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Arrays in rays: terminal addition in echinoderms and its correlation with gene expression

Rich Mooi,[a,]* Bruno David,[b] and Gregory A. Wray[c]

[a]Department of Invertebrate Zoology and Geology, California Academy of Sciences, 875 Howard Street, San Francisco, CA 94103-3009, USA
[b]UMR CNRS Biogéosciences, Université de Bourgogne, Dijon, France
[c]Department of Biology, Duke University, Durham, NC 27708, USA
*Author for correspondence (email: rmooi@calacademy.org)

SUMMARY The echinoderms are deuterostomes that superimpose radial symmetry upon bilateral larval morphology. Consequently, they are not the first animals that come to mind when the concepts of segmentation and terminal addition are being discussed. However, it has long been recognized that echinoderms have serial elements along their radii formed in accordance with the ocular plate rule (OPR). The OPR is a special case of terminal growth, forming elements of the ambulacra that define the rays in echinoderms. New elements are added at the terminus of the ray, which may or may not be marked by a calcified element called the terminal plate (the "ocular" of sea urchins). The OPR operates in every echinoderm, from the occasionally bizarre fossils of the Cambrian to the most familiar extant taxa. Using the OPR and other criteria of recognition, echinoderm body wall can be divided into two main regions: extraxial components are associated with the somatocoels, axial components (formed in accordance with the OPR) with the hydrocoel. We compare patterns of development in axial regions of echinoderms with those found in the anterior–posterior axes of the earliest echinoderms as well as other invertebrates. Although axial and extraxial skeletons appear to be composed of the same biomineral matrix, the genes involved in patterning these two skeletal components are likely distinct. During development of the axial skeleton, for instance, the genes *engrailed* and *orthodenticle* are expressed in spatial and temporal patterns consistent with the OPR. Other genes such as *distal-less* seem to demarcate early ontogenetic boundaries between the axial rudiment and the extraxial larval body. There is a complex and pervasive reorganization of gene expression domains to produce the highly divergent morphologies seen in the Echinodermata. We integrate morphological and genetic information, particularly with respect to the origins of radial symmetry in the rudiment, and the concomitant development of the rays.

INTRODUCTION

Even a cursory review of developmental biology reveals that animals have a myriad of larval forms, many of them of surpassing beauty and intrigue (Young 2002). In seeking to express this diversity, systematists and embryologists have coined and employed an equally diverse terminology for these larval types. Some of these were born as formal taxon names for types known only from early plankton tows. Few phyla rival the Echinodermata in this abundance of nomenclature. The dipleurula, auricularia, pluteus, brachiolaria, doliolaria, and bipinnaria are but a few examples of nomenclature for echinoderm larval types. This makes for a rough road when trying to see common patterns among these types. In the past, systematists in particular have engaged in the somewhat easier pursuit of discerning and describing their differences.

To make matters more complicated, the echinoderms have enormously diverse adult morphologies. With five extant major clades ranging from stalked, multi-armed calyces of crinoids to rigidly sutured, spiny tests of echinoids and even to soft, worm-like bodies of holothuroids, the disparate morphologies are daunting. Throw in another 15 or so even less familiar "classes," such as the hockey puck-like edrioasteroids, enigmatically cigar-shaped helicoplacoids, and taxa, such as the ctenocystoids (with more than a passing resemblance to tiny electric razors), and the search for homologies becomes yet more complex. It is simultaneously fortunate and unfortunate that the fossil record of echinoderms is good—good enough to reveal a half-billion year record of almost unparalleled evolutionary novelty that somehow has to be understood in phylogenetic and developmental contexts (see Mooi 2000 for review). In spite of some advances on molecular (Littlewood et al. 1997; Janies 2000) and morphological

(Sumrall 1997; Mooi and David 1998; David and Mooi 1999) fronts, consensus is still beyond reach for major parts of the tree (particularly at its base), at least in part because the plethora of nomenclature for echinoderm features renders certain morphologies difficult to relate one to another.

Because phylogenetic systematics relies on synapomorphy, a term that in its very etymology requires the sharing of characteristics, finding these commonalities among larval forms has been particularly difficult. A hope arose that the newly discovered tools of developmental genetics would lead to the discovery of overarching genetic systems that could identify or even serve as useful homologies (Finkelstein and Boncinelli 1994; DeRobertis and Sasai 1996). Because these genetic regulatory complexes were, in theory, controlling basic patterning of early morphology, they were usually considered to be evolutionarily conservative. Genes were therefore likely to contain not just phylogenetic signal to support basal nodes, but important general information about how ontogeny was regulated in the otherwise vast array of metazoans.

As more results come to light, it becomes increasingly evident that just as with determination of morphological homologies among major clades, the situation with gene expression is similarly complicated (Wilkins 2002). We are learning that phylogenetic protocols used to establish homologies emerging from the analysis of morphological patterns hold lessons that are equally applicable to comparative studies of homologies in gene expression across phyla. We are also discovering that the Echinodermata is a rich mine of evolutionary developmental data, and the relative ease with which early development can be studied makes them ideal subjects (Raff 1996; Hart et al. 1997; Lowe and Wray 1997; Wray and Lowe 2000). Coupled with the deep time axis afforded by their fossil record, echinoderms should constitute a fruitful laboratory in which to discover basic principles in the appearance of evolutionary novelty, especially how genetic patterning is modified and in some cases heterotopically expressed to generate disparate adult body forms.

Recent theories about patterns found in adult morphologies are also leading to generalizations about ontogenetic pathways (Hotchkiss 1995, 1998; Mooi and David 1997; Peterson et al. 2000). These generalities can be traced all the way back through larval morphologies, even among the first echinoderms (Mooi and David 1998). Renewed emphasis on such commonalities can cut the fog of real and perceived disparity, renewing hopes that even the most radically altered roles of patterning genes in echinoderm evolution can be correlated with both larval and postlarval development.

One of the most significant and promising of these correlations lies in a developmental trajectory that is not intuitively obvious in echinoderms: terminal addition. The most obvious feature of any adult echinoderm, particularly an extant form, is the pentameral, radial organization of its rays. It has long been known that this radiality is derived from a bilaterally symmetric larva (Bury 1895; MacBride 1903), whichever of the abundant larval types might characterize the adult taxon in question. The process by which the transition from bilateral to radial symmetry occurs in echinoderms is accompanied by processes that constrain the ontogenetic pathways (but not necessarily the final morphology). One such pathway, dubbed the "ocular plate rule" (OPR) by Mooi et al. (1994), governs the manner by which new elements are laid down in the rays of all echinoderms, from the most bizarre early fossils to the most highly derived of the irregular sea urchins. It turns out that this rule actually describes a specialized type of terminal addition.

In their capacity as unambiguous synapomorphies for the Echinodermata, properties of OPR-mediated addition sequences reveal caveats for studies that insist on highly conserved genetic expression domains in other parts of the metazoan tree. Intriguingly, certain types of homeoproteins seem to be expressed in accordance with the OPR, sometimes even as gradients along the radii of echinoderms. We will examine this in detail, expanding upon recent discussions by Abouheif et al. (1997), Lowe and Wray (1997), and Wray and Lowe (2000) concerning the perceived conservatism of some regulatory genes during the diversification of metazoans. There is some evidence to suggest that the radical changes that accompanied the origin of the adult body form in echinoderms were paralleled by changes in gene expression not seen in other phyla. The evolutionary lability of these genes and their expression domains during the evolution of the echinoderms contrasts with the highly conserved developmental patterning across the phylum. It also raises a challenge to those who hope that conservatism in homeobox genes will inform basal topologies of animal phylogeny, and changes the perception that homoplasy is more rampant in morphological datasets, because structures are more prone to convergence than the patterning genes. That there remain important early developmental commonalities and even empirically derivable "rules" in adult growth across all the major clades of echinoderms is evidence that homologies can sometimes be more evident by their morphology than by the accompanying patterns of gene expression. By integrating the gene expression data with these overall developmental trajectories, and trying to tease apart the variations in patterns between major clades, some of the complexity can be better understood.

