Planula larva of jellyfish

(After Antoine Morin, Biodidac image data base maintained at the University of Ottawa)

Accessory#1

Uncertainty and Confusion

For all the complexity and wonder of terrestrial animals, the variety of animal life in the oceans is altogether more breathtaking. And where true metamorphosis, in remarkable variety and beauty, is a feature of two major terrestrial classes, the insects and amphibians, metamorphosis, in a bewildering complexity and diversity, is a feature of at least 15 major animal phyla.[1] It is little wonder than that marine invertebrate metamorphosis has continued to intrigue and to baffle scientists long after the hormonal controls in insects were elucidated. In 1972, in the preface to his book on the evolution of the animal life cycle, the Swedish biologist, Gösta Jägersten drew attention to this extravagant variety of marine larval forms, which suggested, on first appraisal, that no general rules could possibly apply to their evolution.[2] As he put it, 'the literature demonstrates

considerable uncertainty and confusion'. Even to this day various authorities defend radically different opinions on the most basic interpretations. This in turn lends itself to a fundamental general question: Why should animal metamorphosis have evolved at all?

Five hundred million years ago, before the rise of the fish, ancient cephalopods dominated the oceans. The term, cephalopod, means a combined "head-and-foot", but even this bizarre appellation belies the richness and complexity of form these animals embrace. There are six hundred species of cephalopods, which in turn are an integral part of the most diverse of all marine phyla, the molluscs. It is more than a little challenging to grasp that the cephalopods are thus related to the humble snails we see in our gardens. With their jet-propelled locomotion, driven by three hearts pumping blue blood, their ability to change colour faster than a chameleon, and their highly developed eyes – as large and sensitive as any mammal – this varied class includes the chambered nautilus, the flashback cuttle fish, the oceanic squid and, the fastest and most intelligent invertebrate animal on the planet, the octopus. The family of octopods takes its name from the fact that the member species possess eight arms, commonly misinterpreted as tentacles. The most familiar of these are the hundred or so members of the *Octopus* genus, which inhabit the ocean floor. In these animals we find that the typical mollusc shell is either lost or internally reduced. Less familiar are the numerous transparent pelagic octopods that inhabit the shifting ocean currents. The giant octopus, *Enteroctopus dofleini*, is the largest in the world, with individuals growing up to 150 pounds. This

remarkable species inhabits the continental shelf of the North Pacific Ocean,

ranging from southern California to Alaska and extending across the Aleutians

and into Asia, as far south as Japan. The mature female lays her 20,000 to

100,000 eggs on the inner side of a rocky den, tending, cleaning and aerating her

brood until they hatch, between 150 to 210 days after fertilisation. It is a poignant

example of maternal sacrifice, since this devoted mother does not eat while she

cares for her young and she dies of malnutrition when the eggs have hatched.

The hatchlings swim towards the surface, where they spend 4 to 12 weeks within

the exotic zoo of planktonic life forms. But though often loosely called larvae, the

hatchlings are not larvae at all. In fact octopuses, like all cephalopods, hatch as

miniature adults. These minuscule octopods inhabit the planktonic layers for 4 to

12 weeks, drifting with oceanic currents, until they reach a mantle length of about

half an inch, at which time the young settle back to the bottom to begin their adult

life history, searching out a new den and growing into fearsome predators of

crabs and scallops.

All authorities agree that the cephalopod molluscs follow a life strategy that

takes advantage of the two ecological phases common to most marine

invertebrates – an early spell exploring surface waters followed by the mature

benthic life style – yet there appears to have been no evolutionary pressure for

the dramatic changes, and cataclysmic sacrifices, of metamorphosis.

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In his ground-breaking book, *The Eighth Day of Creation,* which deals with the

history of the discovery of DNA, and its enormous repercussions throughout

biology, Horace Freeland Judson takes pains to explain how the creative faculty operates in much the same way when a scientist engages in theory as when the musician composes, when the writer puts down his thoughts on paper or the artist experiments with colour and form. One of the most creative ideas in embryology of the nineteenth century was undoubtedly Ernst Haeckel's elegant recapitulationist theory – in his lifetime elevated to a purported law – in which he stated that during embryological development every animal re-enacts its evolutionary history. Haeckel also believed that early development was of such critical importance in the structure and form of an animal that it must remain immutable throughout its subsequent evolution. This theory, which was widely believed in Darwin's lifetime, was extrapolated to include larval development. If the life cycles of marine invertebrates followed Haecklean principles, there would be no mystery about their metamorphosis. At some time in the distant past the ancestor of the species would have resembled the larva. And thus the larva would faithfully and inevitably recapitulate that ancestral stage. Unfortunately, modern developmental biologists have rejected Haeckel's beautiful logic, at least as a universal law. Nevertheless, sometimes the early stages of development does indeed supply useful information on ancestral form and structure. And if, with suitable caution, we can figure out when and how this applies, the study of embryonic and larval forms really can help biologists reconstruct evolutionary history.

