

The evolution of echinoderm development is driven by several distinct factors

Gregory A. Wray and Alexandra E. Bely

Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook, NY 11794, USA

SUMMARY

We analyzed a comparative data base of gene expression, cell fate specification, and morphogenetic movements from several echinoderms to determine why developmental processes do and do not evolve. Mapping this comparative data onto explicit phylogenetic frameworks revealed three distinct evolutionary patterns. First, some evolutionary differences in development correlate well with larval ecology but not with adult morphology. These associations are probably not coincidental because similar developmental changes accompany similar ecological transformations on separate occasions. This suggests that larval ecology has been a potent influence on the evolution of early development in echinoderms. Second, a few changes in early development correlate with transformations in adult morphology. Because most such changes have occurred only once, however, it is difficult to distinguish

chance associations from causal relationships. And third, some changes in development have no apparent phenotypic consequences and do not correlate with obvious features of either life history or morphology. This suggests that some evolutionary changes in development may evolve in a neutral or nearly neutral mode. Importantly, these hypotheses make specific predictions that can be tested with further comparative data and by experimental manipulations. Together, our phylogenetic analyses of comparative data suggest that at least three distinct evolutionary mechanisms have shaped early development in echinoderms.

Key words: evolution of development, regulation of gene expression, cell lineage, gastrulation, sea urchin, comparative method

INTRODUCTION

The processes of early development are often conserved among animals with similar adult body plans. Comparative embryology provides many examples of conserved patterns of cell lineage, gene expression and morphogenetic movement within phyla and classes (e.g., Kumé and Dan, 1968; Anderson, 1973; Patel et al., 1989). These empirical data support a widespread expectation: that any modification to embryogenesis will have grave phenotypic consequences, thereby constraining the evolution of early development (Gould, 1977; Arthur, 1988; Thomson, 1988). Together, the empirical data of comparative embryology and the assumption of constraint have produced a traditional view of how developmental processes evolve. This traditional view posits strong and direct links between early development and adult morphology.

But developmental processes often evolve in ways that violate this traditional view. A large body of empirical evidence demonstrates that the link between embryos and adult body plans is evolutionarily rather labile. First, early development can be conserved in species whose adults are very different. The embryos of clams and earthworms, for example, share distinctive features of axis formation and cell lineage, as well as cell movements during gastrulation and coelom formation (Kumé and Dan, 1968; Freeman and Lundelius, 1992). Similarities in the expression and function of homeotic genes in arthropods and mammals provide another example

(McGinnis and Krumlauf, 1992). If processes of early development were required only to build features of adult morphology, they would not be conserved among phyla with very different body plans. Second, and more telling, early development can differ profoundly among species that are closely related and morphologically very similar as adults. This phenomenon is known from many phyla and encompasses a broad range of developmental processes (e.g., Lillie, 1895; Garstang, 1929; Berrill, 1931; Coe, 1949; Elinson, 1990; Byrne, 1991; Wray and Raff, 1991a; Jeffery and Swalla, 1992; Janies and McEdward, 1993; Henry and Martindale, 1994). If the processes of early development only evolved in order to produce a modified adult morphology, they would not differ among closely related and morphologically similar species.

Together, these two phenomena demonstrate that the relationship between early development and adult morphology is likely to be complex. Certainly, there is no simple one-to-one mapping of embryogenesis onto adult phenotype. But if adult body plans are not sufficient to explain evolutionary similarities and differences in early development, what are the evolutionary mechanisms that conserve and alter developmental processes? In this paper, our objective is to ask *why* developmental processes evolve. We examine the evolution of early development in echinoderms, a clade particularly well suited to addressing this question. We consider several distinct kinds of developmental processes, and posit testable hypotheses to explain why they have or have not evolved.

REPLICATE TRANSFORMATIONS AND HYPOTHESIS TESTING

In trying to understand evolutionary history, it is rarely feasible to carry out actual experiments. Fortunately, evolution has itself provided replicate “experiments” in the form of parallel phenotypic transformations. Examples include transformations between short and long germ band development in insects; between panoistic and meroistic oogenesis in insects; between egg laying and livebearing in vertebrates; between mono- and polyembryony in several phyla; between free-spawning and internal fertilization in most phyla; between parasitic and free-living life cycles in many invertebrates; and between feeding and non-feeding larvae in most phyla.

Besides their parallel origins, these replicate transformations share two important features. First, they are all associated with significant changes in developmental mechanisms. Thus, patterns of gene expression, cell division, and morphogenesis correlate with type of germ band development in insects (Sander, 1983; Patel et al., 1989, 1994; Brown et al., 1994) and with type of larvae (feeding versus non-feeding) in echinoderms (Wray, 1994). And second, these replicate transformations all reflect ecologically significant differences in life history strategies. Whether a vertebrate lays eggs or is live bearing, or whether a nematode is parasitic or free-living has profound ecological consequences. Together, these two features provide a way of relating changes in developmental mechanisms to altered selection regimes.

Parallel phenotypic transformations provide exceptional test cases for teasing apart the various factors that drive evolutionary change and stability in development. Replicate events allow one to determine whether the correlation between developmental change and some external factor, such as an altered life history strategy, is due to chance or reflects a causal relationship. By extending comparisons of development to several replicates, it is possible to rule out chance associations statistically. As illustrated in Fig. 1, comparative data for the same number species may be statistically nonsignificant if only one transformation has occurred, but highly significant when more than one replicate transformation is considered. Thus, studying replicate phenotypic transformations allows one to identify the probable external cause of the evolution of a developmental feature. Functional explanations can then be sought for such cases. We illustrate this approach by examining the evolution of several functionally distinct developmental processes in echinoderms.

ECHINODERMS AS A MODEL CLADE FOR STUDYING DEVELOPMENTAL EVOLUTION

Features that make a single species suitable for studying development are not the same as those that make a group of species suitable for studying the *evolution* of development. Of particular importance in analyzing the evolution of developmental processes are the following: a detailed understanding of phylogenetic relationships, a fossil record, the existence of replicate transformations, and an extensive comparative data base of developmental studies. Echinoderms meet all of these criteria (Fig. 2) (Smith, 1984, 1992; Wray, 1992, 1994).

