: diwilliamson@manx.net*

Larval transfer is one of a number of phenomena that imply a bushier sort of tree, one whose branches occasionally fuse. Although less tidy, the concept is not new. In sexual animals, fertilization routinely fuses genomes, usually those of members of the same species. But genome fusion can even take place between biological kingdoms, as in the formation of the composite life forms called lichens. These gray-green patches on rocks and trees represent fusions between a green alga in the protist kingdom (or often a cyanobacterium in the prokaryote kingdom) with specific ascomycetes in the fungus kingdom. And as this article was going to press, the genome of the fruit fly *Drosophila ananassae* was found to have encapsulated within it the entire genome of the bacterium *Wolbachia,* each generation of insect inheriting the parasite's genome from its parents. The finding, as W. Fred Doolittle of Dalhousie University put it, "establishes the widespread occurrence and high frequency of a process that we would have dismissed as science fiction until just a few years ago"—lateral gene transfers involving higher organisms.
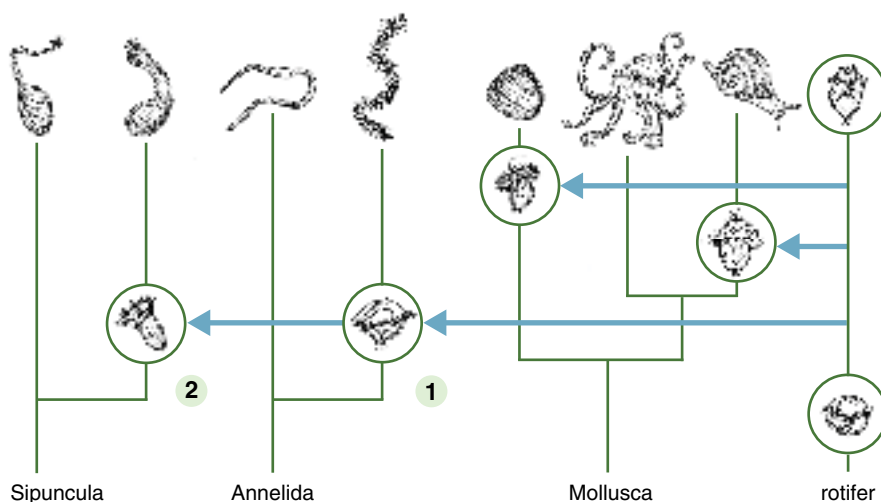
Elsewhere *Geosiphon pyriforme* adults form by the fusion of a fungus with *Nostoc,* a cyanobacterium, every six to eight weeks to form an organism that looks like a small bulbous moss plant. And Lynn Margulis of the University of Massachusetts at Amherst has documented perhaps the ultimate genome fusion: when bacteria that respired atmospheric oxygen merged, perhaps two billion years ago, to produce aerobic protists that permanently contain mitochondria. The novelty of the larval-transfer hypothesis is not the permanent merger of two different genomes, but the fact that each oversees a separate portion of the animal's life history. A complete "changing of the guard" takes place during metamorphosis.

If an adult animal and its larva are a chimera evolved from the fusion of two very different animals, the resulting pattern of life's history should be depicted more like a network than a tree. The larval-transfer hypothesis also provides one possible mechanism for Eldredge and Gould's punctuated equilibrium.

The Larval-Transfer Hypothesis
Williamson recalls the origin of the hypothesis this way:

It all began with sponge crabs and hermit crabs. I first studied hermit crabs, so called because they tuck their abdo-

Figure 3. Rotifers have simple life histories, but these small marine and freshwater animals may have contributed a larval stage to the life histories of other animals, explaining the scattering of so-called trochophore larvae through unrelated phyla. In the above scenario, reading forward in evolutionary time from the bottom, a polychaete worm hybridized with a rotifer, acquiring a trochophore larva (1); this part of the polychaete's genome was acquired by a sipunculan worm in a second hybridization (2). Further hybridizations with rotifers gave trochophore larvae to the ancestors of today's clam-like and snail-like mollusks. Their close relatives, the octopuses and squid, lack larvae. In conventional thinking, larval forms arose over time as young and adult forms within a species became more and more different. The similarities among larvae in distantly related species are thus conventionally explained by convergent evolution—many organisms developing larval stages to solve problems such as dispersal and feeding. (Adapted from diagram by Sonya E. Vickers.)

mens into snail shells, which they carry around with them. (They are not true crabs.) I compared them with sponge crabs, true crabs that carry pieces of sponge on their backs. These adult animals are very different from each other, hardly related at all. The larvae of the two groups, however, look like mysid shrimps and are strikingly similar. It was as if sponge crabs had acquired shrimp-like larvae from hermit crabs—an unexplained curiosity and, according to the thinking of the time, an impossibility.