In the 1870s, Francis Maitland Balfour, brother of the would-be British Prime Minster, James Balfour, took on this challenge while studying marine larvae at

the University of Cambridge. Balfour was almost presciently modern in his thinking and, like his contemporary German colleagues, Wilhelm Roux and August Weismann, he concluded that, contrary to Haeckel's belief, even the earliest embryological stages were capable of evolutionary change. Indeed, animals were not only capable of acquiring new characters that were then interpolated into any stage of their life histories, they were also capable of suppressing earlier developmental stages, so that an entire larval phase could be lost. This made the interpretation of larval-adult relationships a good deal more trickly. Balfour's way of looking at life is also at odds with Williamson's in that he assumed that a "primary" larval stage was universally present early on in animal evolution. In other words, where Williamson classifies "primary direct development" as a life history in which there never was a larval phase, Balfour saw direct development as always secondary, and resulting from the loss of a primary larval stage. But this also implies that, for Balfour, any development that still included a primary larval stage was more likely to repeat ancestral history. And here, in his thinking, was an opportunity for embryologists to unravel the complex links and relationships that gave rise to the evolutionary tree of life.

In cases where Haecklean recapitulation might apply – for example if present day embryos and larvae really did give us useful information about distant ancestral forms – Balfour saw that he needed to ask a pertinent question. What changes to those ancestral forms, if any, had been brought about by evolution over that vast time period? If biologists could answer this question, for example by comparing and contrasting embryological and larval forms across a

phylum, and even across the whole of the animal kingdom, they really could work their way back to discover the true ancestors of phyla, and indeed even the primal ancestor of the entire animal kingdom.[3]

It is important to grasp that Balfour and Haeckel were contemporaries, members of a small intellectual elite that, much as artists, writers and composers, observed and listened to one another, so that global wave of advance, the creative gestalt, involved a similar level of intellectual debate with sometimes the wonderment of mutual enlightenment, and at other times furious disagreement. In the early 1870s, Haeckel had proposed that this primal ancestor of the animals was a gastrula-like organism, which he called the "gastraea".[4] Balfour now extrapolated Haeckel's reasoning to examine embryological and larval forms throughout the animal kingdom, looking for commonalities that would lead him to the ancestral forms. He also compared and contrasted larval forms with living and fossil adults. Where he found similarity of form and structure, say between a larva and an adult, he assumed that the present adult must be closely related to the ancestral stock of the group in which the larva was found. And any such larva could then be regarded as truly ancestral, or "primary".

While Balfour disagreed with Williamson on the nature and origins of direct development, he agreed with Williamson in other, key, respects. For example, he concluded that in addition to his concept of a primary larva, there was also a secondary type of larva – a larva that was newly introduced into a life cycle. And while primary larvae were important clues to distant ancestors of the present

adult forms, secondary larvae provided no ancestral link at all to the adult phyla in which they were found.

The cnidarians, which include the sea anemones, jellyfish, hydras and corals, are among the simplest of animals, in terms of tissue organisation and structure. One of the earliest animals to appear in the fossil record, they follow two very basic body patterns: sometimes they are polypoid, like the hydra, and sometimes they are medusoid, like the jelly fish. They also metamorphose through the simplest of larval forms, a solid ball of cells, known as a "planula". Unlike many other marine invertebrate larvae, the planula is radially symmetrical, with a ciliated epidermis that allows locomotion wrapped around a solid inner endodermal cell mass. It is interesting that these very simple, and primal, animals are radially symmetrical throughout their life cycle. This simple and beautiful metamorphosis was very attractive to Balfour, who saw the planula as a perfect example of his primary larva. Extrapolating his theory of commonalities to the cnidarians, he proposed that the radially symmetrical planula larva must closely resemble the common ancestor of the entire phylum. But when he looked more widely through the many animal phyla, the picture was more complex and uncertain. In Balfour's opinion, the reason for this was that many species had lost their primary larvae during evolution, leading to a period of direct development. The subsequent introduction of a secondary larval form into the life cycle of many of these species further clouded the picture. Here, again, we discover interesting commonalities between Balfour and Williamson. Indeed when, in 2001, Williamson belatedly came across a detailed exposition of

Balfour's full line of thinking – long after he had first proposed his own larval

transfer theory – Williamson regretted he had not discovered this earlier.[5]

When he now read through Balfour's two books, published as long ago as

1880 and 1881, Williamson discovered that this pioneer of animal embryology

had gone so far as to propose that virtually all modern larvae are secondary. In

Balfour's words, 'they have become introduced into the [development] of species,

the young of which were originally hatched with all the characters of the adult'.

Again and again, Williamson discovered commonalities between what Balfour

had been thinking in his lifetime with what he himself was proposing more than a

century later. Balfour shared his belief that there were no echinoderm larvae

until after the establishment of the echinoderm classes, such as sea-urchins, sea

stars and brittle stars. Balfour also rejected Haeckel's opinion, then as now

deeply embedded in orthodox belief, that the original echinoderms were bilateral.

Unaware of Balfour's original ideas, Williamson had arrived at the same

conclusions, based on his own studies, and he had presented them,

independently as he then thought, as new ideas. To his subsequent chagrin,

'biologists might have been more ready to accept them [when I first presented

them] had they known that they were 120 years old'. Balfour went on to

conclude that groups of animals that shared a common primary larval type were

descended from a common stem. This afforded him insight into the very origins

of the animal kingdom. Thus, in his opinion, a medusa-like radially symmetrical

organism, originating with the planula form, had to be a common ancestor of all

animals. Moreover, if we take Balfour's logic a single step further, since the

planula larva is itself the original primary larva, and thus we can take it represent the ancestral form of the medusa-like earliest common ancestor of the animals, then the planula itself – that simple ball of cells wrapped up in a ciliated skin, was the primal animal ancestor.