SERIAL ELEMENTS IN ECHINODERMS AND THE EXTRAXIAL–AXIAL THEORY (EAT)

Not only are the major clades of echinoderms very different from one another, but the phylum is itself very different from all others. Echinoderms are deuterostomes with pentaradial symmetry, and possess a water vascular system, mesodermally derived calcite elements with trabecular architecture (stereom), and developmental modes in which bilateral larvae give rise to radial adults. To this general list of apomorphies might be added the phenomenon of coelomic stacking (David and Mooi 1998; Peterson et al. 2000) in which the initially bilateral, paired somatocoels of the larva are rearranged to lie serially along the anterior–posterior axis of the adult. This serialization is unrelated to that of other phyla, which only serves to highlight the strangeness of echinoderm morphology.

However, there are, within the body wall of echinoderms, many serial elements that recall patterns seen in other phyla. Turner (1998a, b) has referred to this as metamerism and even tagmosis, but it is clear that like the coelomic stacking, these patterns are also not homologous with serialization seen in other phyla. We would regard references to metamerism and tagmosis in echinoderms as manifestations of a more generalized pattern described in the EAT, in which the body wall of echinoderms can be seen as being constructed of two major subregions: axial and extraxial (Mooi et al. 1994).

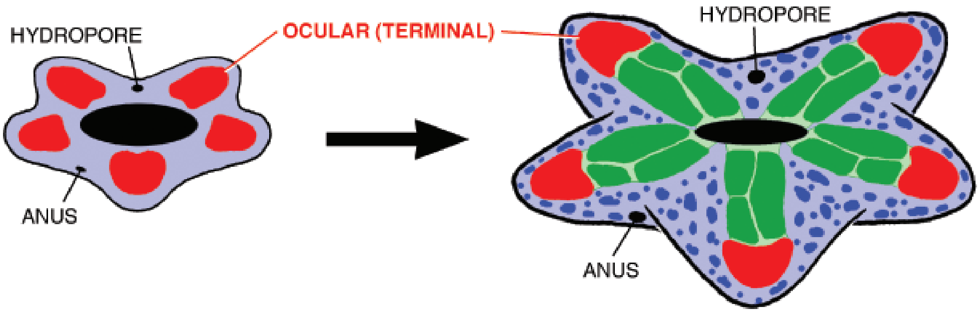
As reviewed in Fig. 1, growth of the axial region is governed by specific patterning rules. In the body wall of a postmetamorphic echinoderm, the axial region grows in accordance with the OPR: new elements are added at the end of each ray (or ambulacrum) in a region that can be interpreted as a blastema. The extraxial region is in strong contrast to this, because in most cases, body wall elements can be added without regard to these constraints (Mooi et al. 1994): new elements are inserted at the points they are required to complete the body wall, without the involvement of a localized blastema.

It has been noted that some plate systems within the extraxial region have a linear arrangement similar to that seen in the ambulacra. It is our contention that these linear systems are not governed by the same ontogenetic constraints that lead to the patterning of the axial region. Smith (1997) assumed that the carinal and marginal plates of asteroids and the dorsal arm plates of ophiuroids were serialized in accordance with the OPR. However, these are special cases of plate ordering in the extraxial skeleton, the growth of which is constrained by space limitations at the tips of the arms. A detailed analysis of processes and topologies involved in the addition of these plates at the arm tips suggests that they are not governed by the OPR (Mooi and David 2000) and are part of the extraxial region.

In extant crinoids, stem growth can be highly organized (Breimer 1978). However, the pattern of appearance of new columnals in the stems is unlike what is seen in the rays, and in modern isocrinids, new elements are added between special columnals known as nodals. In addition, the basal forms of both crinoids and blastozoans had multi-serial or multi-plated stems that predated the highly derived systems of plate addition in more crownward forms (Sprinkle 1973; Ubaghs 1978). This prompted Mooi et al. (1994) to place stems of crinoids and blastozoans in the category of extraxial skeleton.

In addition to skeletal patterning, the axial and extraxial regions are differentiated by the presence or absence of major orifices and perforations (Fig. 1). Furthermore, one of the major precepts of the EAT is that certain coeloms are always associated with specific regions of the body wall. The left and right somatocoels are associated with the extraxial region. Most importantly, the hydrocoel is associated with and directly influences the expression of the axial region. The morphological diversity of the echinoderms is strongly related to the proportions in which extraxial and axial body walls are expressed, as well as to the topology of plate systems within these regions.

This diversity has made the determination of homologies among the major clades the subject of much discussion (see Mooi 2000 for a review). Hypotheses of homology among



	AXIAL REGION	EXTRAXIAL REGION
ORIFICES	mouth	anus gonopores hydropores
PLATE TOPOLOGY	linear, typically biserial radii	isotropic
ONTOGENY	constrained by the OPR	new plates form anywhere

Fig. 1. Comparison of body wall regions described by the extraxial–axial theory as shown in an oblique, oral view of a generalized echinoderm. On left is an early postlarva, on right is a stage at which new axial plates have been added to form the radii and the more aboral regions of the body wall. Ocular, or terminal plate in red, extraxial regions in blue, axial regions in green, orifices and body outline in black.

serialized regions of echinoderm body wall have been particularly alluring, in part because they are distinctive and relatively easily delineated as unified systems. It is important to review some of these systems in order to focus on the one that concerns us the most—the axial system associated with the hydrocoel and ambulacra.

THE OPR IN ADULT ECHINODERMS

Terminal addition in growth zones of extant echinoderms

When Agassiz (1841: 100) coined the term "plaques ocellaires" in an obscure part of a monograph on sand dollar echinoids, he was making reference to a suggestion by Forbes that there were "eyes" in these plates. This reinforced a resemblance to the terminal plate at the ends of the rays on starfish, to which these oculars were evidently homologous. In some ways, it is unfortunate that echinoids became the "model organism" for plate growth in echinoderms, because urchins are highly autapomorphic, and not especially good representatives of the general pattern in part because of the involvement of interambulacral plates in the formation of the test. Agassiz (1841) was most perspicacious in his observations of teratological post-Paleozoic echinoids because he noticed right away that the coronal skeleton of an urchin is composed of five growth zones, each of which is made up of a biserial column of ambulacral plates plus two columns of interambulacral plates, one on each side of the central ambulacrum.

Jackson (1912) showed that there is a fundamental ontogenetic relationship between the ocular plates in the apical system near the summit of the test and both the ambulacra and interambulacra of echinoids, even in Paleozoic forms. During ontogeny, plates in the ambulacra and interambulacra alike are continuously added to the test in a region adjacent to the adoral edge of the ocular plates (Fig. 2). In the ambulacra themselves, a plate is added first to one of the columns in the pair, and then to the other, yielding a distinctive zig-zag pattern. An important consequence of this sequence of plate addition is that there is a chronological gradient within the entire set of plate columns in the growth zone. With the exception of the ocular (terminal in nonechinoids), the youngest plates are always found adjacent to the apical system, and the oldest next to the mouth, with an ocular plate at the head of each growth zone. Because plates appear first in one column, and then the other, the chronological gradient also follows a line that undulates from side to side as well as from the mouth to the ocular.

Interambulacral plates of echinoids are part of the axial body wall. Therefore, the concept of a growth zone has been advanced (Mooi et al. 1994), based in part on the original observations of Agassiz (1841). Mooi and David (1997) developed the concept further in establishing that the interambulacral regions of echinoids are not homologous with the interradii of other echinoderms.