In particular, echinoderms provide a variety of replicate

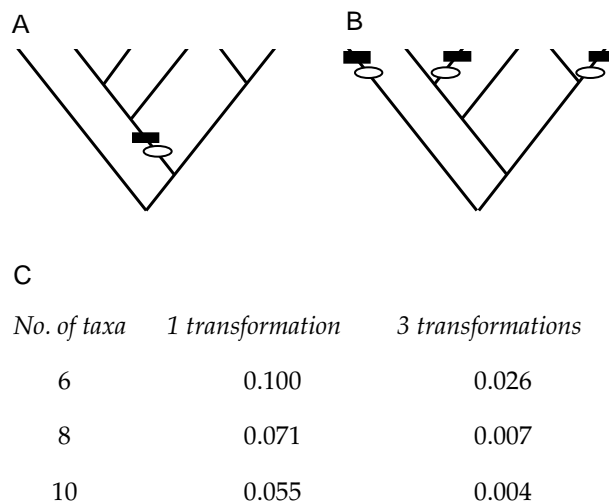


Fig. 1. The importance of replicate transformations in testing for the association between an evolutionary change in two characters. (A,B) Two characters that change in concert are mapped onto a hypothetical phylogeny in two ways. The associated changes have occurred once in phylogeny A and three times independently in phylogeny B. Note that both phylogenies have the same number of taxa representing each character state. The concentrated changes test (Maddison, 1990) indicates that the association between the changes is significant in phylogeny B ($P=0.02$) but not phylogeny A ($P=0.1$). (C) The strength of association between two characters is affected by the number of replicate transformations and the number of taxa included in the analysis. P -values from the concentrated changes test are given for a series of hypothetical phylogenies. The association becomes more robust when replicate transformations have occurred and when more taxa are included in the analysis. These values emphasize the importance of examining developmental processes in several species and across replicate evolutionary transformations.

transformations in life history strategies (Strathmann, 1978; Raff, 1987; McEdward and Janies, 1993). Of these, the switch from feeding to non-feeding larvae has been studied in the most detail (Byrne, 1991; Wray and Raff, 1991a; Raff, 1992). Feeding and non-feeding larvae differ substantially in their morphology, behaviour and ecology (Strathmann, 1985; Wray, 1992; McEdward and Janies, 1993). In particular, non-feeding larvae nearly always lack a mouth and functional digestive tract, the arms used for feeding are lost or reduced, the supporting endoskeleton is lost or reduced, and the time to metamorphosis is greatly accelerated. Differences in the embryonic development of species with feeding and non-feeding larvae are extensive, and include functionally profound changes in oogenesis, axis formation, cell fate specification, cleavage geometry, gene expression, and morphogenetic movements (Wray and Raff, 1989, 1990, 1991b; Henry and Raff, 1990; Scott et al., 1990; Raff, 1992).

Mapping feeding versus non-feeding life history strategies onto a cladogram for echinoderms tells us three important things. First, the polarity of the transformation is invariably from feeding to non-feeding larvae (Strathmann, 1978; Raff, 1987). Second, lecithotrophy has evolved literally dozens of times from planktotrophy (Emlet, 1990; our unpublished tallies). Among sea urchins, the group we will focus on, this transformation has evolved at least 20 times. And third, this

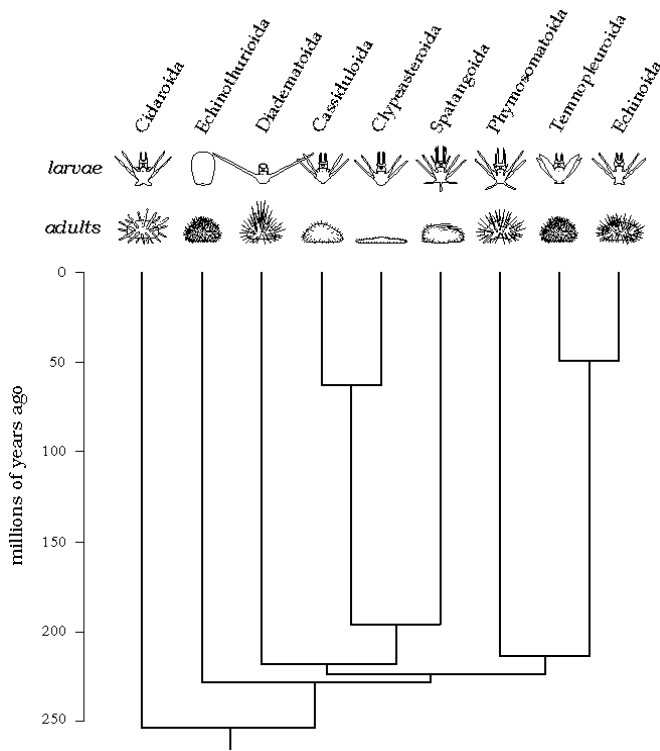


Fig. 2. Phylogenetic relationships, divergence times, and characteristic morphologies of major sea urchin orders. The estimated phylogenetic relationships of sea urchin orders is shown, following recent cladistic analyses based on adult morphology (Smith, 1984, 1988; Smith et al., 1993). Estimated divergence times are based on Smith (1984).

transformation is not associated with any specific changes in adult morphology (Wray and Lowe, unpublished). In particular, very closely related and morphologically similar species can differ in larval type.

WHY ECHINODERM DEVELOPMENT EVOLVES

In this section, we examine the various evolutionary mechanisms that influence the evolution of developmental mechanisms in echinoderms (Table 1). The examples cover three fundamental and functionally distinct developmental processes:

regulation of embryonic gene expression, specification of cell fates, and morphogenetic movements.

Gene expression

The first example concerns evolutionary changes in the regulation of early zygotic gene expression. The expression of the gene *msp130* has been studied by antibody localization in several species (Parks et al., 1988, 1989; Wray and McClay, 1988, 1989; Amemiya and Emler, 1992). *msp130* protein is part of the mesodermally derived envelope that surrounds the endoskeleton (Decker et al., 1988; Parr et al., 1990). Although the endoskeleton is biochemically similar throughout the life cycle (Benson et al., 1987; Drager et al., 1989), it plays functionally distinct roles in larvae and adults: it supports arms that are used to capture food in larvae, while it provides protection and rigidity to the adult. Importantly, the endoskeleton is lacking or reduced in non-feeding larvae (Wray and Raff, 1991a). This makes *msp130* interesting from an evolutionary perspective: it plays a direct role in producing phenotypic features, these features have clearly defined functions, and they vary in functionally significant ways among species. These characteristics are important in understanding why evolutionary changes have occurred in gene expression.

The timing of *msp130* expression varies widely among the dozen species of sea urchin that have been examined. These differences in timing are mapped onto a cladogram of the study species in Fig. 3. The changes fall into two distinct categories. The first class of changes (white bars) have the following two features in common: they are relatively small timing shifts (1-2 hours), and they all involve an earlier onset of expression. The second class of changes in *msp130* expression (black bars) are quite different: they are relatively large timing shifts (10-20 hours), and they are all delays in the onset of expression.

Why have these changes in early zygotic gene expression evolved? And why do they fall into two such distinct classes? We can begin by tentatively ruling out a relationship with adult morphology. Both kinds of changes are scattered across the cladogram, and do not correlate in any understandable way with changes in adult morphology. However, there does seem to be a correlation with life history strategy: all the large delays in expression coincide with the transformation to non-feeding larvae (Fig. 3, black ovals).

To decide whether this association could be the result of chance, we can compute the probability that three independent changes in one feature (timing of gene expression) would by chance change in concert with another feature (life history

Table 1. Reasons why developmental processes do and do not evolve

Reason for change	Example in text
Generate derived larval morphology	Timing of <i>msp130</i> expression (major changes) Cleavage geometry
Generate derived life history feature	Specification of adult cell fates Morphogenesis of adult imaginal rudiment
Compensate for other changes	Gastrulation cell movements (for yolker egg) Fate specification (for gastrulation movements)
Drift (nearly neutral changes)	Timing of <i>msp130</i> expression (minor changes) Position of first cleavage plane (most cases)
Generate derived adult morphology	Mode of rudiment formation ?? Skeletogenic cell movements ??