What then did his contemporaries make of Balfour's pioneering ideas?

Charles Darwin, a friend and admirer, wrote a letter to Fritz Müller, dated January 5, 1882, in which he stated:

Your appreciation of Balfour's books has pleased me excessively, for though I could not properly judge of it, yet it seemed to me one of the most remarkable books which have been published for some considerable time. He is quite a young man, and if he keeps his health, will do splendid work… He is very modest and very pleasant, and often visits here and we like him very much.[6]

However, on February 13, 1992, Darwin wrote a new letter to Dr Dohrn:

I have got one very bad piece of news to tell you, that F. Balfour is very ill at Cambridge with typhoid fever… I hope that he is not in a very dangerous state; but the fever is severe. Good heavens, what a loss he would be to Science, and to his many loving friends![7]

This presaged a sad, and somewhat ironic, ending to this story. Balfour recovered from his typhoid fever and travelled to Switzerland for a period of convalescence. Unfortunately, while there in 1882, he died from a fall while attempting to climb the unconquered Aiguille Blanche slope on Mont Blanc. He was just 31 years old. And as Darwin anticipated, his death, barely a year after first publication of his pioneering ideas, was a great loss to the world of science, and to the burgeoning science of embryology in particular. Despite his young age, he was already an international authority on the development of animals. In the words of Don Williamson, 'Had he lived, he would have defended his revolutionary ideas and expanded on them. In practice, his ideas have been completely ignored.' How poignant also that on Wednesday 19 April 1882, just two months after writing his letter of affectionate concern to Dr Dohrn, Darwin himself died.

For Brian K Hall, a professor in biology at Dalhousie University, Balfour's conclusions, however speculative, still provide an important guide for continued embryological research even today.[8] We have seen how, in Balfour's day, most biologists accepted the Haecklean viewpoint that larvae represented ancient adult forms and thus biologists wishing to understand subsequent evolution should focus on adult changes. However, as early as the 1920s, the marine biologist, Walter Garstang, challenged the universal application of Haeckel's law, and in particular its application to larvae.[9] Garstang, who was the professor of biology at Leeds University, appears to have been an engaging individual, who composed comic verses as an aid to memory. But now, in a landmark

presentation and paper, he proposed that biologists should take a fresh look at the real nature and evolution of larvae. Larvae were capable of dramatic evolutionary change in their own right. Indeed, he argued – and in this he would have found much agreement with Balfour – that most of the modern planktonic larval features are secondary adaptations to larval life. As such they tell us nothing about primitive or ancestral adult types. Indeed, rather than simply recapitulating prior evolution, development, whether at the embryological or larval stage, is a creative evolutionary arena in its own right. For Garstang, this has revolutionary significance, in a way his biological colleagues just had not considered before. Because it meant that evolutionary changes that took place at the embryological or at the larval stages were capable of changing the evolution of the entire animal life cycle and form.

One way in which this happened was through "paedomorphosis", a term he coined for the retention of a larval form into the adult stage. It was obvious when you thought about it that the retention of the larval form into the adult would radically change the form, and subsequent evolution, of the adult. This idea is now incorporated into modern evolutionary development, where it is variously known as "paedomorphosis", "paedogenesis", or "neoteny".

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In 1972, Gösta Jägersten, Professor of Zoology at the University of Uppsala, Sweden, published a book, *The Evolution of the Metazoan Life* Cycle, in which he proposed his own theory for the evolution of animal life cycles.[10] Jägersten was not well known outside Scandinavian circles but he was already an

established authority on marine larvae.[11] In his preface, he explained that, in attempting to rationalise the various animal groups, he found it impossible to arrive at satisfactory conclusions because a comprehensive overview of the evolution of the animal life cycle did not exist. Jägersten accepted the Darwinian perspective that evolutionary forces operated at all stages of the animal life cycle and these gave rise to the hereditary changes essential for the evolution of animal body forms. But this left much to be explained. Did evolutionary changes take place in a haphazard way? Or were they governed by rules that were essentially the same in all the different groups. 'Zoologists,' he stated, 'do not, on the whole, appear to have recognised that they are confronted with a fundamental problem.'

What then is the nature of Jägersten's fundamental problem?

'The great variety of larval forms, often within uniform and well defined groups, suggests that no general rules are involved.'

No wonder there was so much uncertainty and confusion in the scientific literature. Certainly, as Jägersten now recounted, there had been great strides in understanding development, both at the embryological and larval level – but this had not led to any better understanding of the *interrelationships* of the animal life cycle, still less to a comprehensive theory of marine metamorphosis. Jägersten felt obliged to elaborate his own theory, one that some of his readers might find 'daring, even provocative'. When confronted with problems on which his theory might shed new light, he would not hesitate to draw whatever conclusions were

demanded. 'If no attempt is made to solve the problems, understanding will certainly not deepen.'