In echinoids, a growth zone comprises an ocular plate and its associated serial columns of ambulacral plates plus adjacent columns of interambulacral plates on each side of the central ambulacrum. In other words, a growth zone encompasses all the plates ontogenetically related to a given ocular, plus the ocular itself. This definition takes into account the pluriserial ambulacra and interambulacra present in some Paleozoic echinoids. However, ambulacral columns are plesiomorphically biserial in all echinoderm clades, and departures from the biserial, zig-zag pattern are derived. Monoserial ambulacra in some edrioasteroids (Bell 1976), the pluriserial condition in certain Paleozoic echinoids (Kier 1965), and the opposite pairing found in ophiuroids and asteroids (Mooi and David 2000) are demonstrably apomorphic within the clades in which these features appear.

Ophiuroids and asteroids have a fully calcified element at the distal end of the growth zone, homologous to the ocular in

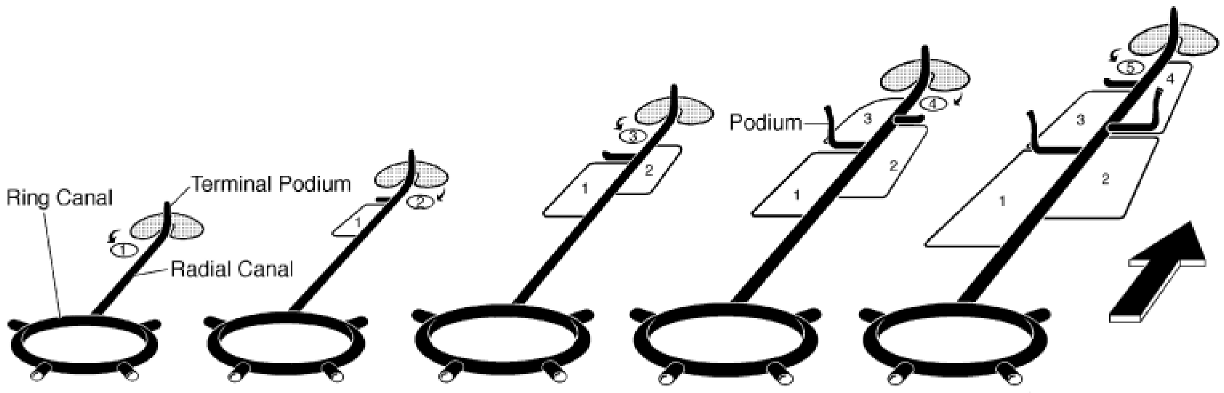


Fig. 2. Terminal addition in echinoderm rays according to the ocular plate rule. Ocular (or terminal) plate shaded, water vascular system black, order of appearance of plates indicated by numerals, large arrow to right indicates elongation of ray, new plates (unfilled ellipses) shown added on that side of ocular indicated by small, curved arrows.

echinoids (Agassiz 1841). No calcified element has been identified as a terminal homologue in holothuroids. Nevertheless, there is a localized region of growth associated with the base of each terminal tentacle of a sea cucumber, and new podia are added proximal to this region (Mooi and David 1997; David and Mooi 1998). It has yet to be demonstrated that there is a calcified terminal element at the tip of each of the many-branched arms of crinoids. Because the axial region of extant crinoids lacks calcified ambulacral plates, it is possible that the terminal is also uncalcified in modern crinoids.

Podia and other axial elements

The fundamental, ambulacral elements of the axial region are accompanied by podia (tube feet). The podium associated with the ambulacral skeletal element appears after the element itself (Fig. 2). There are important developmental events associated with this pattern (see "Commonality of the echinoderm developmental pattern"). The podium can pierce the plate, as in echinoids, or can occur between adjacent ambulacral elements as in basal echinoderms. In the latter, ambulacrals have become known as flooring plates because many of the earliest forms lived with the oral surface upward, and the plates formed the "floor" of the ray (Fig. 3). In general, the plesiomorphic pattern serves to keep one podium per flooring plate.

Podia are extremely rarely preserved in fossil echinoderms. Fortunately, the positions of tube feet are easily determined from attachment structures such as pores and podial "basins" (Spencer and Wright 1966). Only when heavily plated, as in ophiocistioids (e.g., Jell 1983), or under extraordinary taphonomic conditions (Glass and Blake 2004) are podia themselves preserved. Sprinkle (1973) questioned whether blastozoans possessed podia at all, but the general consensus is that they did have them, as evidenced by a study of blastoids (Breimer and Macurda 1972), and that basal echinoderms such as *Camptostroma*, *Stromatocystites*, and *Edrioasteroids* likely had podia (Bell 1976; Smith 1985; Mooi and David 1998).

When the podium is mounted on a pore passing through the plate, this is a derived condition. Such piercing has occurred independently in the echinoids and the asteroids, because in the basal forms of the latter, the podia emerge between the ambulacrals. In addition, the connection through the ambulacra is to an ampulla, and not to a radial water vessel, which is truly internal only in the echinoids (Mooi and David 1997). In ophiuroids, the podia are mounted on highly modified "vertebral" ossicles that are homologous to ambulacrals. In this case, in both basal forms and early postmetamorphic juveniles, the podia emerge between ossicles, not from pores through them (Mooi and David 2000). As expected, fossilized podia on early ophiuroids are between or directly adjacent to plates (Glass and Blake 2004).

The cover plates found in more basal echinoderms such as edrioasteroid-like taxa and blastozoans are associated with axial derivatives. However, the cover plates do not seem to display the ordered and highly conserved terminal addition sequence shown by the ambulacral or flooring plates, and the intercalation of new plates in these series suggests that they are not fully governed by the OPR.

There remains controversy over whether the adambulacral plates of asteroids and the lateral plates of ophiuroids are axial or extraxial (Smith 1997). Whether axial or extraxial, adambulacrals or laterals are not homologous to cover plates (Mooi and David 2000). The serialization of elements directly adjacent to the ambulacral plates and in parts of the body even more distal to the axial region raises questions about the boundary between axial and extraxial regions in some echinoderm groups. If molecular markers can be developed that unequivocally distinguish axial from extraxial regions, many questions concerning terminal addition in echinoderms could be precisely addressed.

Commonality of the OPR in echinoderms

The modified form of terminal addition as expressed by the OPR exists in all echinoderms, fossil and extant. It is, in fact, a synapomorphy for the Echinodermata, and has been used as such to establish the membership within that phylum of a variety of problematic taxa, notably the homalozoans (David et al. 2000). Smith (1985) depicted the pattern in *Stromatocystites* and other early edrioasteroid-like forms. Our analysis of the basal forms *Camptostroma*, *Kinzercystis*, and *Lepidocystis* show the OPR in the flooring plates (Fig. 3, A, B and D). Because the latter two taxa are basal to blastozoans, the pattern is considered ancestral to the patterns seen in more crownward forms such as blastoids and other brachiole-bearing taxa. These taxa demonstrate the same biserial arrangement of axial structures in the brachioles as in ambulacra of other echinoderms (Fig. 3, D and F).