How, I wondered, could a familiar shrimp metamorphose into two different types of crabs? I began to notice other anomalies. Radially symmetrical starfish and bilaterally symmetrical acorn worms also have very similar larvae. The conventional explanation, advanced by Ernst Haeckel in 1866, imagined a common ancestor with bilateral symmetry. Ancestral starfish, the story goes, anchored themselves to a substratum and gradually developed a radial symmetry more efficient for fixed forms, while the free-floating larvae retained the primitive symmetry. I questioned this far-fetched tale and the conventional wisdom that it was impossible to transfer larvae or their genetic recipes. I eventually decided that cross-fertilization, or hybridization, was the method of transfer.

It gradually became apparent to me that the hypothesis could be applied to all larvae, and that all larvae have (or once had) an adult counterpart—an animal that does not metamorphose. The whole genome of this animal is transferred, but the hybrid uses only part.

The Caterpillar and the Snail
Imagine taking a larva's view of the organization of life. To us landlubbers, the most familiar larvae are the caterpillars of lepidopterans—butterflies and moths—so we can begin there. Caterpillars are larvae with three pairs of legs extending from the thorax and a variable number of small extra legs, or prolegs, attached to the abdomen. An expandable "eating machine," a caterpillar can only crawl, never fly.

Caterpillar larvae, however, are not confined to the order Lepidoptera. They also occur in scorpionflies in the order Mecoptera and in woodwasps and sawflies in Hymenoptera. Other hymenopterans, including ants, bees and wasps, have legless grubs as larvae.

If you were to classify the types of insect larvae, in fact, you would come up with a pattern quite independent of the classification of the adults. Although this pattern is problematic for the theorist looking for a common an-

Figure 4. Echinoderms are radially symmetrical marine animals, including starfish, sea urchins and sea cucumbers. Although the radial symmetry of adults is traditionally thought to have evolved in response to their sedentary lifestyle, the evidence is mixed. Radial adults can be found deep in the fossil record; adult sea cucumbers show a mix of bilateral and radial features, and their auricularia larvae (left) more closely resemble the tornaria larvae of creatures such as the acorn worm (right) than the larvae of other echinoderms. Williamson proposes that the acorn worm acquired its larva by hybridization with an ancient planctosphere, then hybridized with a sea cucumber.

cestor, it is explicable if larvae were later additions to life histories.

Williamson's larval-transfer hypothesis holds that the original caterpillar larvae were transferred from adults resembling present-day velvet worms of the genus *Peripatus*. This is the adult counterpart for all caterpillar larvae. This worm lives in the organic-rich soils of tropical America, South Africa and Australia. These worms-with-legs thus belong to the phylum Onychophora, entirely separate from insects or even earthworms. When hybridization between different insects and velvet worms took place, the surviving chimera enjoyed the best of both worlds: a larval form specialized for feeding and a flying adult adept at spreading its genes. Both onychophorans and flying insects have survived rigorous natural selection for millions of years, yet hybridization generated the novelty that neither could manage alone.

Broadening one's focus from caterpillars to look at other larval types upends conventional views of life's organization. The Mollusca (mollusks) are a large phylum that includes clams, snails, octopuses and squid. Annelids are another large phylum of segmented worms that includes polychaetes and earthworms. Members of these two major groups are as distantly related as are rabbits and butterflies.

Within these groups one sees a curious distribution of larval forms. Most clams and sea snails develop from small, translucent *trochophore* larvae, characterized by one or more bands of hairlike appendages (cilia) and sharing no morphological traits with the adults into which they grow. Octopuses and squids, also Mollusca, entirely lack larvae.

Many polychaete worms have trochophore larvae similar to those of clams and snails. Yet in the same phylum, earthworms have no larvae. Some but not all members of several lesser-known marine phyla, including some sipunculan worms (the "peanut worms"), also have trochophore larvae. The conventional explanation of the link between these phyla is that they all descended from ancestors with trochophore larvae. Following this logic, groups such as octopuses, earthworms and some sipunculans evolved by *loss* of larvae.

The larval-transfer hypothesis implies, then, that similar larvae will turn up in the life histories of distantly related and very different animals—and that closely related animals will have quite different larvae or diverging life histories because only some acquired larvae. It also implies that there must be adult forms similar to larvae.

And indeed, in addition to the velvet worm mentioned above, there are.

To take another example, the rotifers or "wheel animalcules" are a phylum of small marine and freshwater animals that have cilia and a simple life cycle. *Trochosphaera* is a rotifer with a marked resemblance to trochophore larvae. We are convinced that clams, snails and polychaete worms and some sipunculans acquired trochophore larvae by hybridizing with rotifers. No octopuses, squids, earthworms or other sipunculans ever hybridized.