Ever since Haeckel, extreme recapitulationists had taken the view that larvae provided a direct picture of the ancestral adults of each evolutionary group. But few evolutionary biologists took this extreme view any more. Some experts favoured a directly developing ancestor of all the animals, so that marine larvae, however ancient, were introduced into the life cycle as adaptations affecting pre-existing juvenile forms. By this he did not mean larval transfer, but the neo-Darwinian alternative to Williamson's theory. According to this viewpoint, the wide variety of larval forms came about as a result of different patterns of adaptation, through mutation-plus-natural selection, taking place within pre-existing life cycles in different circumstances. Many neo-Darwinians took this view further in suggesting that where similar larvae were found in different taxonomic groups, this was merely the result of convergent evolution. For Fell and Willmer, who took this perspective, larval morphology should be put aside from the broader evolutionary perspective because larval adaptations and convergence had no part to play in drawing up the tree of life. But Jägersten, as Williamson twenty years later, rejected this argument, which seemed just as extreme as views of the most fervent recapitulationists. The extreme neo-Darwinian viewpoint was not the solution, but part of the problem. As he now saw it, the classification of life had shifted far too much towards the exclusive consideration of adult morphology. People were dismissing larvae as no more than adapted forms, their modern appearances and structures so changed by

natural selection that it was impossible to draw any conclusions about ancestral

relationships. Surely it was obvious that the adults were just as likely to be

adapted by natural selection, so that, if the adaptationist argument was taken to

its logical conclusion, biologists should equally dismiss them from any ancestral

considerations. For Jägersten, taking as broad as possible a perspective, it

made a lot more sense to pay attention to the entire life cycle, and in particular,

to the relationships between its different stages.

Jägersten was perfectly happy with Haeckel's idea of the "gastraea" as the

ancestor of all animals. Long ago these simple non-metamorphosing and radially

symmetrical creatures had inhabited the three-dimensional waters of the oceans.

When, during the course of their further evolution, they had settled to the bottom

to adapt to the constraints of two-dimensional locomotion, they had evolved into

bilaterally symmetrical animals. Jägersten could imagine how this gave rise to a

simple two-phase life cycle in which the fertilised eggs of these bottom-dwelling

bilateral forms floated to the surface, where, in a free floating juvenile phase, they

followed their original radial symmetry; then, when they subsequently settled to

the bottom, they continued their development to the bilateral adult phase. As he

saw it, this involved nothing more than a continuance of development after

hatching. Through natural selection, the planktonic juvenile and the benthic adult

continued to adapt to their very different ecologies so that, in time, the

divergence became so marked that the pelagic juvenile phase became the larva

and the benthic adult phase became the adult. In this way metamorphosis

became established. Jägersten called this his "Bilaterogastraea theory".[12] From

here he went on to develop a more complex all-embracing theory of animal life cycles.

He drew a number of overall conclusions. For example, the pelago-benthic life cycle was so ancient that it dated back to close to the common ancestral form of all animals. He rejected any notion of life beginning as a simple and direct evolution entirely on the ocean floor. He agreed with Balfour that all of the direct life cycles we see today must be secondary, with loss of the original larval metamorphosis evolving independently in different circumstances. Jägersten also introduced a few specific terms to explain his ideas and he proposed several new conceptual theses in support of his theory.

We can see that, like Balfour, Jägersten conceived two different forms of larvae, primary and secondary. And in agreement with Balfour, his "primary larva" was one that evolved directly from the juveniles of those first multicellular animals as they established their planktonic and benthic life cycles. Jägersten saw no reason to couple primary larvae with commonalities to adult shape. In his view, there had been so much adaptation through natural selection over the vast time periods since the larva had first evolved that any such assumption was likely to mislead. The key to proving primary status had to be the demonstration that the larval type had persisted as part of the developmental life cycle without interruption since its first appearance. This, in practice, led to his disagreeing with Balfour over the primary nature of many of the common larval types. For example, Jägersten included many familiar forms, including the acorn-shaped trochophore, the spinning tornaria and ear-like auricularia. He faced a difficulty

when two or more larval forms appeared as part of the same animal's development, for instance the trochophore and veliger in the gastropods, but he got around this by proposing that these could be accommodated if both were considered primary.

Another key extrapolation of Jägersten's theory was that fully developed larvae should be considered a final stage in their own evolutionary development. In this sense, larvae might be viewed as life forms in their own right, a startlingly novel concept. Once again, we encounter an idea, arrived at from a radically different perspective to Williamson's, yet in which we encounter striking commonalities with aspects of his theory. Marine larvae, while being much smaller and simpler in their anatomical structures and physiology than the corresponding animals, possess highly developed organs and tissues, and thus, in Jägersten's words, their 'embryogenesis gives rise to an individual living a life that is often essentially different from that of the adult, with different locomotion, feeding, etc.' This is in perfect keeping with the remarkable developments we have witnessed in larvae undergoing cataclysmic metamorphosis, where two entirely different body plans, giving rise to different axes of symmetry, form, tissues and internal organs, are seen to develop in parallel from the same fertilised egg.

Jägersten arrived at a particularly dramatic insight. Larvae sometimes show evolutionary changes that also appear in the adult development. This led him to invoke a radically new developmental concept, and one that is the very opposite of Garstang's paedomorphosis – "adultation". He suggested that some

internal genomic mechanism might somehow accelerate development during the larval stage, resulting in a shift of adult features into the larval development. Who was to say that such adultations had not taken place repeatedly throughout evolution? Jägersten extended his concept of adultation to explain all "directly developing" species, which must, in his opinion, have arisen from the complete adultation of what had previously been indirectly developing species. Convinced as he was that this had occurred many times in different circumstances, it came as no surprise that it followed a great many variations, with different degrees of conservation of the former larval development. But while all of this is reasonably plausible as a rounded theory, he muddied the water somewhat with his own definition of "primary" and "secondary" direct development.