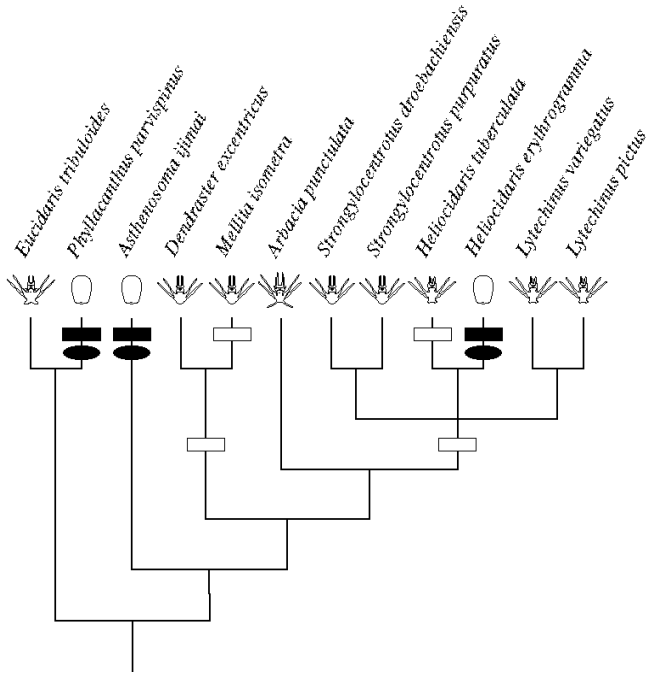


Fig. 3. Evolutionary transformations in gene expression. Changes in timing of *msp130* expression and life history are mapped, using parsimony, onto a cladogram of sea urchin species. Black ovals represent a change from feeding to non-feeding larvae. Large delays in onset of *msp130* expression (black bars) are significantly associated with changes in life history mode ($P=0.002$; concentrated changes test, Maddison and Maddison, 1992) and may be driven by the switch to non-feeding larvae. Changes to slightly earlier expression of *msp130* (white bars) do not correlate with either morphology or life history, and may represent effectively neutral changes. *Phylogenetic methods:* Phylogenetic relationships among sea urchins are based on parsimony analyses of adult morphology and 18S rRNA sequence data by Smith (1984, 1988, 1993). Topologies based on molecular and morphological data are congruent. None of the characters we map were used in generating the cladogram.

strategy). The concentrated changes test (Maddison, 1990) calculates this probability, given a particular phylogeny and a particular distribution of evolutionary changes in two traits. This statistical test, as well as other analytic tools for addressing similar issues, is implemented in the computer program MacClade (Maddison and Maddison, 1992). The concentrated changes test indicates that the observed association between the two traits would arise less than 1% of the time by chance alone. The correlation is therefore statistically robust.

The large changes in the timing of *msp130* expression might be driven by changes in life history strategy. This interpretation makes sense functionally. *msp130* protein is a structural component of the skeleton, which supports larval arms that are used for feeding (Hart, 1991; Wray, 1992). In species with feeding larvae, *msp130* protein must be present very early in development in order to build these arms. Species whose larvae do not feed no longer require arms, and most have lost not only the arms but the larval skeleton that supports them (Wray, 1992). In these species, therefore, *msp130* protein is not required until later in development when the adult skeleton is made.

The small changes in the onset of *msp130* expression (Fig. 3, white bars) are not associated with life history differences or adult morphology, and must be explained in another way. In most species, *msp130* protein appears before skeletogenesis actually begins (Wray and McClay, 1989). Activating *msp130* transcription slightly before it is required may not be detrimental. It is possible, therefore, that these minor differences in the timing of expression are functionally neutral.

There are several ways to test these hypotheses rigorously. The hypothesis that large delays in *msp130* expression are driven by life history can be tested in two ways. First, there is a strong prediction about what *msp130* expression patterns should look like in the other 5,000 or so echinoderm species that have not yet been examined. To the extent that the timing of *msp130* expression continues to correlate with life history strategy as additional species are examined, the hypothesis is increasingly supported. Second, a similar evolutionary correlation should exist for other genes involved in skeletogenesis. Several other structural components of the echinoderm endoskeleton have been characterized molecularly (Harkey and Whiteley, 1983; Benson et al., 1987; Livingston et al., 1991), and their expression could be examined in a comparative way. The hypothesis that the minor timing changes in *msp130* expression are neutral or nearly so could be tested in the following ways. First, it is possible to experimentally manipulate the timing and level of gene expression in echinoderm embryos (e.g., Franks et al., 1990). A close examination of larvae reared from embryos where the onset of *msp130* expression was altered slightly should reveal no effects. Even if this is not the ideal confirmatory test (subtle but functionally significant effects could be overlooked), it is a means of potentially falsifying the hypothesis. Second, there should exist heritable variation for minor differences in the timing of *msp130* expression within natural populations. If the smaller changes in gene expression are indeed evolving neutrally, they should at least occasionally appear as intraspecific variation.

Patterns of early zygotic gene expression are sometimes very highly conserved evolutionarily (Patel et al., 1989, 1994; Püschel et al., 1992; Brown et al., 1994). When the gene being compared across taxa has a direct role in pattern formation, and the study taxa share basic elements of body organization, this is to be expected. Indeed, any other result would suggest that the developmental role of the gene has changed, and a few examples of this are known (e.g., Patel et al., 1992). However, when the gene operates downstream of pattern formation, as most do, evolutionary changes may be much more numerous. As the foregoing example illustrates, these changes can be quite sensitive to evolutionary changes in the ecology of an organism.

Cell fate specification

The second set of examples concerns evolutionary changes in the processes that establish axes and cell fates. Comparative data exist for fate specification mechanisms, cell lineages, and cleavage geometry for several sea urchin and a few starfishes (see Wray, 1994). Echinoderms have stereotypic patterns of cell division and cell lineage segregation during cleavage (Hörstadius, 1973; Cameron and Davidson, 1991; Wray, 1994), as is the case for most metazoans (Davidson, 1990, 1991). Specification begins within the first few cleavage divisions, as demonstrated by clonal establishment of cell fates