Paired with natural selection, the hypothesis allows the distribution of larvae to be explained by the occurrence or nonoccurrence of hybridization, but also by the loss of larvae in species that once hybridized. Some sea snails and polychaete worms that lack larvae but are closely related to species with larvae have probably lost their larvae. The pattern of cell division within the egg in these animals is similar to that in species with larvae. The pattern of cell division in octopuses, squids and earthworms is quite different, in line with the view that these animals never had larvae.

The competing explanation under the same-stock theory is that the adult rotifers are "persistent larvae"— descendants of forms that matured in the larval state. Were this so, we should expect the genes of the lost adult to be preserved as the "junk DNA" found in many genomes; but in fact rotifers have remarkably few junk genes.

Stars on the Bottom of the Sea

The echinoderms are a phylum of radially symmetrical animals that includes starfish, brittle stars, feather stars, sea urchins and sea cucumbers. Echinoderm larvae have a symmetry different from adults. Not only are they bilaterally symmetrical, but with their mixture of convoluted and straight bands of cilia, some also resemble the *tornaria* larvae found in the Hemichordata, the phylum of acorn worms. The widely accepted explanation of this anomaly, the one advanced by Haeckel, states that the bilateral larvae of echinoderms and hemichordates evolved from a common ancestor with tornaria larvae. An ancestor of modern echinoderms evolved radial symmetry, he claimed, in response to sedentary life.

Fossils and larvae described since Haeckel do not support his views on the origins of echinoderms. Radial echinoderm adults can be found in the fossil record going back at least 540 million years. Some hemichordate larvae

resemble trochophores, not tornarias. Of all echinoderm larvae, the sea cucumber larva most closely resembles a tornaria. Adult sea cucumbers show a mixture of bilateral and radial features.

A Haeckelian interpretation suggests that sea cucumbers are the nearest living echinoderms to the ancestral form. Paleontology, however, tells us that sea cucumbers evolved comparatively late in echinoderm phylogeny. Williamson proposes, then, that larvae were later additions to this branch on the tangled tree of life. The adult from which echinoderm tornaria larvae evolved, in this view, is *Planctosphaera pelagica*. This is the only known planctosphere—a spherical planktonic animal (hence the name) that is up to 25 millimeters in diameter and propelled by convoluted bands of cilia.

This animal is classified as a hemichordate because of its resemblance to a tornaria larva, but we regard it as an adult member of the group that gave rise to tornaria larvae by hybrid transfer. That is, an ancestor of *Planctosphaera* hybridized with an acorn worm to produce an acorn worm with a tornaria larva. This type of larva was then spread by cross-fertilization between an acorn worm and a sea cucumber. Further hybridizations between sea cucumbers and starfish, starfish and sea urchins, and sea urchins and brittle stars, would explain the larval forms found among these echinoderms.

The doliolaria larva of one group of echinoderm, the feather stars, cannot be traced back to a planctosphere. And such connections are not necessary with larval transfer, which suggests that the evolutionary dispersal of tornaria-like larvae should be independent of adult echinoderm evolution.

Testing the Hypothesis

As Michael W. Hart of Simon Fraser University pointed out in 1996, the larval-transfer hypothesis is so heretical that a single positive example would bolster it. As a test, Williamson devised an experiment aimed at inducing hybridization in the laboratory. In 1990, he fertilized eggs of a sea squirt (a urochordate), *Ascidia mentula,* with sperm of a sea urchin, the echinoderm *Echinus esculentus.* The results were reported in Williamson's 1992 book *Larvae and Evolution: Toward a New Zoology.*

What happened? Although, as expected, the *Ascidia* eggs failed to divide in the majority of such experiments—

the hypothesis views successful hybridization as a very rare event—in one experiment more than 3,000 eggs hatched and produced *pluteus* larvae, the typical larval shape of the paternal sea urchin, whose slender arms are supported by calcareous rods. Well over 90 percent of these resorbed their pluteal arms to become spheroids, which lived for more than a month but did not develop further. The other 7 to 9 percent of the plutei metamorphosed into sea urchins, four of which survived for more than a year. Two survivors had the fivefold radial symmetry of a typical sea urchin, but the other two displayed fourfold symmetry. The three largest survived beyond four years until the seawater circulation failed in the laboratory, all of them producing eggs in their later life.

Hart, reviewing this evidence later, proposed that if the survivors were hybrids, genetic analysis should detect mitochondrial DNA from the female parent, the sea squirt *A. mentula*, as well as nuclear DNA from both parents. Three years after the death of the larvae, he extracted DNA from frozen tube feet of the three surviving urchins for comparison with the DNA of wild individuals of the two species involved.