Crinoids seem to be the exception to the rule. The brachial elements supporting the arms of fossil crinoids are plesiomorphically uniserial, and generally contain a deep furrow that was interpreted as the channel in which lay a radial water vessel (Fig. 3G). However, these uniserial elements are undoubtedly part of the extraxial skeleton. The furrow they support houses an extension of the coelom overlain by a soft tissue shelf that supports the radial water vessel (Mooi and David 1997). That this shelf is homologous to the flooring plates of other echinoderms was borne out by recent description of the most basal crinoids (Guensberg and Sprinkle 2001). These early crinoids had flooring plates underlying a radial vessel running up the arms (Fig. 3G), suggesting that crownward crinoids have decalcified the flooring plates, perhaps to save weight in the arms as they are held erect for feeding. Podia are added in accordance with the OPR, but in

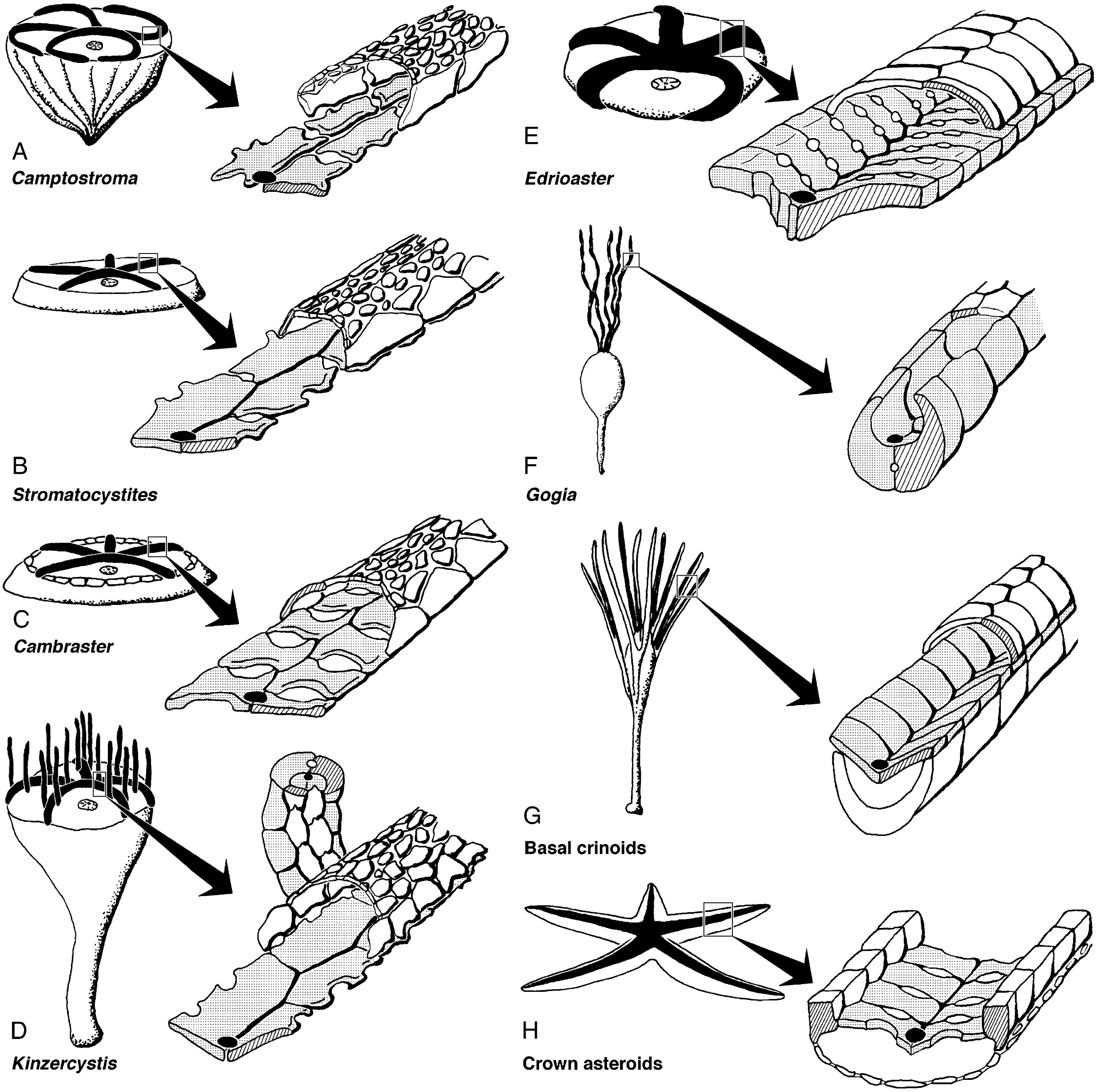


Fig. 3. Arrangements of serial elements formed according to the ocular plate rule in various echinoderms. In each case, the figure drawing to the left shows the axial region (black), and the approximate area shown in detail on the right. Flooring (ambulacral) plates are shaded. Hatched regions show cut edges of plates. Cross-sectional position of radial water vessel indicated by black ellipse. (A) *Camptostroma roddyi*, based on National Museum of Natural History, Washington, holotype NMNH 85181, Lower Cambrian. (B) *Stromatocystites walcotti*, based on Smith (1985), Lower Cambrian. (C) *Cambraster cannati*, based on Smith (1985) Middle Cambrian. (D) *Kinzercystis durhami*, based on Museum of Comparative Zoology, Harvard, paratypes MCZ 582A+B, Lower Cambrian. (E) *Edrioaster* sp., based on Bell (1976), Middle Ordovician. (F) *Gogia* sp., based on Sprinkle (1973), Lower Cambrian. (G) Basal crinoid, based on Guensberg and Sprinkle (2001), Lower Ordovician. (H) Crown asteroid, based on Mooi and David (2000), post-Paleozoic.

the absence of calcified elements. Therefore, this "exception" developed into a prediction that was eventually upheld by the discovery of a fossil form.

Although it was long assumed that holothuroids were a kind of decalcified echinoid, and that the buccal podia of the latter were hypertrophied in holothuroids to become the feeding tentacles, there is abundant evidence to contradict this interpretation (David and Mooi 1996; Mooi and David 1997). Some holothuroid clades possess podia running in bands along the worm-like body from the oral region to the posterior end. Repeated, linearly arranged podia should suggest the operation of the OPR. However, several clades completely lack these bands. Moreover, podia along the body of sea cucumbers do not appear in a chronological gradient, nor is there a blastema ("terminal") near the posterior pole. This led to a new interpretation of the body wall homologies in holothuroids (Mooi and David 1997; David and Mooi 1998), and removed the longitudinal bands of podia from the consideration of terminal addition in echinoderms.

By definition, the staggered addition of plates in each biserial ray of the axial system implies that one of the two first plates must appear before the other in the early juvenile. There is a specific order that determines which plate is laid down first in each ambulacrum, creating a pattern that allows unequivocal homologies for ambulacra throughout the phylum. This is known as Lovén's rule. It has been found in a basal, edrioasteroid-like form (*Stromatocystites*), ophiuroids, and echinoids. There are some hints that it is expressed in holothuroids as well (R. Mooi and B. David, unpublished). Most of these taxa have relatively easily observed plate patterns around the mouth. However, Lovén's rule is not always easy to detect. David et al. (1995) reformulated Lovén's rule as a sequence of plate addition, establishing ontogenetic criteria by which it might be detected in other echinoderms. The wide phylogenetic distribution of Lovén's rule suggests a strongly conserved pattern of plate addition rooted in the ontogeny of all echinoderms. The implications of Lovén's rule have been discussed in the context of echinoderm evolution by David et al. (1995) and Hotchkiss (1995).

ECHINODERM DEVELOPMENTAL PATTERNS

Embryology and the EAT

The major precepts of the EAT are strongly correlated with the development of echinoderms (David and Mooi 1996). It is unfortunate that the "model organism" for studies of the development of echinoderms has often been an echinoid. Sea urchins are highly derived with respect to the relative amounts of axial and extraxial body wall in the adult, and therefore in developmental events that lead to these distinctive apomorphies (Mooi and David 1998). Echinoderm larvae have experienced diversification of life histories obscuring commonalities that exist, and reliance on only a few examples can be problematic when trying to elucidate the evolutionary history of echinoderm embryology (Smith 1997; Lowe et al. 2002).