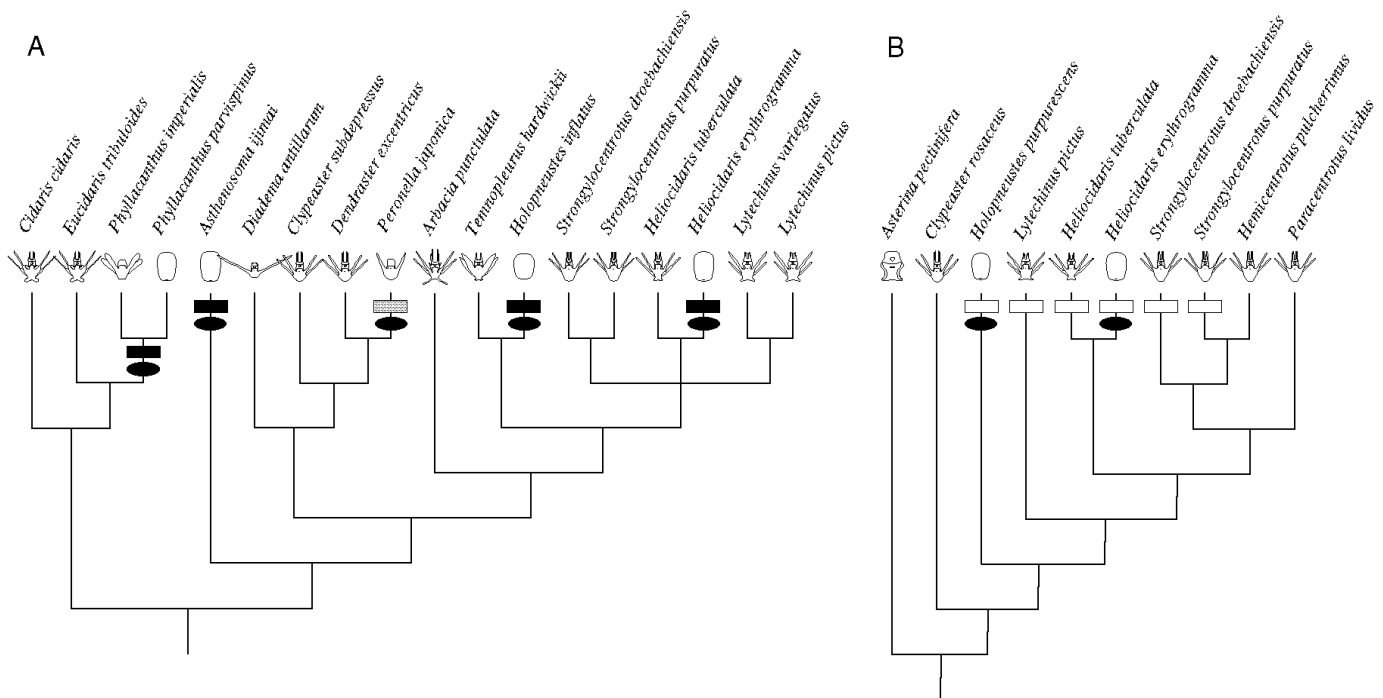


Fig. 4. Evolutionary transformations in cell fate specification. (A) Cleavage geometry. Black bars mark changes from the ancestral cleavage geometry (see text) to a two-tiered, 16-cell embryo with blastomeres of equal size. The grey bar marks a change from the ancestral condition to a 16-cell embryo with enlarged micromeres. These modifications in cleavage geometry are significantly associated with the switch to non-feeding larvae (black ovals) ($P=0.00002$). (B) Axial establishment. Changes in the relative position of the first cleavage plane to the dorsoventral axis (white bars) show no obvious association with either morphology or derived life history mode (black ovals). These transformations may represent neutral changes. Alternative, equally parsimonious reconstructions of axis formation exist, but do not alter our conclusions. Methods for phylogenetic reconstruction are described in the legend for Fig. 3.

(Cameron et al., 1987; Wray and Raff, 1990) and by direct experimental manipulations (Hörstadius, 1973; Henry et al., 1989; Ransick and Davidson, 1993). Because specification begins so early, most evolutionary changes in cleavage geometry will alter the timing of fate specification, or the number or position of descendent cells.

Mapping overall cleavage geometry onto a cladogram reveals several changes among sea urchins (Fig. 4A). A characteristic, but not universal, feature is a 16-cell embryo with three tiers of cells: mesomeres, macromeres, and micromeres (Hörstadius, 1973). This feature is reconstructed as the ancestral geometry, and has been retained in many phylogenetically diverse species. Since species with this cleavage geometry diverged as long ago as 250 million years (see Fig. 2), it is clearly an ancient feature of sea urchin development. On at least four separate occasions, however, there has been a parallel modification to a 2-tiered, 16-cell embryo with all blastomeres of equal size (Fig. 4A, black bars) (Wray, 1994). An additional case seems to be intermediate, with enlarged micromeres (Fig. 4A, grey bar) (Okazaki, 1975). Each of these modifications in cleavage geometry is associated with the switch to non-feeding larvae. This association is highly significant statistically ($P = 0.00002$) using the concentrated changes test. Because the intermediate case occurs in a species that already has non-feeding larvae, it suggests that the change in cleavage geometry is a consequence, rather than a cause, of the change in larval ecology.

It is not immediately obvious why this particular change in

cleavage geometry should be favored in non-feeding larvae. However, a detailed analysis of modified cell lineages and fate specification in *Heliocidaris erythrogramma*, one of the species with non-feeding larvae, provides some clues (Wray and Raff, 1989, 1990; Henry and Raff, 1990). For example, adult ectoderm is specified much earlier and in much larger quantity in *H. erythrogramma* than in species that produce feeding larvae. Instead of involving induction between mesoderm and ectoderm, as is the case in feeding larvae (Okazaki, 1975), specification takes place at the 8-cell stage through interactions among blastomeres (Wray and Raff, 1990). This change in fate specification allows much more rapid development through metamorphosis, by avoiding the prolonged period of proliferation of adult ectodermal cells that occurs in feeding larvae. Accelerated development of non-feeding larvae is probably favored by selection. A non-feeding larva does not need to spend much time in the plankton, where mortality rates are of the order of 10–20% per day (Rumrill and Chia, 1984). Thus, there should be strong selection to minimize the time to metamorphosis. This is borne out by empirical data: all species with non-feeding larvae complete metamorphosis faster than their closest relatives with feeding larvae. Whether other species with non-feeding larvae use the same modifications in fate specification as *H. erythrogramma* in order to achieve this end remains to be seen.

If some aspects of fate specification evolve in response to altered life history strategies, this may not be true of all. The spatial relationship between the first cleavage plane and the

dorsoventral axis varies among echinoderms with no obvious relationship to either morphology or life history mode (Fig. 4B; Hörstadius, 1973; Kominami, 1983, 1988; Wray and Raff, 1989; Cameron et al., 1989; Henry et al., 1992). This spatial relationship is variable even within several species. The functional significance of this variation is a change in the timing of dorsoventral axis formation (Henry et al., 1992). Evidently, the exact time of dorsoventral axis specification is not crucial to building particular phenotypes in echinoderms. Therefore, minor differences in the timing of this developmental process may be evolving in a nearly neutral mode.

Other differences in axis formation may, however, be selected for. In *H. erythrogramma*, the dorsoventral axis is not only specified earlier, it is determined much earlier (Henry and Raff, 1990). This earlier determination in a species with non-feeding larvae may have evolved in response to selection for a general acceleration of premetamorphic development. Until comparative data for other species with non-feeding larvae become available, this hypothesis remains untested.

The hypotheses just discussed are all testable. The hypothesis that changes in cleavage geometry and specification of adult ectoderm are driven by changes in life history strategy is testable with further comparative data. There is a strong prediction that the ancestral cleavage geometry will always be retained in species with feeding larvae, but usually modified in those whose larvae do not feed. Further, there should be evolutionary changes in fate specification that reduce the time to metamorphosis in sea urchins with non-feeding larvae. The hypothesis that the position of the first two cleavage planes is nearly neutral can be tested functionally by artificially altering their position (e.g., Hörstadius, 1973), or comparatively, by examining additional species. This hypothesis predicts that changes in the position of the first cleavage planes should neither cause nor correlate with differences in morphology. Finally, the hypothesis that accelerated determination of the dorsoventral axis is driven by the loss of larval feeding can be tested by examining this process in additional species with feeding and non-feeding larvae.