Hart's comparison of aligned nucleotide sequences and a portion of the nuclear 28S ribosomal RNA gene did not turn up evidence of sea-squirt genetic material in the survivors of the experiment. Hart speculated that the experiment might have been contaminated with sea-urchin eggs, despite the precautions taken. Along with Richard Strathmann of the University of Washington, he wondered whether there might have been hermaphrodites—known to be rare—in the sea-urchin population used. Williamson totally rejects these explanations and gave his reasons in his 2003 book, *The Origins of Larvae.*

Although the search for sea-squirt DNA in Williamson's tissue samples was unsuccessful, molecular-biological evidence for larval transfer is now appearing. The Canadian zoologist Ernest W. MacBride, who in 1914 tackled the problem of echinoderm forms, recognized a sequence in the appearance of larvae in the evolutionary record, from tornaria (acorn worms), to auricularia (sea cucumbers), to bipinnaria (starfish) and finally to pluteus (sea urchins and brittle stars). This same sequence, consistent with larval transfer, has now appeared in the distribution of a ribosomal gene.
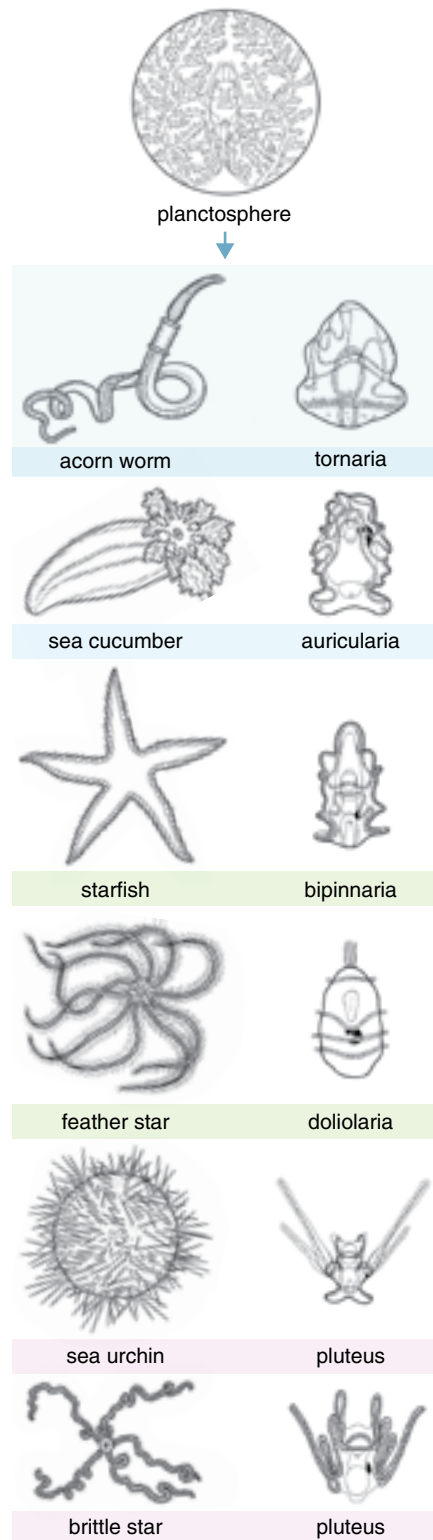

planctosphere

acorn worm | tornaria

sea cucumber | auricularia

starfish | bipinnaria

feather star | doliolaria

sea urchin | pluteus

brittle star | pluteus

Figure 5. Dissimilarities between adults (*left*) **and larvae** (*right*) **of the acorn worm (a hemichordate) and the echinoderms are striking. All the larvae shown may have been acquired through a series of hybridizations that began when the acorn worm acquired an ancient planctosphere** (*top*). **In this drawing adults are reduced in size and larvae enlarged. Planctosphere and tornaria redrawn from Barnes** *et al.* **2001. All others redrawn from Williamson 2003, with permission of Springer Science and Business Media.**

Figure 6. In a laboratory experiment, Williamson fertilized eggs of a sea squirt, *Ascidia mentula,* with sperm of a sea urchin, *Echinus esculentus.* Surviving three-year-old sea urchins from this experiment—two showing fivefold radial symmetry typical of a sea urchin, but the other two displaying fourfold symmetry—are shown in a 1993 photograph. At right is a pluteus larva from the experiment, photographed at one month. (Photographs courtesy of the author, republished by permission of Springer Science and Business Media.)