Although many differences in development have been recorded among echinoderm groups (Smiley 1988; Giese et al. 1991), they tend to be superficial in many cases, and the major events in development, particularly early embryogenesis, are very similar among the major clades. For that reason, generalization of the sea urchin pattern is applicable to events characterizing other clades. Focusing on these events will help in interpreting genetic expression patterns.

After gastrulation, the larva begins to show a bilaterally symmetric arrangement (Fig. 4A). There exist left and right axocoels, a pair of mesocoels fated to become the hydrocoel (left) and the "dorsal sac" (right), and paired right and left somatocoels. In more crownward forms (acroechinoids), early stages of development, particularly those in connection with the hydrocoel, occur within an ectodermally derived chamber called the vestibule. Basal echinoids such as cidaroids and echinothurioids lack the vestibule (Emlet 1988; Amemiya and Emlet 1992). That this is the plesiomorphic condition is also supported by the fact that asteroids also lack a vestibule, and that it is often poorly developed in ophiuroids and holothuroids.

In terms of the OPR, events on the left side of the larva, particularly involving the left mesocoel, are the most important (see David and Mooi 1996 for a detailed description). The ectodermal region over this mesocoel thickens and comes into contact with it, and the left mesocoel becomes recognizable as the hydrocoel (Fig. 4B). Along with a portion of the left somatocoel, which comes to lie under the developing hydrocoel, a complex of tissues interact to form the "adult rudiment," in which critical events occur to establish the rays. The rudiment is fated to comprise the axial portions of the body wall of the adult (David and Mooi 1996). The nonrudiment regions of the larva, particularly those associated with the right somatocoel, will become the extraxial region (Fig. 4C). Therefore, axial and extraxial skeletal elements display radically different embryological origins.

The hydrocoel begins to form an incomplete torus that encircles the future position of the esophagus. Before the torus closes, five equally spaced extensions develop on the outer edge of the crescent. These are known as the primary lobes (Fig. 4B). The primary lobes project outwards and slightly towards the ectoderm. If there is a vestibule, they project slightly into the vestibular cup.

The primary lobes induce formation of the primary podia—combinations of ectoderm and mesoderm that will become the "terminal tentacles" (Fig. 4C). The terminal tentacles represent the ends of the growing radial water vessels. As summarized in David and Mooi (1996), a series of events in the region overlying the hydrocoel leads to the formation of epineural sinuses and nerve tracts that pass along the ray. The patterning of these nerves can be important insofar as gene expression domains are concerned.

Metamorphosis in echinoderms causes the primary larval tissues to be rearranged to accommodate the growing predominance of the rudiment. In asteroids, ophiuroids, and echinoids, metamorphosis is pronounced because so much of the larval body has to be reduced to result in the relatively high proportion of the axial region in the adult. The conspicuous evagination of the rudiment (in forms with a vestibule), combined with the addition of new plates at the edges of the terminals, establishes the increasingly comprehensive

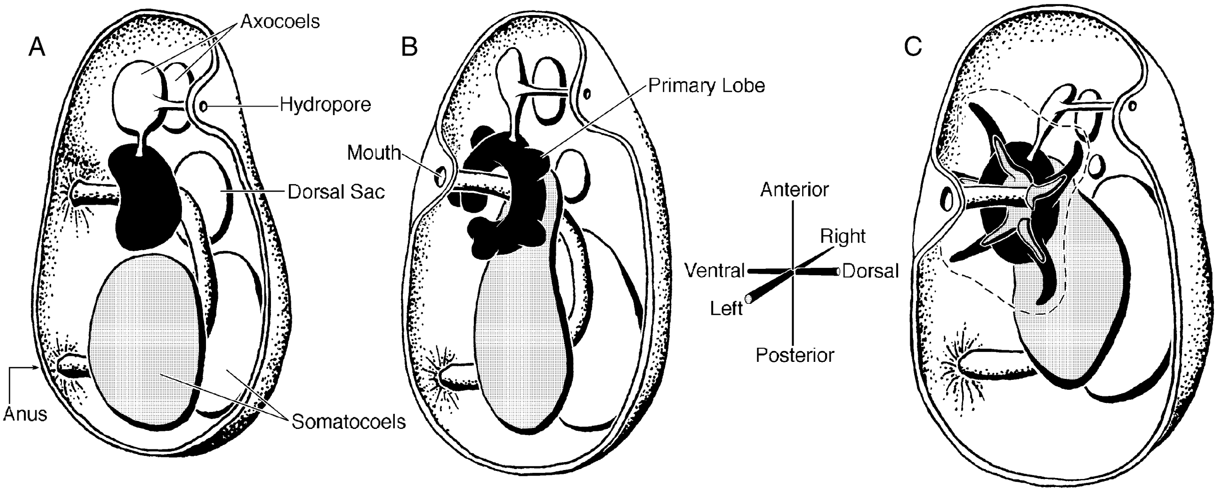


Fig. 4. Diagrammatic, cut-away views of three stages in the early development of the rudiment in echinoderm larvae. Hydrocoel in black, left somatocoel shaded. (A) Early larvae showing arrangement of three pairs of coeloms along the anterior–posterior axis. (B) The hydrocoel torus begins to differentiate into a ring with primary lobes, but is not yet closed. (C) The rudiment becomes distinct through interaction with the left somatocoel, and the appearance of primary podia. Structures within the broken line are destined to become part of the axial region in adults, those outside the line will become extraxial. Coelomic stacking is initiated.

part played by the axial skeleton in the body wall of crownward echinoderms. Plesiomorphic taxa, such as crinoids, and paedomorphic forms, such as holothuroids, retain significant amounts of the extraxial region into adulthood. Because the extraxial region is correlated with the larval body, less metamorphosis is required to produce the adult proportions (Mooi and David 1998).

Just prior to metamorphosis, skeletal elements begin to develop. The order of appearance of the skeletal elements can vary depending on the taxon, but the positions in which they are initiated are relatively constant. In echinoids, some of the genital plates develop in association with the rods that support the larval arms. The terminal plates start to form in the rudiment on the outer side of the primary podia, before encircling their bases. The first ambulacral plates arise close to the terminals, just proximal to them and adjacent to the position at which the mouth will open. These are the first elements to form according to the OPR. In echinoids, supplementary ambulacral and interambulacral plates are produced at the proximal edges of the terminals according to the OPR. Insertion of plates next to the oculars induces expansion of the test, or lengthening of the rays. New podia appear according to the OPR, either just distal to the accompanying plate, or through a pore in the plate itself (Fig. 2).

In a critique of the EAT with respect to larval evolution in echinoderms, Smith (1997) claimed that some axial elements are derived from elements in the larval (pluteal) body of echinoids. In particular, two of the five ocular plates originate in association with the skeletal rods. To Smith (p. 234), this suggested that "either the correspondence between larval or rudiment tissue and axial/extraxial skeleton is imprecise, or ocular plates are part of the extraxial system."

Similarities between the two types of pluteus larvae, the echinopluteus of echinoids and the ophiopluteus of ophiuroids, are purely superficial (Byrne and Selvakumaraswamy 2002). Differences in skeletogenesis between the two types of plutei indicate that skeletal elements and overall "pluteus" organization of these forms are not homologous. The manner of production of the rods attached to the oculars of developing echinoids is best interpreted as autapomorphic. In addition, calcified terminal plates do not exist in all echinoderm clades (only in asteroids, ophiuroids, ophiocistioids, and echinoids). A subset of the ocular plates in echinoids have been co-opted in the development of the larval rods so that they can serve as anchor points in the early ontogeny of the planktotrophic larva. In direct developers, the oculars appear in the oral mesoderm, completely independent of any larval rods. Specialized structures found only in the feeding larvae of echinoids, and then on only a minor subset of oculars that otherwise form in exactly the same way in all other echinoderms are not compelling challenges to the EAT.