Cell lineage and axis formation are often considered to be evolutionarily conservative developmental processes. As the foregoing examples demonstrate, however, this is not necessarily the case. Further, these are not isolated cases. There are other examples of both minor and major evolutionary changes in cell lineage among related animals, including nematodes (Skiba and Schierenberg, 1992), mollusks

(Lillie, 1895; Freeman and Lundelius, 1992), nemerteans (Henry and Martindale, 1994), and ascidians (Berrill, 1931; Jeffery and Swalla, 1991). If studies in these other phyla could be expanded to cover a greater range of related species, it should be possible to begin to understand why these changes have evolved.

Morphogenesis

The third and last set of examples concerns evolutionary changes in the cell movements that drive morphogenesis. Morphogenesis has been extensively studied in echinoderm embryos because of their exceptional clarity, simple organization, and robust constitution (Gustafson and Wolpert, 1967; Wilt, 1987; Ettensohn and Ingersoll, 1992). The comparative data base on echinoderm morphogenesis is rapidly expanding. It is now possible to begin drawing some conclusions about why certain features have changed or remained the same, and to begin framing specific hypotheses for further testing. We consider two distinct morphogenetic processes: gastrulation and formation of the adult body rudiment.

Gastrulation cell movements have been studied in several echinoderms (Fig. 5A). In sea urchins with feeding larvae, gas-

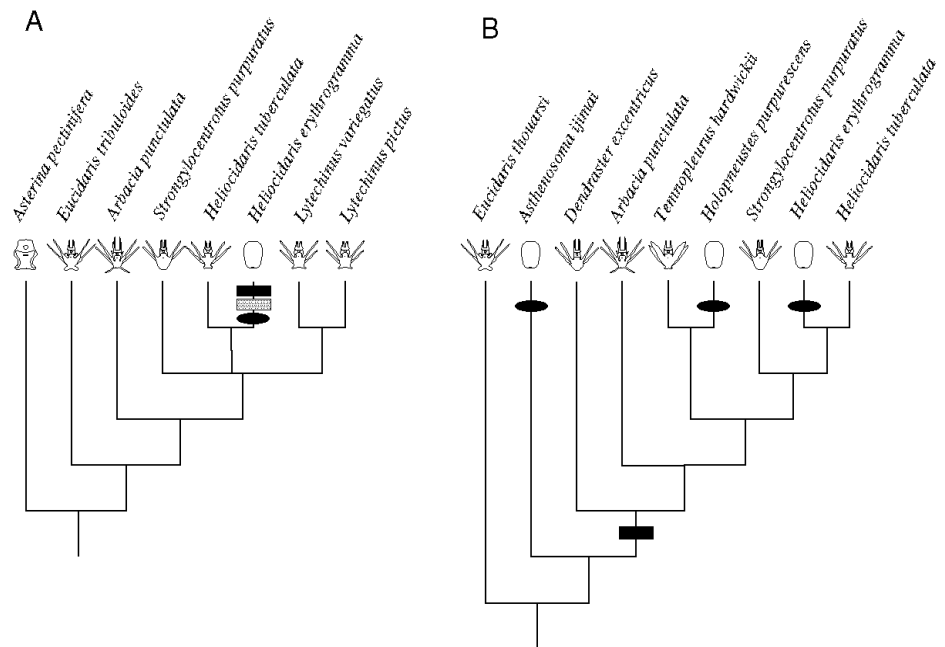


Fig. 5. Evolutionary transformations in morphogenesis. (A) Cell movements during gastrulation. Two significant changes in cell movements are the origin of extensive involution during gastrulation (black bar) and the imposition of a strong dorsoventral asymmetry in cell movements (grey bar). Both correlate with the life history transformation from feeding to non-feeding larvae (black oval). This association is not statistically significant by the concentrated changes test ($P=0.07$), but suggests that additional species with the derived life history pattern should be examined for parallel changes in morphogenesis. (B) Rudiment formation. During sea urchin development, the adult body forms as an imaginal rudiment on the left side of the larval body. In some species, this rudiment is exposed on the surface of the larva, but in one morphologically derived clade, it is enclosed within an invagination called the vestibule (black bar). Vestibule formation (black bar) does not correlate with a switch in life history (black ovals) but is significantly associated with adult morphology ($P=0.03$). (Note: some species included in the statistical analysis are not shown here.) It is possible that this developmental change is part of the reason that this derived clade of sea urchins looks different as adults. Methods for phylogenetic reconstruction are described in the legend for Fig. 3.

distinct phases: a brief period of involution, an extended period of convergent extension involving cell rearrangements and shape changes but no further involution, and a final period of "target recognition" during which filopodial traction guides the tip of the archenteron to the site of the future mouth (Ettensohn, 1984, 1985; Hardin and Cheng, 1986; Hardin, 1988; Hardin and McClay, 1990; Burke et al., 1991). Gastrulation has been less thoroughly studied in starfishes; it apparently involves a longer period of involution, but otherwise may be quite similar (Kuraishi and Osanai, 1992). This suggests a very lengthy conservation in the basic cell movements of echinoderm gastrulation, since starfishes and sea urchins diverged over 400 million years ago (Smith, 1988).

In *H. erythrogramma*, which has non-feeding larvae, gastrulation cell movements are modified in several important ways (Wray and Raff, 1991b). First, involution persists throughout gastrulation, helping to drive archenteron extension. Second, cell movements rapidly become asymmetric, with involution occurring primarily over the ventral lip of the blastopore. And third, the last phase of gastrulation is absent, in that no larval mouth is produced. Although gastrulation cell movements have not been studied in any other species with non-feeding larvae, the larval mouth is usually absent, suggesting that the third modification has evolved in parallel several times.

Two distinct evolutionary mechanisms may account for these changes in gastrulation cell movements. Loss of the larval mouth is consistent with generally accelerating premetamorphic development (see previous section), since it is a feeding structure that is no longer needed. The other changes, prolonged and asymmetric involution, may have a very different cause. Sea urchins with non-feeding larvae all have greatly enlarged, lipid-rich eggs. In vertebrates, large increases in egg size require changes in gastrulation cell movements due to mechanical constraints (Ballard, 1976; del Piño and Elinson, 1983), and the same may be true of echinoderms. If this interpretation is correct, it would constitute an example of yet another reason for evolutionary changes in development: to accommodate, or compensate for, another change in development. Indeed, the asymmetry of cell movements during gastrulation in *H. erythrogramma* may in turn account for the dorsoventral asymmetry in cell fate specification present in this species (Fig. 5A). Thus, evolutionary changes in two mechanistically distinct developmental processes, cell fate specification and cell movements, may be evolutionarily tracking changes in egg size that are in turn being driven by ecological factors.

Morphogenesis of the adult rudiment is similarly affected by several evolutionary mechanisms. The adult sea urchin body develops on the left side of the larva from imaginal cell populations (Okazaki, 1975). The arrangement of this adult rudiment varies among sea urchin species (Fig. 5B): ancestrally, the rudiment develops on the surface of the larva (Emler, 1988; Amemiya and Emler, 1992), while in a single derived clade (the non-echinothuriid euechinoids) it is enclosed in a vestibule (Bury, 1895; Okazaki, 1975). This change in the morphogenesis of the rudiment could be directly related to producing a derived adult morphology: the statistical correlation is robust ($P = 0.03$). However, there are no replicate origins of the derived adult morphology, making this interpretation difficult to test directly with further comparative data.