Ribosomal RNA genes are the most conserved genetic material across all of life's kingdoms, and they have come to be used in building "molecular phylogenies" that trace lineages and estimate the rates at which new species diverge. Sometimes the diagrams that emerge are different from those based on other observations, such as fossils, life histories or the body shapes of adult organisms.

Michael Syvanen, currently a medical microbiologist at the University of California, Davis, has been analyzing large-scale patterns in the 18S ribosomal RNA gene for more than two decades. Geneticists have deposited about 4,500 18S sequences for multicellular animals into a gene database, and Syvanen has shared with Williamson some of the phylogenetic patterns seen in his analysis. A cladogram, or phylogenetic diagram, derived from the 18S sequences shows the same time series of emergence: acorn worm, sea cucumber, starfish, sea urchin. Although this order of appearance is the same shown by the larvae of these groups, it bears no relation to the evolutionary history of the *adults* told by other evidence.

The larval-transfer hypothesis explains this pattern simply: The 18S ribosomal RNA has been transferred several times between taxa. In the case of hemichordates and echinoderms, it appears to have been transferred at the same time as genes specifying larval form. Hybridization is the most plausible method for the simultaneous transfer of these ribosomal and other nuclear genes. Although the hypothesis was founded on

animal morphology, it is also compatible with this molecular evidence.

Syvanen's RNA analysis, it should be added, provides genetic evidence of "widespread parallelisms" across major lineages of higher organisms. Parallel patterns can often be interpreted as convergent evolution; dolphins and fish, for example, both have body plans suited for swimming. But Syvanen's work strongly suggests that genes are in fact transferred across great taxonomic distances in multicellular plants and animals. Larval transfer is probably one of several mechanisms of genome fusion that will eventually be discovered.

The Star of the Larval-Transfer Story
Once you start taking phylogenetic diagrams apart and looking at them from the larva's viewpoint, other anomalies in echinoderm development begin to find explanation. Brittle stars and sea urchins are very different as adults but share the unique pluteus form of larva. Why would similar larvae produce dissimilar adults? Larval transfer proposes that the basic pluteus larva evolved only once, in a sea urchin, and was retained in this sea urchin's descendants. An ancestor of most existing brittle stars then acquired a pluteus larva by hybridizing with a sea urchin.

One member of this group, Kirk's brittle star, develops directly from the fertilized egg, with no trace of a bilateral larva, and its blastopore becomes a mouth. It is, therefore, a *protostome*. All echinoderm larvae are in a different developmental class, the *deuterostomes*, in which the mouth is

a new opening; several echinoderms that have lost their larvae develop as deuterostomes. If Kirk's brittle star lost its larva, it must have also adopted a fundamentally different pattern of cell division in the embryo. We believe, however, that Kirk's brittle star has no larva because none of its ancestors hybridized with a sea urchin. The heart urchin *Abatus cordatus* and the three known species of sea daisies also have no larvae and develop as protostomes. We suggest that this is the ancestral method of echinoderm development, and the pattern called deuterostomy came with the transferred larvae.

Two sea urchins, *Lytechinus variegatus* and *Lytechinus verruculatus*, are of the same genus, but the similar adults each develop from very different pluteus larvae. Such cases are difficult to explain if larvae and their corresponding adults evolved from one common ancestor. They are explainable, however, if the larvae were acquired by hybridization, and the two similar adults hybridized comparatively recently with different species.

Perhaps the strangest anomaly is the starfish *Luidia sarsi*, which decorates the cover of Williamson's 2003 book. As in other starfish, the fertilized egg develops into a bilateral larva with a small radial juvenile inside. The juvenile then migrates to the outside of the larva. In most starfish the larva would then settle and degenerate, leaving the juvenile to crawl away. In *L. sarsi*, however, the juvenile drops off the swimming larva, and both continue to live independently for months.

These two very unlike organisms are the same individual, hatched from the same fertilized egg! How can a single individual split into dissimilar animals, both having the same genome? According to the larval-transfer hypothesis, the coexisting swimming larva and crawling starfish of *Luidia sarsi* evolved by the fusion of genomes of two animals that hybridized to produce one animal with a larva. The two genomes, however, have retained a considerable degree of independence, and both are expressed together during development. This is an example of overlapping metamorphosis, which also takes place in other echinoderms, in doliolid salps and in polychaete worms, although the overlap does not last as long as in *Luidia sarsi*. Because the larva and juvenile exist side by side, the larva does not "develop into" the juvenile, as would be expected if adults and larvae had evolved from a common ancestor.

Other Views on Larval Evolution

If you haven't tuned in to debates on the evolution-of-development ("evo-devo") frontier lately, you might expect that the major questions about larval evolution have accepted answers. In fact the larval-transfer hypothesis is one of several proposals in an area of biology where lively debate may continue for some time.