For Williamson, direct development implies that the developing animal goes directly from the egg to the miniature adult. It follows two very different patterns. In "primary direct development", the life history has never had a larval stage, and in "secondary direct development", the life history once had a larval stage, and vestiges of this still appear in the embryo or in the life cycle. But Jägersten, who believed all animal histories had originally incorporated a larval stage, "primary direct development" was one in which this larval stage had been suppressed as a result of adultation. Meanwhile, "secondary direct development" followed the same pattern of suppression of the primary larva, but in this case it was followed by the introduction of an entirely new larva, the "secondary larva", which often looked and behaved very differently from the primary larva. Williamson, of course, would describe this as "secondary indirect development".

In the interests of clarification, we might look to the intriguing tale of a very unusual development.
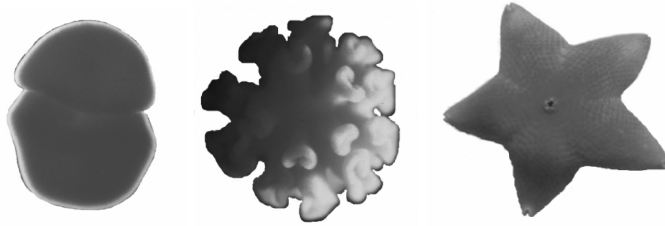
The cushion star metamorphosis

(after McEdward, Department of Zoology of the University of Florida)

Accessory#2

A Brooding Starfish

With a wide geographic range along the Pacific Coast of North America, from the Bering Sea to Carmel Bay, California, the five-armed starfish, *Pteraster tesselatus*, is a subtidal, often deep-water predator. More popularly, and engagingly, known as the "cushion star", it grows to about six inches in diameter, and has a number of decidedly unusual features. In appearance, it is a yellow-brown or pale orange, and has a rather chunky look, with wide, inflated-looking arms. In fact the entire upper surface is covered with a thick, loose and fleshy membrane, giving it a fairly smooth texture for an echinoderm, and a soft rather than a prickly feel to the touch. Disturb it in its natural habitat and it is likely to surprise you with a sudden squirt of large amounts of a thick, viscous slime – hence it's less engaging appellation, the "slime star". But it is in the development

of its pelagic larva, and its metamorphosis into the juvenile stage, that the cushion star is highly unusual. For this description I rely on the vivid and meticulous investigation of the late Larry R McEdward, of the Department of Zoology of the University of Florida.[1]

Pteraster starfish possess an unusual structure, known as the supradorsal membrane. This is an additional membrane over the back surface, about two millimetres thick, and which forms a secondary covering over the entire body, including the arms and central disk. This gives the starfish its cushion-like appearance and feel. It also creates a broad flat chamber between the membrane and the prickly skin of the back. The membrane contains mucus cells and muscles that perform a variety of functions. It is perforated by numerous tiny openings, or spiracles, and at the dead centre is a larger opening, or "osculum". This curious membrane, which is unique to this family of starfish, has three known functions. Alternating muscular contractions make it work like a bellows, so that a current of seawater enters the chamber and flows over the respiratory papules, exiting via the osculum. This helps with respiration. The mucous secreting cells are closely associated with the spiracles. When it needs to defend itself, it produces tremendous quantities of slime, which are ejected from the spiracles through bellows-like contraction of the chamber. This is directed not so much at curious humans but at two even more fiercely predatorial starfish, Solaster dawsoni and Pycnopodia helianthoides, when they pounce on the cushion star in search of a meal. But it is to the third function of the membrane that McEdward postulates a key evolutionary transition: the enclosed chamber

enables *Pteraster* to brood its young throughout development to the juvenile stage, offering an unusual degree of maternal protection.

It is probable that most, though not all, species of the genus *Pteraster*, are brooders. But until McEdward's study of *Pteraster tesselatus* very little was known about the morphology and development of *Pteraster* larvae. Major evolutionary transitions in the patterns of animal development are rare in the history of biological investigation and the evolution of a new star fish larval development as a result of maternal brooding had never been documented before his study. It also seemed likely that *Pteraster* had moved on from brooding to develop a novel pattern of metamorphosis. So it was with considerable interest and excitement that the marine biologist began a series of experiments to examine this further.

After inducing females to spawn, with a hormone injection, he collected the eggs as the bellows forced them out through the central opening in the fleshy membrane. At this stage he experienced his first surprise. McEdward was preparing to artificially fertilise the eggs with appropriate sperm. But these eggs contained zygotes that were already dividing. Somehow they had been fertilised while still within the brood chamber. At the time, McEdward could offer no satisfactory explanation for this. But his postgraduate student, Jonathan Cowart, would subsequently solve the mystery as part of his master's thesis. Cowart set himself three questions. Was *Pteraster tesselatus* developing through hermaphroditic self-fertilisation? Was it developing through maternal to identical daughter reproduction, without the need for sperm fertilisation – a process known

as parthenogenesis? Or was it developing through normal sexual fertilisation, which would in turn imply that the female had been storing the sperm of the male in the brood chamber? To test these three possibilities, Cowart induced females to spawn, then split the spawn into two experimental groups, one of which he fertilised using sperm from another male. The other group was left "unfertilised" – although he knew, from McEdward's observations, that a proportion of these would go on to develop. He took tissue specimens for genetic analysis from the maternal sources, the sperm-providing males and the resultant offspring, including both the fertilised and unfertilised groups. Genetic analysis ruled out parthenogenesis and it also ruled out hermaphroditic self-fertilisation. In fact the offspring in both the experimental groups were the result of normal male-female fertilisation. For Cowart, the explanation for the prior fertilisation was now abundantly clear. The female *Pteraster* anticipated reproduction by storing sperm from conspecific males as well as her own eggs in her brood chamber.[2]