Commonality of the echinoderm developmental pattern

Many past treatises on the early development of echinoderms emphasized the disparity among the ontogenies of the major echinoderm clades (see David and Mooi 1996 for discussion). These differences are important, and are paralleled by differences among the overall larval morphologies in the groups involved. However, fundamental similarities in the sequences of events that mark the development of the rudiment are considerably more important in understanding how terminal addition in the rays is initiated and in correlating these events with gene expression domains.

Echinoderm groups studied so far can be seen to share several fundamental events: (i) the hydrocoel encircles the position of the future digestive tract; (ii) five primary lobes appear on the outside rim of the toroidal hydrocoel that will form the ring canal; (iii) the ectoderm, primary lobes, and, in part, the left somatocoel work in concert to form the rudiment (sensu David and Mooi 1996, 1998); (iv) in forms with a calcified terminal, the latter forms just distal to the terminal tentacle; (v) new elements in the axial region form just proximal to the terminal tentacle, along with their accompanying podia.

Exceptions to this pattern are rare, and even when they do occur, serve to highlight the seminal nature of these events to the expression of the OPR in the rudiment. For example, it can be shown that holothuroids undergo almost no metamorphosis—a situation underlying the fact that the term "rudiment" has seldom been used in reference to holothuroids. Nevertheless, holothuroids do possess a rudiment in which all of the commonalities described above occur. As discussed in David and Mooi (1996, 1998) and Mooi and David (1997), in sea cucumbers the rudiment does not develop beyond the region within the tentacular circlet. The process of terminal addition according to the OPR is suppressed, restricting the primordial rays to one end of the elongated body. Holothuroids appear to be strongly paedomorphic in this regard.

"Paleo-ontogeny"

The above descriptions of echinoderm morphology and embryology indicate that there are several places in which systems of serial elements can be found in echinoderms. The first order of seriality occurs in the coelomic arrangement in the larva, and later in adults as the coelomic stacking of hydrocoel, left somatocoel, and right somatocoel becomes more pronounced. The second order is in the axial region, in which terminal addition is most strongly expressed by the way in which new elements are added according to the OPR. The third and less significant order can be found in far less universal ordering of elements in the extraxial body wall region.

The ubiquity of the basic patterning in echinoderm development in extant forms, especially with respect to terminal addition, strongly suggests that the earliest echinoderms shared these patterns. The divergence of crinoids likely occurred during the Middle to Late Cambrian (Smith 1988; Guensberg and Sprinkle 2001). Therefore, the fundamental events described above were well established by that time. They also likely pertained to the edrioasteroid-like forms that predated the emergence of both blastozoans and crinozoans (Mooi and David 1998).

Much has been made of the so-called "departures" from "perfect" pentaradial symmetry shown by taxa such as asteroids and echinoids in which each ray proceeds outward directly from the mouth (Fig. 3H). One such important "departure" is evinced by what has become known as the 2–1–2 pattern of basal edrioasteroid-like taxa (reviewed in Hotchkiss 1998), in which only three main rays proceed outward from the mouth. Two of these rays then bifurcate to form a total of five rays (Fig. 3, A–E). This pattern is plesiomorphic for the Echinodermata, and the situation in the asteroids, ophiuroids, echinoids, and holothuroids is highly derived and somewhat atypical when viewed against the backdrop of the entire evolutionary history of the phylum.

Because modern crinoids show the 2–1–2 pattern, it is possible that we have access to embryological data for this pattern. If the 2–1–2 pattern seen in crinoids today represents the plesiomorphic state for echinoderms in general, it would appear that it does not preclude the existence of five distinct primordial lobes of the kind seen in larval development of extant forms. Crinoids do not seem to have any developmental modifications that would reflect the 2–1–2 arrangement of arrays in the adult. Even more remarkable, Lovén's rule, which is a direct consequence of the way in which plates are laid down according to the OPR, is found in very early forms with 2–1–2 symmetry such as *Stromatocystites*.

Some of the most disparate of echinoderms are found among the blastozoan clades and especially among the homalozoans. Some homalozoans such as stylophorans are best considered as crinozoans with reduced arm number. Others, such as the homoiosteleans, have a brachiole that places them in the blastozoan clade. Given that crinozoans and blastozoans have axial regions that grow in accordance with the OPR, even these very early, highly divergent taxa with reduced numbers of rays and modified plate architectures show serialization of the rays according to the OPR (David et al. 2000). There is no evidence to suggest that a rudiment with a primordial lobe or lobes could not have existed.

Therefore, there are several lines of argument that can be used to support the idea that even the most unfamiliar fossil echinoderms had developmental patterns essentially like those of the modern forms. Most importantly, the developmental events that lead to the expression of terminal addition according to the OPR were well established in the earliest echinoderms. The supposition that eleutherozoans (echinoids, asteroids, ophiuroids, holothuroids) and cystoids have "different expressions of pentamery" (Smith 1997: 237; see also Hotchkiss 1998) overlooks the fact that these fundamental developmental pathways are well established in all echinoderms, whether they have 2–1–2 symmetry, or any other arrangement of rays around the mouth. Attempts to place blastozoans basally and edrioasteroid-like forms more crownward with eleutherozoans rest almost solely on the inability to establish the existence of Lovén's rule in the blastozoans

(Smith 1997) and crinozoans. So much in the ontogeny of echinoderms is conserved that we expect some manifestation of Lovén's rule to be found in these clades as well. This would remove another barrier to the idea that the ancient, edrioasteroid-like echinoderms do not share most recent common ancestry with the eleutherozoans, but instead represent the earliest echinoderm body form (Mooi and David 1998).

GENE EXPRESSION PATTERNS AND THE OPR

The contrast of morphology with gene expression

When we compare echinoderms with other phyla, we see that the "reorganization of body architecture involved extensive changes in the deployment and roles of homeobox genes" (Lowe and Wray 1997: 718). Lowe and Wray highlighted instances by which the highly derived, radially symmetric adult morphology of echinoderms evolved by modifications of the roles and expression domains of genes that they likely inherited from ancestors shared with bilateral sister taxa such as chordates.

We have shown that in spite of divergence among final adult forms, the fundamental properties of the axial region, the OPR, and the ontogenetic pathways by which these properties develop are common to all echinoderms. One might expect that evolutionary morphological conservatism be correlated with relative conservatism in gene expression. Is this prediction borne out within the Echinodermata? The answer seems to be both yes and no.

Wray and Lowe (2000) discussed evidence that many of the genes studied so far have variable expression in the extant clades of echinoderms. Most of these vary because their roles have been recruited and then altered from that in the ancestor with other echinoderm groups. Lowe et al. (2002) performed a survey of expression patterns for *distal-less* and *orthodenticle* in several taxa, each of holothuroids, echinoids, and asteroids. Just as has been observed in other phyla (Wilkins 2002), details in patterns of expression vary within each of these clades. This is also not overtly surprising, given the morphological disparity in adult echinoderms.

By examining a specific case from adult morphology, the terminal addition sequences in the rays, we have found that very important commonalities in gene expression patterns do occur in both the rudiment and along the rays. We can now determine if they are correlated with the strongly canalized processes that lead to the OPR and the terminal addition in rays of echinoderms.