As mentioned above, most literature on the evolution of animals with larvae assumes that an organism started out without a larval stage, then through evolution developed one as a means of survival and propagation. For example, the nematode *Caenorhabditis elegans* is capable of producing a slightly different body plan if stressed during development. It will revert to the normal body plan when the stress is removed. Genes have been located for this divergent body plan, and it has been hypothesized by Birgit Gerisch and her colleagues at the Max Planck Institute for Molecular Genetics in Berlin and by John Wang and Stuart K. Kim at Stanford University that continued environmental changes might cause the two body plans to diverge so much that one eventually becomes the larva that will later develop into the adult body plan. Such plasticity reminds us of how flexible animal genomes can be, and how much diversity is possible.

The most common explanation for the similarities among larvae of such different phyla as mollusks and annelids is convergent evolution: The similar larvae of diverse groups look similar because they face the same survival tasks. But there is no perfect planktonic shape to converge to. Marine plankton take on an amazing diversity of shapes, all apparently adapted to the environment. One wonders how *Luidia sarsi*, the little starfish that separates from its larva and both continue to live, fits the paradigm of a single organism evolving a larva. This starfish seems to proclaim its double parentage for all to see.

Another hypothesis might be called a larvae-came-first proposal. (It is more commonly called a "larva-like ancestor" hypothesis because it is not accepted that larvae can be acquired through hybridization.) This proposal, from Kevin J. Peterson, Eric H. Davidson and colleagues at the California Institute of Technology, arises from the fact that at the cellular level larvae can be seen to be constructed of larval-only cells (limited in their ability to divide and unable to differentiate) and patches of distinct "set-aside cells" that will create the rudiment of the adult body. In what is called indirect development—a life history that essentially jumps the track to pass through a larval stage—the larva provides a life-support system for the rudiment, which develops within the larva or appended to it. After metamorphosis the larva-specific structures are lost.

Examining patterns of set-aside cells and genes across several phyla, the Caltech group concluded in 1997 that larval forms are widely homologous—evolutionarily closely related—across several phyla, representing regulatory programs for development that are still being used by their modern descendants. Applying conventional evolutionary logic, their analysis suggests that larval forms were part of the primitive ancestry of creatures from
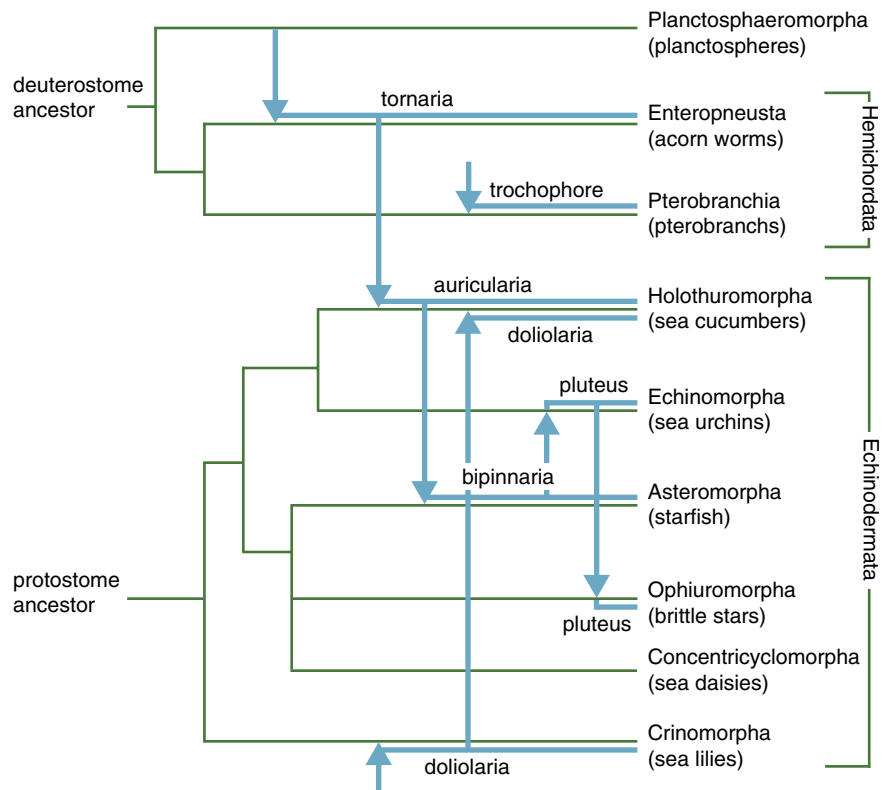