McEdward, in his series of pioneering experiments, noted that the fertilised eggs were light yellow to dark red in colour, and rather large for a starfish, at between 1.0 and 1.4 mm in diameter. They were also significantly opaque and yolky, making them positively buoyant. Large and yolky marine invertebrate eggs are often associated with direct development. The eggs were sheathed in a thick jelly coat that dissolved away prior to hatching, typically within 48 hours after laying. Inspecting the first changes of embryogenesis inside the eggs, McEdward expected to see the typical radial cleavage pattern of starfish. Instead he saw a variable and irregular cleavage pattern, which led to a blastula with a

smooth wall. In time, through continued division, the blastula wall became

deeply folded until it developed an unusually wrinkled appearance. This

developed into a gastrula while still within the egg and the large blastopore (the

opening that becomes mouth, or anus, or both) opened into a broad shallow

archenteron, or proto-gut. Subsequently the blastopore began to close down – it

would never be functional in the larva. Instead, it closed off completely soon

after hatching and the now "mouthless" larva failed to develop any functional gut.

This is a common finding in direct development where the "secondary" and often

obtunded larva derives its nourishment entirely from the yolk. McEdward

continued to observe the hatchlings as they emerged from the eggs as simple,

oval-shaped larvae, which swam through the water column through coordinated

movements of their surface cilia. But from here on, he was astonished to witness

a unique transformation.

Within a couple of days of hatching, a waist-like groove encircled the

larva, about a third of the way back from the front end. This groove tightened up,

dividing the larva into two regions, one at the front that contained mainly food

stores, to be absorbed during further development, while the larger region at the

back end developed into the juvenile starfish. 'In striking contrast to most other

asteroid larvae, specialised settlement structures did not form.' Indeed, the larva

failed to develop any of the normal larval structures associated with starfish

development, such as ciliated bands, or brachiolar arms. Instead the rear end

shortened from front to back and spread out laterally, five broad bulges, or lobes,

sprouting around its circumference, and foot-like processes, called "podia",

popping up in the grooves between the bulges. Eventually these podia came to

be distributed in a ring around the circumference of the larva, within the groove

that separated the front half from the back. These appeared to be entirely larval

structures, designed for attachment to the substratum, and played no part in the

subsequent development of the arms. A second set of lobes formed at the rear

end of the developing juvenile. The further development of these lobes and

podia are too complex to describe in detail, but in essence the second set of

lobes developed into the fleshy membrane that would cover the back – this being

so unexpected, it probably misled earlier investigators. Meanwhile, the five arms

only developed at a later stage of the development. McEdward realised that

what he was witnessing was so unusual it simply did not fit with any of the normal

definitions of a starfish larva. He could only use the term "larva" in a kind of

ecological sense, as a stage between hatching and juvenile that accompanied a

free-swimming life cycle. There was no clear-cut metamorphosis to juvenile.

Instead the "larva" settled to the bottom and gradually grew and changed, over

weeks or months, to the fully formed juvenile adult. Only later, at about two

months into the development, did the real mouth first appear, and a month after

that the distinctive five arms of the adult starfish. There were other, equally

striking, anomalies.

In all other starfish, metamorphosis takes place through a bilaterally

symmetrical bipinnarian larva, which has two distinctive rings of cilia (see

illustration, Chapter 7). This often metamorphoses to a second larval form,

known as a "brachiolaria", which is equipped with short arms and organs of

attachment that enable it to tether itself to the substratum in anticipation of metamorphosing to the adult. The adult starfish grows within one of the coelomic sacs of the larva, where it ignores the larval tissue structures, including the entire body orientation, adopting a new body axis at a right angle to that of the larva. The disk of the juvenile starfish lies in the so-called sagittal plane, with the mouth of the juvenile facing the left side of the larva. In order to settle to the bottom, the larva has to bend its body to one side so mouth of the juvenile disk faces the substratum. In *Pteraster*, the development is radial from start to finish. And the axis of the juvenile follows precisely that of the larva and so it requires no flexion through 90° to attain the correct orientation when the life cycle switched from planktonic to benthic.

It is clear that *Pteraster tesselatus* confronts us with a number of anomalies that are difficult to explain, whatever one's developmental perspective. Indeed, it is fascinating to compare and contrast this curious development with that of Kirk's brittle star. Both are highly anomalous. In neither development do we find any evidence for metamorphosis through a bilaterian larval stage. In both developments there is a brief appearance of a first blastopore, which quickly closes off and is made redundant. But from hereon in, the differences greatly exceed the similarities. In the case of Kirk's brittle-star, if we recall, the blastopore becomes the mouth and the internal body cavity, or coelom, develops from a tear in the central tissues, known as the mesenchyme. In other words, it develops as a schizocoelous protostome. I was fascinated to know whether *Pteraster* developed as a protostome or as a deuterostome, like all other starfish.