Gene expression in the rudiment

Even before formation of the rudiment begins through differentiation of the hydrocoel (Fig. 4), there is much going on within the larva that lays the foundation for the establishment not only of the rudiment, but of the essential radial symmetry shown by the adult echinoderm. Most importantly, *hox* genes have been detected that are expressed during the establishment of the somatocoels and the hydrocoel (Arenas-Mena et al. 1997, 2000). There are other genes that are expressed early enough in the ontogeny of echinoderms to suggest boundaries between the rudiment and the rest of the larval body. Among these are *not* and *brachyury* (Peterson et al. 1999). In the rudiment, both are expressed in the prospective adult ectoderm of echinoids. Because the rudiment is an apomorphy unique to the Echinodermata, the modification of these genes to perform patterning roles in this structure must also be apomorphic. The *wnt-5* gene is expressed in the radial nerves of postmetamorphic echinoids (Ferkowitz and Raff 2001), but it is not yet known whether this is analogous to the expression of *engrailed* in ophiuroids, or what it might mean in terms of the OPR.

distal-less is among the most distinctively expressed genes in the rudiment itself. As summarized by Lowe et al. (2002), *distal-less* is expressed in three main phases in several echinoderm groups. For example, in echinoids it is found in ciliary bands of early larvae. A second phase reveals expression only on the left side of the larva, almost perfectly delineating the boundary between the vestibular and larval ectoderm (Fig. 5). This pattern highlights the boundary between the axial region represented by the rudiment, and the extraxial region of the rest of the pluteal body. Vestibular ectoderm in contact with the hydrocoel continues to express *distal-less*, notably as the primary lobes are formed. In a third phase, the protein products of the gene persist in the hydrocoel as it differentiates into the water vascular system.

As discussed earlier, the vestibule appears to be apomorphic for acroechinoids. Therefore, expression of *distal-less* specifically within the vestibule might reflect cooption of the gene for a patterning role in the formation of that structure within the crownward echinoids, as suggested by Wray and Lowe (2000). However, the assumption that *distal-less* is specific for the formation of the vestibule requires further examination. Because the gene seems to be expressed in association with the rudiment in other forms (Fig. 5), whether or not there is a vestibule, it might constitute a more general marker for rudiment formation, and not just for the vestibule. For example, in holothuroids, Distal-less proteins are present in scattered nuclei of presumed nerve cells in the five primary lobes of the rudiment.

distal-less might be inferred to have a role in establishing radial symmetry in echinoderms. The expression of *orthodenticle* and *distal-less* is closely correlated in some forms (Lowe et al. 2002). It would appear that the latter in particular has a close association with the hydrocoel, and therefore with structures of axial origin, including the podia. The gene is expressed early enough to show up in the primary lobes in the rudiment of both asteroids and holothuroids as well as in echinoids (Lowe et al. 2002). These structures initiate the

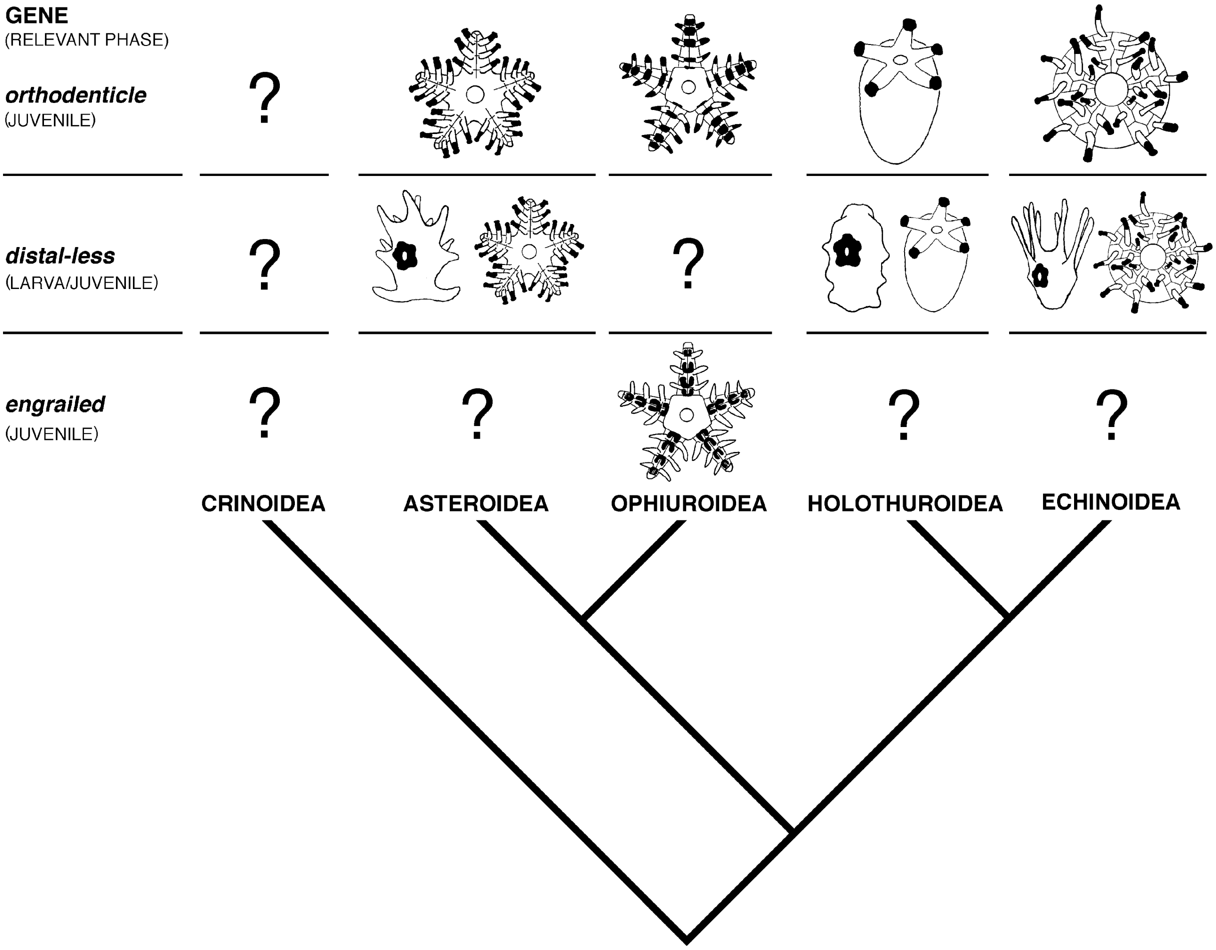


Fig. 5. Summary of expression domains (black regions) for three genes related to the extraxial–axial theory and ocular plate rule expressed in the axial region of echinoderms. See text for explanation. Question marks indicate lack of data.

pentaradial axial morphology of all echinoderms. Therefore, it is essential that more work be done with genes such as *distal-less* in order to determine their correlation with events in the establishment of adult echinoderm symmetry. The expression domains of other genes studied to date appear too late in the development of the postlarva to suggest involvement with symmetry determination (Wray and Lowe 2000), or so early that they can only be inferred to have a role in establishing basic patterns in coelom arrangement (Arenas-Mena et al. 1997, 2000). Of course, this does not preclude the likelihood that many other genes are involved in the establishment of radial symmetry, notably *hox* genes, but only general patterns are emerging, and the precise mechanisms are not yet well understood.

Gene expression along the rays

The *engrailed* gene has been well studied in ophiuroids (Lowe and Wray 1997; Wray and Lowe 2000), and seems to hold the most promise in terms of its correlation with formation of axial structures. It is of interest in the context of the OPR because of its expression pattern in postmetamorphic juveniles. *engrailed* appears in the neurons of the radial nerves of ophiurioids, as well as in regions between elements in the ray (Fig. 5). More specifically, the Engrailed protein shows up in the nuclei of cells in areas of active skeletogenesis, notably in bands that seem to correspond to the edges of the paired ambulacral plates in the axial skeleton (Lowe and Wray 1997). In ophiuroids, these paired plates later fuse to form the “vertebral” ossicles that lend the brittlestar arm such unparalleled flexibility. The degree to which *engrailed* is expressed in the boundaries between elements at the tips of the rays is reduced proximally (i.e., in older parts of the ray), but neuronal expression remains strong even in “the older, more proximal segments” (Lowe and Wray 1997: 721).