Figure 7. Although a test for genetic evidence of hybridization from the experiment in Figure 6 did not find telltale sequences of sea-squirt DNA, more general molecular evidence supporting a sequence of larval transfers across hemichordate and echinoderm species comes from Michael Syvanen's analysis of large-scale patterns in 18S ribosomal RNA, which provides a way to trace lineages and estimate divergence. The resulting cladogram, or tree of hypothetical relationships, indicates the same order of species appearance that Williamson's proposed larval transfers, shown by blue arrows in this diagram, would suggest. This can be explained if the 18S ribosomal RNA was transferred at the same time as genes specifying larval form. More generally, Syvanen's work strongly suggests that genes are transferred across large taxonomic distances in higher animals. Protostomes and deuterostomes are groups of organisms classified by their developmental pattern. In the former the larval blastopore becomes the animal's mouth; in the latter the mouth is a new opening. (Adapted from diagram by Donald I. Williamson.)
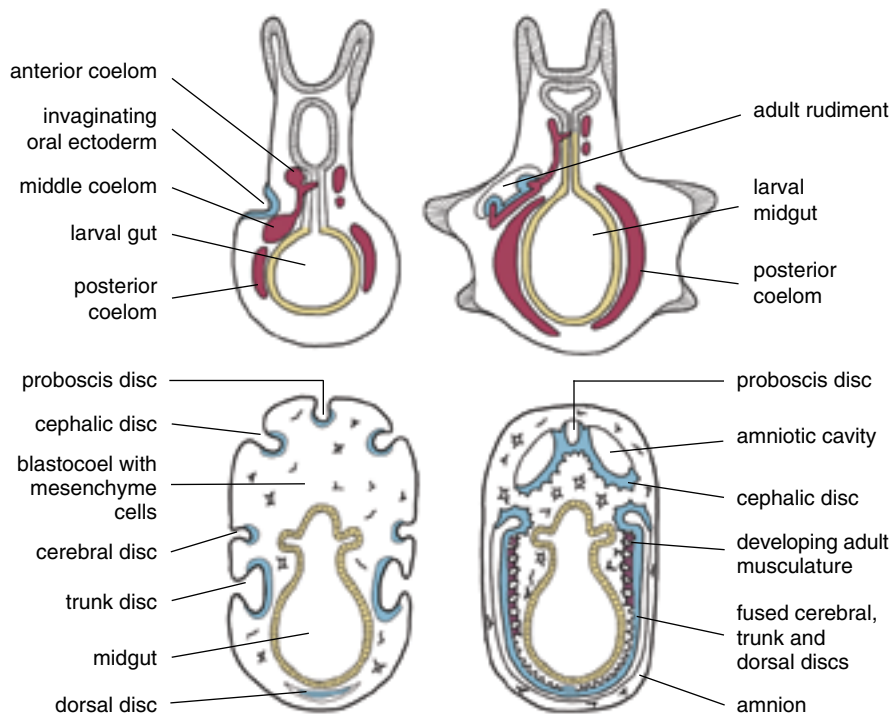
Figure 8. Among the recent proposals for answering questions in larval evolution is one involving so-called set-aside cells. Kevin J. Peterson, Eric H. Davidson and their colleagues point out that larvae are made up of larval-only cells and patches of distinct cells (the "set-aside cells") that are capable of differentiating and will create the rudiment of the adult body. Here are shown in color the areas of set-aside cells in cross-sectional diagrams of a sea-urchin larva (*top*) and a nemertean worm (*bottom*). Peterson and colleagues suggest that patterns of set-aside cells and related genes support the idea that larvae are primitive, part of the deep ancestry of many phyla. Williamson sees these cells differently—in the case of the sea urchin, as a legacy that came with the acquisition of tornaria-like larvae. (Adapted from Peterson *et al.* 1997, used with permission of Wiley-Liss/John Wiley & Sons.)

sea urchins to leeches to insects. In 2000, Peterson and Davidson proposed that the evolution of bilateral symmetry must have followed "a long prior history of bilateral micrometazoans similar in grade of organism to the primary larvae of modern bilaterians."

In 2003, Belinda J. Sly and her colleagues at Indiana University presented a sharp critique of the larval-form-first idea and noted evidence showing that larvae appear over short spans of evolutionary time. In Sly's scenario, larvae have evolved by co-opting genes from the adult genome to function in the larva. Many of the same patterns in genomes and in the evolutionary record that support Sly's proposal could be explained by larval transfer.