A more extensive change in rudiment morphogenesis is, however, amenable to hypothesis testing. In sea urchins with feeding larvae, the rudiment appears as a tiny invagination that grows by cell proliferation over the course of days or weeks (Okazaki, 1975). In *H. erythrogramma*, in contrast, the rudiment forms at very nearly full size (Williams and Anderson, 1975). Instead of a tiny invagination a few tens of cells in diameter, the vestibular invagination is hundreds of cells in diameter. This change in morphogenesis may be the result of selection for a shortened time to metamorphosis, as discussed earlier. If so, other species with non-feeding larvae should have enlarged vestibular invaginations. Because there exist replicates of this ecological shift, it is possible to test this hypothesis with comparative data.

Gastrulation and formation of the adult body plan are among the most fundamental morphogenetic movements during development. As such, they are not usually considered to be evolutionarily labile among closely related species (Thomson, 1988; Hall, 1992). However, the examples just discussed suggest that morphogenetic processes may change for reasons other than producing a different adult morphology. Because mechanistically distinct underlying cell movements can generate very similar phenotypic outcomes, morphological comparisons alone are not sufficient to rule out extensive evolutionary changes in morphogenetic mechanisms. Understanding why morphogenesis evolves will therefore require detailed comparisons of cell movements across a broader array of species.

ELEMENTS OF A COMPARATIVE ANALYSIS OF DEVELOPMENT

The preceding examples suggest several reasons why developmental processes have or have not changed within a clade. These evolutionary mechanisms are summarized in Table 1. The examples also suggest that the evolution of a particular developmental process can be influenced by different factors. Discriminating between competing explanations will not always be easy or even possible. However, comparative and experimental evidence will together often support or reject one or more of these alternatives. Such an analysis requires three elements.

Extensive and judicious sampling of taxa

Many published studies of developmental evolution compare a feature in two species. This approach provides a valuable starting point. When a developmental process is evolutionarily conserved, the divergence time between the two species provides a minimum estimate of its antiquity. When the process differs, it clearly can change without incurring a drastic functional cost.

Sampling additional taxa can provide a great deal more information. First, it becomes possible to reconstruct the polarity of the change: which is the ancestral and which the derived state of the developmental process. This requires sampling at least three species of known phylogenetic relationship, and preferably more (Maddison and Maddison, 1992). Second, sampling additional species provides a measure of how widely conserved the developmental process is within a clade. The more species that are examined, the stronger the support for conservation or variation. And third, discriminat-

Table 2. Probabilities of evolutionary associations*

Hypothesized factor	Developmental correlate	Number of changes [†]	Number of taxa [‡]	Probability [§] (<i>P</i> - value)
Non-feeding larva	Delay msp130 expression	3	12	0.002
	Loss of larval skeleton	5	19	0.00002
	Alter cleavage geometry	5	18	0.00002
	Alter gastrulation movements	1	8	0.07
	Larger egg	5	19	0.00002
Adult morphology	Skeletogenic cell movements	1	15	0.04
	Mode of rudiment formation	1	19	0.03

*Probabilities < 0.05 suggest that the association between a hypothesized influence and a developmental process is non-random.

[†]Determined from maximum parsimony reconstructions with MacClade 3.0 (Maddison and Maddison, 1992) using the phylogenetic relationships shown in Figs 3-5.

[‡]Each analysis is based on a phylogeny containing only those species for which the particular trait has been studied.

[§]Calculated with MacClade 3.0 (Maddison and Maddison, 1992) using Maddison's (1990) concentrated changes test. Expressed as the probability that the hypothesized association could have arisen by chance.

ing among potential evolutionary mechanisms with any statistical confidence may require sampling on the order of ten species (Fig. 1). In each case, then, conclusions will become more robust when more taxa are examined.

Quantity is not, however, everything. Judicious choices about study taxa can considerably increase the information that can be extracted from any comparative analysis. If the issue is reconstructing the polarity of the change, it is best to choose closely related taxa. If the issue is how widely a particular developmental process is conserved, it is important that a representative of each major subgroup within a clade be examined, as well as any species that are distinguished by unusual morphology or life history. Many supposedly invariant features of development have later been found to vary within a group when the number of examined species rises to three or four. Study taxa are often chosen primarily for convenience; however, the extra effort of studying non-standard species is often well worthwhile, and can provide particularly illuminating comparisons.

A robust phylogenetic framework

Understanding the phylogenetic relationships of study taxa is crucial for two reasons. First, it provides the only rigorous way to reconstruct the evolutionary history of development. By mapping differences in developmental processes onto a cladogram, it is possible to reconstruct the polarity of changes, how many times they have arisen independently, the sequence in which they have occurred, and what clades they define (for examples, see Figs 3, 4, and 5). By combining this information with divergence times between study taxa, it is possible to determine when particular changes occurred or how old a conserved feature is. Simple reconstructions can be done by eye, but large data sets require the assistance of computer-based algorithms. These programs are particularly important for exploring alternative, equally parsimonious reconstructions, and for assessing the impact of uncertainties in cladogram topology on the evolution of the characters being studied. Methods for reconstructing evolutionary changes are reviewed in detail by Harvey and Pagel (1991) and by Maddison and Maddison (1992).

Second, a phylogeny provides a framework for testing associations between developmental changes and other features, such as environment, life history, or morphology. The fact that many species share a derived feature of development and

derived life history strategy may be a coincidence if each change has only evolved once and these species form a single clade. An explicit statistical test for associations, such as the concentrated changes test (Maddison, 1990), is therefore essential. Statistical significance will typically require sample sizes of more than six species, and two or more replicate transformations (Fig. 1; Table 2). Brooks and McLennan (1991) and Harvey and Pagel (1991) provide general discussions about using phylogenetic information to frame and test evolutionary hypotheses.

An understanding of the functional consequences of variation

Finally, it is important to understand the consequences of differences in developmental processes, because, ultimately, it is phenotype that interacts with the environment and largely determines which genetic variants become fixed in populations. In some cases, a change in development may have no phenotypic effect: it may be canalized, or may be compensated for by other changes in development, as in the gastrulation example discussed above. However, most changes in development probably do have phenotypic consequences. These may be manifest in the morphology of adults or preadult phases such as larvae, or they may be limited to changing a non-morphological aspect of phenotype such as the rate of development or behavior.

Understanding why a particular developmental process has changed or remained the same requires distinguishing among these alternatives. Developmental processes can often be manipulated experimentally or genetically. Such perturbation experiments provide useful information for understanding not only how a single species develops, but why different species use the same or different developmental processes.