Sadly, Larry McEdward had died prematurely, so I wrote to his friend and colleague, Richard Strathmann, and asked if he knew. He wrote back: 'The blastopore definitely becomes the anus in Pteraster. That show's in Larry and Dan's figures, with the blastopore a dimple on the aboral side of the developing starfish.[3] Later those odd flaps fuse over the aboral [back] surface, with an osculum as the visible opening and anus hidden below.' Pteraster, like other starfish, develops as a deuterostome.

What a wonderful life history! And how it helps dispel any residual confusion!

Where Kirk's brittle star illustrates what may be a true direct development, in the sense that Williamson uses the term, *Pteraster* perfectly illustrates a secondary pattern of development – the development one would expect in an animal that formerly incorporated a larval stage, and metamorphosis, and then lost it. This secondary larva of *Pteraster* – whether, like Jägersten, we term it "secondary direct", or, like Williamson, "secondary indirect" – offers no clue to starfish ancestry or to the echinoderm evolutionary tree of life. What then are we witnessing in the obtunded larva and the very unusual development?

The conventional view is that the *Pteraster tesselatus* larva must be a partially suppressed brachiolarian larva.[4] But Strathmann suggests an intriguing alternative possibility: that the unusual features of this larva might imply a novel evolution resulting from benthic brooding, rather than modification of a previous planktonic larva.[5] McEdward is inclined to agree. Refuting the conventional explanation, which demands modification of fundamental larval features at a time

they are functional in the larval life cycle, he proposes that this curious development might best be interpreted as a rare evolutionary transition in animal development – the re-evolution of pelagic larval development arising in a benthic brooding species.[6] At some stage in the past, brooding, with its extreme form of protection, might have led to the reduction and eventual loss of larval characteristics in the offspring. Subsequent re-evolution of planktonic development, probably in a shallow-water ancestor of *P.* Tesselatus, resulted in the appearance of a larval form that was distinctly different from that of all other starfish.

There is, however, an additional interesting possibility.

The more McEdwards examined the development of *Pteraster,* the more variable he found it to be, with marked differences, for example, in the formation of the rear lobes in larvae from different geographic regions. Perhaps these differences signify different species within the strange genus. But it is also possible that what he was witnessing was truly marked differences arising in the same species. Other anomalous features that McEdwards found in *Pteraster* included greatly accelerated development of the water vascular system and the use of podia, normally reserved for the adult, for benthic attachment during the "larval" phase. The jargon for this is "heterochrony". Heterochrony implies an evolutionarily significant change in the timing of a programme during development. Add to this the unique fleshy membrane, the fact that some *Pterasters* are brooders while others are not, the exceedingly slow rate of development, and the extremely variable rate of development within and between

species, as witnessed in animals found in different geographic locations – all of this brings to mind the variable developments seen in hybrid experiments. Could it be that *Pteraster* is indeed an example of hybridisation in nature giving rise to significant evolutionary transition: the re-evolution of a pelagic larval form arising from a previous hybrid union, with disparate parental species at present unknown? Of course I cannot definitively suggest this is true, but I would suggest that future researchers might at least consider it, along with other possibilities, for further investigation.

Let us, for the moment, put this fascinating brooding starfish to one side and return to the lively arena of alternative metamorphosis theories.

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Doctor Claus Nielsen, at the Zoological Museum of the University of Copenhagen, has been inspired by the theories of Jägersten to look afresh at the origin of metamorphosis. He has extended and refined Jägersten's theory while making a number of salient observations of his own.[7] It seems to him that the sheer variety of different types of metamorphosis suggests that they could not be the result of one and the same evolutionary mechanism. Nevertheless it is likely that many metamorphoses, especially those seen in marine invertebrates, may nevertheless be explained by a common principle. Metamorphosis is often found where the animal life cycle embraces a dramatic change in ecology. This change entails revision of locomotory and feeding strategies. For example, in marine invertebrates it involves the switch from a free-swimming, often planktonic, filter-feeding larva in the three-dimensional ocean currents to a carnivorous adult

crawling over the two-dimensional ocean bottom. In insects it involves the switch from the herbivorous larva creeping over its two-dimensional terrestrial habitat to a frequently non-feeding reproductively orientated adult, flying through the three-dimensional ecology of the air. In amphibians, it involves the switch from a filter-feeding tadpole, swimming through the three-dimensional habitat of water, to a carnivorous adult, such as a frog, hopping over its two-dimensional terrestrial habitat. In each case the switch demands dramatic changes to accommodate new strategies of locomotion, feeding and reproduction. Nielsen also agrees with Wigglesworth that the catastrophic or cataclysmic changes seen in many metamorphoses, which appear at first to be so dramatic and extraordinary, can mostly be explained by "acceleration" or "compaction" of changes that first evolved in a more gradual fashion. In such circumstances, cataclysmic change may have been selected by nature because it carried survival advantages: after all the transitory stage cannot be well adapted to both the larval and adult life strategy. This is a more scientific explanation, based on sounder evolutionary principles, than any "need" for rapid change: evolution is not foreword planning and does not respond to needs or desires. Nielsen also accepts Jägersten's contention that the earliest animals developed through an indirect, or metamorphic, life cycle. For him, this is more likely than the alternative scenario, proposed by Williamson and Balfour, which demands the interposition of a planktotrophic larva within a pre-existing direct development. This, in his view, would involve the evolution of a complicated feeding structure, which has no adaptational value before it is fully formed. But Nielsen's view does not take

hybridisation into account. While it is hard to envisage the introduction of a larva into a pre-existing life cycle arising from the slow and incremental process of mutation-plus-selection, hybridisation offers a radically different solution. New structures deriving from hybrid evolution would not require the evolution of a complicated feeding structure from scratch. Rather the appearance of modified feeding structures from pre-evolved forbears, deriving from the blending of the developmental programme of the hybrid partners.