Peterson and his colleagues developed their "set aside" hypothesis from the development of echinoderms. Williamson explains echinoderm metamorphosis quite differently. The cells in question are undifferentiated mesoderm cells—stem cells—lining the *coelomic sacs* (body cavities) in echinoderm larvae. The larval-transfer hypothesis identi-

fies these as a legacy acquired with tornaria-like larvae, transferred by hybridization from an acorn worm. Lacking larvae, ancestral echinoderms would have extended their five arms from the *gastrula*, a structure formed during an intermediate stage in embryonic development, just as Kirk's brittle star does today. Modern echinoderms with larvae substitute a larval coelomic sac of appropriate size for a gastrula. The cells from which a radial echinoderm grows were not "set aside" but resulted from a chance hybridization between an acorn worm and an echinoderm.

A way to differentiate between these views and those that involve hybridization might be a further comparison of the DNA sequences of larval and adult forms. Similar larvae of different adults would be expected to have similar genes that they received from their common ancestor during hybridization. Closely related adults that have different larvae, on the other hand, should be expected to show a disparity in the genes expressed in the larval stage if they indeed came from two different sources through hy-

bridization. This is not easy work to do; the picture is complicated by the formation of serial chimeras, since larval transfer suggests that many present-day animals came about through not just one hybridization, but perhaps many as seen in the echinoderms.

If the tree of life is not a tree but a tangled web of intertwining relationships, the DNA evidence might be as confusing to evolutionists as the 18S ribosomal RNA data. But powerful tools are now available to apply to such questions. Just last year the sequencing of the genome of the purple sea urchin was completed—a full century after Theodor Boveri, using sea-urchin eggs, determined that normal embryonic development requires that every cell have a full complement of chromosomes. We can only hope that the search for larval origins in this exquisite echinoderm will not take another century.

Problems

Like the hotly debated hypotheses above, larval transfer raises many questions that do not yet have answers. Strathmann has pointed out the grave difficulties impeding hybridization. These obstacles include sperm-egg binding and the influence of the egg cytoplasm on gene expression during early development. Indeed the mechanisms of fertilization and embryogenesis differ from one organism to another, and animal genomes do not readily fuse between taxonomic groups.

These barriers, like much else in evolution, are unlikely to be insurmountable, although they can certainly explain why it would be exceedingly hard to achieve hybridization and subsequent survival to adulthood in a laboratory experiment. Chance fertilization between unlike animals is probably infrequent, but it has taken place. Unfertilized insect eggs are occasionally spawned by sperm of different species. Onychophoran fertilization is likewise external. In the sea, eggs and sperm are often cast into open water where fertilizations between unlike animals seem more probable. Most of the evidence for the larval-transfer hypothesis comes from these marine animals, and it is not hard to come up with an equally long list of problems with same-stock theories. As Brian K. Hall and Marvalee H. Wake wrote in introducing their 1999 volume on larval forms,

Despite a long history of research, however, even such fundamental questions as "what is a larva?" and

"what is metamorphosis?" occupy us today as they occupied naturalists, zoologists, marine biologists, and evolutionary biologists over a century and a half ago.

What Might Larval Transfer Imply?

If, as Williamson proposes, larvae were later additions to life histories, the earliest animals cannot have had larvae. When a successful hybridization occurred, the resulting chimera had the benefits that each animal had acquired through years of natural selection, along with the new benefits of an early feeding stage coupled with a later reproductive stage. These benefits gave it the potential to make many more of its kind, resulting in the diversity seen today of animals with larvae. Occasional successful hybridizations between adults occurred in the seas where egg and sperm were randomly distributed, but this activity was not limited to the distant past. Brittle stars in the same genus displaying different larval forms indicate a recent hybridization. This would indicate that even close relatives may not have a common ancestor. The web, not tree, of life has fusing branches deep in time that involve many phyla, and fusions that have occurred recently that separate even species within a genus.

Larval transfer shows how larvae originated, and it explains the distribution of types of larvae in the animal kingdom. This distribution is independent of the phylogeny of adults, so the phylogenetic trees that have been almost universally accepted since the early 20th century are fatally flawed. Because the larvae were later additions to their life histories, mollusks, annelids and other so-called trochophorate phyla did not, in this revised view, evolve from a common ancestor with trochophore larvae, and, similarly, echinoderms and chordates did not spring from a common ancestor with tornaria larvae.

We can now answer the question posed by Sly and her colleagues: "Who came first—larvae or adults?" We are convinced that larvae were later additions to life-histories, so there were mollusks and echinoderms before either had a larva. The basic *features* of larvae, however, must have evolved long before animals with larvae existed, just as Margulis pointed out that "the functions now performed by cell organelles are thought to have evolved long before eukaryotic cells existed." In the case of larvae, there were rotifers before

mollusks and annelids acquired trochophore larvae. Rotifers, planctospheres and onychophorans are not persistent larvae; rather, they have been the source of larvae acquired by other organisms.

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