CONCLUSIONS

In 1975, VanValen wrote that "evolution is the control of development by ecology". To a large extent, this may be true. The available empirical evidence from echinoderms, when explicitly analyzed within a phylogenetic framework, suggests that ecology can exert a profound influence on a variety of important developmental processes. In sea urchins, the ecologically significant shift from feeding to non-feeding larvae

has evolved on several separate occasions. This change in life history strategy seems to be driving functionally significant changes in the regulation of gene expression, cell fate specification, and cell movements during morphogenesis. A second way in which ecology controls development is through more conventional aspects of phenotype. Some evolutionary changes in development are probably fixed because their phenotypic consequences improve locomotion or feeding efficiency or provide additional protection from predation. However, it is unlikely that all evolutionary changes in development are driven directly by ecology. A third class of modifications appears to be driven indirectly, evolving in order to compensate for other changes in development. And, fourth, some changes may not be related to ecology at all; instead, these changes may evolve simply because they produce neutral or nearly neutral phenotypic effects. This fourth kind of change is possible when developmental processes are canalized and buffer some perturbations.

We thank Chris Lowe and two anonymous reviewers for helpful comments on the manuscript. The National Science Foundation and the National Institutes of Health supported G. A. W.'s research.

REFERENCES

- Amemiya, S. and Emler, R. B.** (1992). The development and larval form of an echinothurioid echinoid, *Asthenosomal ijimai*, revisited. *Biol. Bull.* **182**, 15-30.
- Anderson, D. T.** (1973). *Embryology and Phylogeny in Annelids and Arthropods*. Oxford: Pergamon Press.
- Arthur, W.** (1988). *A Theory of the Evolution of Development*. Chichester: Wiley.
- Ballard, W. W.** (1976). Problems of gastrulation: real and verbal. *BioScience* **26**, 36-39.
- Benson, S., Suvoc, H., Stephens, L., Davidson, E. and Wilt, F.** (1987). A lineage-specific gene encoding a major matrix protein of the sea urchin embryo spicule. I. Authentication of the cloned gene and its developmental expression. *Dev. Biol.* **120**, 499-506.
- Berrill, N. J.** (1931). Studies in tunicate development. II. Abbreviation of development in the Molgulidae. *Phil. Trans. R. Soc. Lond.* **B219**, 281-346.
- Brooks, D. R. and McLennan, D. A.** (1991). *Phylogeny, Ecology, and Behavior: A Research Program in Comparative Biology*. Chicago: University of Chicago Press.
- Brown, S. J., Patel, N. H. and Denell, R. E.** (1994). Embryonic expression of the single *Tribolium engrailed* homolog. *Dev. Genet.* **15**, 7-18.
- Burke, R. D., Myers, R. L., Sexton, T. L. and Jackson, C.** (1991). Cell movements during the initial phase of gastrulation in the sea urchin embryo. *Dev. Biol.* **146**, 542-557.
- Bury, H.** (1895). The metamorphosis of echinoderms. *Quart. J. Microsc. Sci., N.S.* **38**, 45-135.
- Byrne, M.** (1991). Developmental diversity in the starfish genus *Patiriella* (Asteroidea: Asterinidae). In *Proceedings of the 7th International Echinoderm Conference* (ed. T. Yanagisawa, I. Yasumasu, C. Oguro, N. Suzuki, and T. Motokawa), pp. 499-508. Rotterdam: Balkema.
- Cameron, R. A. and Davidson, E. H.** (1991). Cell type specification during sea urchin development. *Trends Genet.* **7**, 212-218.
- Cameron, R. A., Fraser, S. E., Britten, R. J. and Davidson, E. H.** (1989). The oral-aboral axis of a sea urchin embryo is specified by first cleavage. *Development* **106**, 641-647.
- Cameron, R. A., Hough-Evans, B., Britten, R. J. and Davidson, E. H.** (1987). Lineage and fate of each blastomere of the eight-cell sea urchin embryo. *Genes Dev.* **1**, 75-84.
- Coe, W. R.** (1949). Divergent methods of development in morphologically similar species of prosobranch gastropods. *J. Morph.* **84**, 383-399.
- Davidson, E. H.** (1990). How embryos work: a comparative view of diverse modes of cell fate specification. *Development* **108**, 365-389.
- Davidson, E. H.** (1991). Spatial mechanisms of gene regulation in metazoan embryos. *Development* **113**, 1-26.
- Decker, G. L., Valdizan, M. C., Wessel, G. M. and Lennarz, W. J.** (1988). Developmental distribution of a cell surface glycoprotein in the sea urchin *Strongylocentrotus purpuratus*. *Dev. Biol.* **129**, 339-349.
- del Piño, E. M. and Elinson, R. P.** (1983). A novel development pattern for frogs: gastrulation produces an embryonic disk. *Nature* **306**, 589-591.
- Drager, B. J., Harkey, M. A., Iwata, M. and Whiteley, A. H.** (1989). The expression of embryonic primary mesenchyme genes of the sea urchin, *Strongylocentrotus purpuratus*, in the adult skeletogenic tissues of this and other echinoderms. *Dev. Biol.* **133**, 14-23.
- Elinson, R. P.** (1990). Direct development in frogs: wiping the recapitulationist slate clean. *Sem. Dev. Biol.* **1**, 263-270.
- Emler, R. B.** (1988). Larval form and metamorphosis of a "primitive" sea urchin, *Eucidaris thouarsi* (Echinodermata: Echinoidea: Cidaroida), with implications for developmental and phylogenetic studies. *Biol. Bull.* **174**, 4-19.
- Emler, R. B.** (1990). World patterns of developmental mode in echinoid echinoderms. In *Advances in Invertebrate Reproduction* (ed. M. Hoshi and O. Yamashita), pp. 329-335. Amsterdam: Elsevier.
- Ettensohn, C. A.** (1984). Primary invagination of the vegetal plate during sea urchin gastrulation. *Amer. Zool.* **24**, 571-588.
- Ettensohn, C. A.** (1985). Mechanisms of epithelial invagination. *Quart. Rev. Biol.* **60**, 289-307.
- Ettensohn, C. A. and Ingersoll, E. P.** (1992). Morphogenesis of the sea urchin embryo. In *Morphogenesis: An Analysis of the Development of Biological Form* (ed. E. F. Rossomando and S. Alexander), pp. 189-262. New York: Marcel Dekker Press.
- Franks, R. R., Anderson, R., Moore, J. G., Hough-Evans, B. R., Britten, R. J. and Davidson, E. H.** (1990). Competitive titration in living sea urchin embryos of regulatory factors required for expression of the CyIIIa actin gene. *Development* **110**, 31-40.
- Freeman, G. and Lundelius, J. W.** (1992). Evolutionary implications of the mode of D quadrant specification in coelomates with spiral cleavage. *J. Evol. Biol.* **5**, 205-247.
- Garstang, W.** (1929). The origin and evolution of larval forms. *British Association for the Advancement of Science* 1929, 77-98.
- Gould, S. J.** (1977). *Ontogeny and Phylogeny*. Cambridge, MA: Belknap Press.
- Gustafson, T. and Wolpert, L.** (1967). Cellular movement and contact in sea urchin morphogenesis. *Biol. Rev.* **42**, 442-498.
- Hall, B. K.** (1992). *Evolutionary Developmental Biology*. London: Chapman & Hall.
- Hardin, J. D.** (1988). The role of secondary mesenchyme cells during sea urchin gastrulation studied by laser ablation. *Development* **103**, 317-324.
- Hardin, J. D. and Cheng, L. Y.** (1986). The mechanisms and mechanics of archenteron elongation during sea urchin gastrulation. *Dev. Biol.* **115**, 490-501.
- Hardin, J. and McClay, D. R.** (1990). Target recognition by the archenteron during sea urchin gastrulation. *Dev. Biol.* **142**, 86-102.
- Harkey, M. A. and Whiteley, A. H.** (1983). The program of protein synthesis during the development of the micromere-primary mesenchyme cell line in the sea urchin embryo. *Dev. Biol.* **100**, 12-28.
- Hart, M.** (1991). Particle capture and the method of suspension feeding by echinoderm larvae. *Biol. Bull.* **180**, 12-27.
- Harvey, P. H. and Pagel, M. D.** (1991). *The Comparative Method in Evolutionary Biology*. New York: Oxford University Press.
- Henry, J. J., Amemiya, S., Wray, G. A. and Raff, R. A.** (1989). Early inductive interactions are involved in restricting cell fates of mesomeres in sea urchin embryos. *Dev. Biol.* **136**, 140-153.
- Henry, J. J., Klueg, K. M. and Raff, R. A.** (1992). Evolutionary dissociation between cleavage, cell lineage and embryonic axes in sea urchin embryos. *Development* **114**, 931-938.
- Henry, J. J. and Martindale, M. Q.** (1994). Establishment of the dorsoventral axis in nemertean embryos: evolutionary considerations of spiralian development. *Dev. Genet.* **15**, 64-78.
- Henry, J. J. and Raff, R. A.** (1990). Evolutionary change in the process of dorsoventral axis determination in the direct developing sea urchin, *Heliocidaris erythrogramma*. *Dev. Biol.* **141**, 55-69.
- Hörstadius, S.** (1973). *Experimental Embryology of Echinoderms*. London: Clarendon, Oxford Univ. Press.
- Janies, D. A. and McEdward, L. R.** (1993). Highly derived coelomic and water-vascular morphogenesis in a starfish with pelagic direct development. *Biol. Bull.* **185**, 56-76.
- Jeffery, W. R. and Swalla, B. J.** (1991). An evolutionary change in the muscle lineage of an anural ascidian embryo is restored by interspecific hybridization with a urodele ascidian. *Dev. Biol.* **145**, 328-337.