In this neuronal pattern of expression, *engrailed* appears in paired ganglia along the ophiuroid arm (Fig. 5). This is reminiscent of the pattern seen in bilateral phyla, in which it likely plays a role in neural differentiation. However, as Lowe and Wray (1997) noted, the nerves running along the axis of echinoderm rays plesiomorphically followed the same zig-zag pattern exhibited by the plates. Both ophiuroids and asteroids have derived ambulacral plating in which the paired plates are directly opposite one another across the line marked by the radial water vessel (Mooi and David 2000). The nerve tracts also follow this modification. Although Turner (1998a) relied on the similarity of this pattern to that in bilateral phyla to emphasize metamerism in echinoderms, it is clear that mirror-image bilateral symmetry along the proximal–distal axis of the arms in both ophiuroids and asteroids is a derived condition among echinoderms (Mooi and David 2000). Therefore, any similarities in either morphology or expression of *engrailed* to the conditions found in bilateral metazoans are superficial and because of convergence (Lowe and Wray 1997).

Unfortunately, *engrailed* expression remains unstudied in other echinoderm clades (Fig. 5), except for some preliminary results in asteroids (M. Byrne, personal communication) in which *engrailed* is expressed in a manner similar to that in ophiuroids. Antibodies used to highlight the protein in ophiuroids do not bind to the protein in echinoids (Lowe and Wray 1997). If technical problems such as this can be overcome, *engrailed* holds promise in studies of OPR patterning in postmetamorphic echinoderms, particularly in delineating the boundaries between axial and extraxial components of the body wall. Examination of the patterning in echinoids should support the assertion that interambulacral plates are axial derivatives (Mooi et al. 1994), and whether marginals, carinals, and even adambulacrals of asteroids are extraxial, as suggested by Mooi and David (2000).

Proteins coded by the gene *distal-less* are also present in axial elements of echinoderms. *distal-less* is expressed in the podia of echinoids, asteroids, and the buccal tentacles of holothuroids, but has yet to be found in the podia of ophiuroids (Lowe and Wray 1997) (Fig. 5). The continuity of expression from the rudiment and into the axial elements of the adult suggests that this gene is strongly associated with the development of the hydrocoel, and that it could inform analyses of expression domains in the context of the OPR.

orthodenticle is expressed in a wide variety of regions in developing echinoderms (Fig. 5). In the embryos and larvae of echinoids and holothuroids, it is found in the ectoderm associated with the mouth and ciliated bands. However, in postmetamorphic juveniles of ophiuroids, asteroids, echinoids, and holothuroids, it is most strongly expressed in the ectoderm of the podia (Wray and Lowe 2000). Interestingly, *orthodenticle* is expressed in the buccal tentacles of holothuroids as well as in the podia of the other major clades. As stated earlier, the EAT has predicted homology between the tentacles of holothuroids and podia of other groups.

In ophiuroids, *orthodenticle* is expressed at high levels at the tips of the arms, particularly in the ectoderm overlying the terminal (Fig. 5). The strength of the expression declines proximally (Lowe and Wray 1997). Therefore, there is a correlation of the degree to which the gene is present with the age of the axial elements. Whether or not this is a causal correlation remains to be determined, but the pattern is very suggestive of the OPR. However, *orthodenticle* has not yet been detected in association with the oculars of echinoids.

CONCLUSIONS

Even among the disparate morphologies we see in echinoderms past and present, important commonalities exist in both adult form and developmental trajectories. This conservatism is not always shared by gene expression patterns. Although some correlations exist among these genes, they are not universal even among the small numbers of taxa sampled so far. Important exceptions exist, and it remains to be seen whether these are artifacts of the methods being used, sampling error, or real differences in the ways the genes are employed during the diversification of the Echinodermata. The assumption that genetic patterns are relatively free of homoplasy, and therefore more replete with phylogenetic signal, is not borne out by attempts to correlate their roles with early development and the patterns dictated by the OPR, both of which are universal throughout the Echinodermata.

The field has not yet reached full understanding of genetic markers for axial or extraxial skeletons. However, it does appear that certain proteins are expressed in connection with some of the most distinct synapomorphies for the Echinodermata. Several patterning genes accompany the initiation of the rudiment in the majority of extant clades. The earliest events in the operation of the OPR (notably the formation of the primary lobes) are also accompanied by the expression of specific genes, particularly *distal-less*.

Expression patterns in genes such as *engrailed* and the *hox* genes studied by Arenas-Mena et al. (1997, 2000) will likely prove useful in determining the presence or absence of an anterior–posterior axis in echinoderms. Peterson et al. (2000) used the EAT and evidence from homeobox genes to discuss coelomic stacking, and combined several lines of evidence to show that the left and right somatocoels are posterior in extant forms. This conforms to conclusions presented by David and Mooi (1998). The "rays as appendages" hypothesis (Hotchkiss 1998) seemed to have some merit in terms of this primary axis. The assumptions of homology between expression of *engrailed* in the dorsal nerve chord of chordates and that in radial nerves of echinoderms are not well founded, and we prefer to regard its patterning in ophiuroids as indicative of serialization of repeated units that could shed light on this question.

However, the idea that there are analogies between appendages of bilateral forms and the rays of echinoderms (in part because they are arranged orthogonal to the anterior–posterior axis of the animal) is fraught with difficulties. The derivation of the "appendages" from a region elaborated from a coelom only on the left side of the larva cannot be indicative of a detailed relationship to appendages of other phyla. As an analogy, it might be useful in determining general patterns in origination (Hotchkiss 1998), but in detail, the implications for phylogeny are problematic, and the patterns of gene expression seem to bear that out. In the absence of anything remotely like it in other phyla, we regard the pentaradial development of the primary lobes in the rudiment as a deeply rooted autapomorphy of the Echinodermata. Labeling a ray as some form of appendage could be misleading without consideration of this unique condition.

Figure 5 highlights another important feature of the research to date on gene expression from rudiment to ray in echinoderms. Unfortunately, we must note the lack of data for what will likely prove to be pivotal taxa. There are no data for any of these genes for crinoids, the basalmost living form, and the only extant group with 2–1–2 symmetry of the rays. Knowledge of expression regimes in the Crinoidea would constitute an ideal opportunity to test the relative apomorphy of many of the patterns so far determined in other taxa. There are major questions concerning the distribution of axial and extraxial skeletons along the rays of crinoids that could be explored if data become available. For example, the determination of spatial and temporal patterning for genes such as *orthodenticle* along crinoid arms could be helpful in determining whether serialized elements occur along the oral surfaces of the rays in crinoids. Expression patterns for *engrailed* are so far known only in ophiuroids. Given the strong OPR-related patterns displayed by this gene, overcoming related technical problems will prove rewarding.

The known roles for *engrailed* and *orthodenticle* seem to be bearing important information with respect to the OPR, and the EAT in general. That *engrailed* is expressed along the boundaries between axial elements is intriguing, but further research is required to determine whether it is evident during skeletogenesis of repeated extraxial elements as well. *orthodenticle* is clearly a constrained marker for the axial skeleton. Honing the observation of expression topologies is necessary to determine how useful it might be in finding the boundary between axial and extraxial body wall regions. In any case, *distal-less* highlights the differences in developmental trajectories between axial and extraxial regions. In postmetamorphic juveniles, it seems to be restricted to axial derivatives, because it is found not only in the rudiment, but in the developing podia as well.

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