Nielsen has many interesting ideas. For example, the dramatic 90 degree change of axis from larva to adult that is seen in most starfish may have evolved because of the switch in locomotory strategies between free-swimming in three dimensions to crawling in two dimensions. Here again, Nielsen does not offer a precise evolutionary mechanism for this dramatic developmental reorganisation, assuming, perhaps, it arose through mutation-plus-selection.

Hall and Wake appear to support the Jägersten-Nielsen contention that 'larvae may be as ancient as the metazoan'.[8] Such ancient origins may also be supported by the observations of Chinese palaeontologists, who have identified what they believe to be embryos and planula larvae in pre-Cambrian rocks dating to approximately 600 million years ago.[9] However, the early origin of the planula larva would not discomfit Balfour or Williamson – we recall that Balfour saw the planula as a perfect example of his primary larva and he assumed that this radially symmetrical form must closely resemble the common ancestor of the cnidarians. In 1993, the same year he reviewed Williamson's book, Richard Strathmann also published a landmark review of all known hypotheses on marine

larval origins.[10] In his opening lines, he agrees with Nielsen that larval forms and

metamorphosis are almost certainly of varied origins. But he also makes a telling

admission of the prevailing state of confusion: 'There is no generally accepted

comprehensive account of the origin of marine larval forms, and such an account

is not imminent.' Strathmann goes on to list the many different hypotheses and

theories put forward by different biologists. Much as in his review of Williamson's

book, he does the world of evolutionary biology a much-needed service in

describing how 'some hypotheses on larval origins can be tested, others laid to

rest, and new ones generated.' Like Jägersten and Nielsen, he emphasizes the

need to consider larval feeding and locomotion in any explanation of larval

origins and metamorphosis. He also agrees with the Scandinavians in

concluding that the evolution of complex larvae, such as the echinoderm pluteus,

appears to combine adult and larval traits, suggesting that Jägersten was

prophetic in his concept of adultation. Strathmann contradicts Williamson in

accepting that processes such as adultation may have accelerated what was

formerly a gradual process of change and this offers a reasonable explanation of

what now appears to be cataclysmic metamorphosis. He also differs with

Williamson in accepting a convergent explanation of similar larval forms

appearing in different taxa. Indeed, he restates his criticisms of Williamson's

hypothesis, highlighting the lack of genetic confirmation of parentage in the

hybrid offspring. Nevertheless, he remains sufficiently broad-minded to admit

that hybridisation, if it could be conclusively proven, would change the ballgame:

the question of genetic transfers between distantly related animals… 'could [if convincingly determined] have interesting morphogenetic effects'.

However, Strathmann also disagrees with any exclusive reliance on the ancestral nature of metamorphosis. In his view, the vast range of life cycle strategies is too diverse to allow any such generalisation. Why, as some biologists insist, should we believe that octopuses had to have gone through some former larval phase? The only reason for such an insistence is dogma. Since many molluscs undergo metamorphosis, and since some biologists take the view that a metamorphic life cycle was primal, they extrapolate this to imply that a larval stage, for which there is no evidence whatsoever in the present life history. Indeed, Strathmann finds no evidence to assume that metamorphosis was ancestral to all animals. Direct development may just as well be just the ancestral condition. Strathmann's lucid thinking strikes the imagination like a refreshing cold shower. Indeed, he highlights an important question, and one that is of critical importance to Williamson's theory. Which really did come first in the origin of marine invertebrate animals, a direct development into the adult or a development through the metamorphic life cycle, with its all-important larval stage?

Nothing could better illustrate the continuing uncertainty and debate than two papers emanating from the laboratories of leading proponents of modern evolutionary development. In 2000, Eric H Davidson and his colleagues published a review in which they argued, on the basis of systematic, molecular and palaeontological lines of evidence, that the latest common ancestors of the

echinoderms and the hemichordates used a maximal indirect mode of development.[11] Three years later, Rudolf A Raff and his colleagues challenged this with a paper entitled, 'Who came first – larvae or adults?', in which they championed the very opposite opinion with an evolutionary model involving the 'intercalation of larval features into the ontogeny of an ancestral direct-developing bilaterian.'[12] Suddenly we realise the importance of the brooding starfish, *Pteraster tesselatus.* What else is the ciliated planktonic stage of *Pteraster* if not the equivalent of a new planktonic larva? In Strathmann's opinion, you just had to imagine some similar evolution of the octopus juvenile, which already inhabits the planktonic ecology, to glimpse how an utterly new larva phase could readily arise in a life cycle that had formerly been direct.

Why so much heat over the esoteric topic of the ancestry of larvae? The reason is as simple as it is profound. How the earliest marine animals evolved may be something close to the holy grail – the key to understanding all subsequent animal evolution and diversification.

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