- Jeffery, W. R. and Swalla, B. J.** (1992). Evolution of alternate modes of development in ascidians. *Bioessays* **14**, 219-226.
- Kominami, T.** (1983). Establishment of the embryonic axes in larvae of the starfish, *Asterina pectinifera*. *J. Embryol. Exp. Morphol.* **75**, 87-100.
- Kominami, T.** (1988). Determination of dorso-ventral axis in early embryos of the sea urchin, *Hemicentrotus pulcherrimus*. *Dev. Biol.* **127**, 187-196.
- Kumé, M. and Dan, K.** (1968). *Invertebrate Embryology*. Belgrade: NOLIT Press.
- Kuraishi, R. and Osanai, K.** (1992). Cell movements during gastrulation of starfish larvae. *Biol. Bull.* **183**, 258-268.
- Lillie, F.** (1895). The embryology of the Unionidae. *J. Morph.* **10**, 1-100.
- Livingston, B. T., Shaw, R., Bailey, A. and Wilt, F.** (1991). Characterization of a cDNA encoding a protein involved in formation of the skeleton during development of the sea urchin *Lytechinus pictus*. *Dev. Biol.* **148**, 473-480.
- Maddison, W. P.** (1990). A method for testing the correlated evolution of two binary characters: are gains or losses concentrated on certain branches of a phylogenetic tree? *Evolution* **44**, 539-557.
- Maddison, W. P. and Maddison, D. R.** (1992). MacClade. v.3.01. Sunderland, MA: Sinauer Associates.
- McEdward, L. R. and Janies, D. A.** (1993). Life cycle evolution in asteroids: what is a larva? *Biol. Bull.* **184**, 255-268.
- McGinnis, W. and Krumlauf, R.** (1992). Homeobox genes and axial patterning. *Cell* **68**, 283-302.
- Okazaki, K.** (1975). Normal development to metamorphosis. In *The Sea Urchin Embryo* (ed. G. Czihak), pp. 177-232. Berlin: Springer-Verlag.
- Parks, A. L., Bisgrove, B. W., Wray, G. A. and Raff, R. A.** (1989). Direct development in the sea urchin *Phyllacanthus parvispinus* (Cidaroida): phylogenetic history and functional modification. *Biol. Bull.* **177**, 96-109.
- Parks, A. L., Parr, B. A., Chin, J., Leaf, D. S. and Raff, R. A.** (1988). Molecular analysis of heterochronic changes in the evolution of direct developing sea urchins. *J. Evol. Biol.* **1**, 27-44.
- Parr, B. A., Parks, A. L. and Raff, R. A.** (1990). Promoter structure and protein sequence of msp130, a lipid-anchored sea urchin glycoprotein. *J. Biol. Chem.* **265**, 1408-1413.
- Patel, N. H., Ball, E. E. and Goodman, C. S.** (1992). Changing role of *even-skipped* during the evolution of insect pattern formation. *Nature* **357**, 339-342.
- Patel, N. H., Condrón, B. G. and Zinn, K.** (1994). Pair-rule expression patterns of *even-skipped* are found in both short- and long-germ beetles. *Nature* **367**, 429-434.
- Patel, N. H., Martín-Blanco, E., Coleman, K. G., Poole, S. J., Ellis, M. C., Kornberg, T. B. and Goodman, C. S.** (1989). Expression of *engrailed* proteins in arthropods, annelids, and chordates. *Cell* **58**, 955-968.
- Püschel, A. W., Westerfield, M. and Dressler, G. R.** (1992). Comparative analysis of Pax-2 protein distributions during neurulation in mice and zebrafish. *Mech. Dev.* **38**, 197-208.
- Raff, R. A.** (1987). Constraint, flexibility, and phylogenetic history in the evolution of direct development in sea urchins. *Dev. Biol.* **119**, 6-19.
- Raff, R. A.** (1992). Direct-developing sea urchins and the evolutionary reorganization of early development. *BioEssays* **14**, 1-8.
- Ransick, A. and Davidson, E. H.** (1993). A complete second gut induced by transplanted micromeres in the sea urchin embryo. *Science* **259**, 1134-1138.
- Rumrill, S. and Chia, F.** (1984). Differential mortality during the embryonic and larval lives of northeast Pacific echinoids. In *Echinodermata: Proceedings of the Fifth International Echinoderm Conference* (ed. B. F. Keegan and B. D. S. O'Connor), pp. 333-338. Amsterdam: Balkema.
- Sander, K.** (1983). The evolution of patterning mechanisms: gleanings from insect embryogenesis and spermatogenesis. In *Development and Evolution* (ed. B. C. Goodwin, N. Holder, and C. C. Wylie), pp. 137-159. Cambridge, UK: Cambridge Univ. Press.
- Scott, L. B., Lennarz, W. J., Raff, R. A. and Wray, G. A.** (1990). The "lecithotrophic" sea urchin *Heliocidaris erythrogramma* lacks typical yolk platelets and yolk glycoproteins. *Dev. Biol.* **138**, 188-193.
- Skiba, F. and Schierenberg, E.** (1992). Cell lineages, developmental timing, and spatial pattern formation in embryos of free-living soil nematodes. *Dev. Biol.* **151**, 597-610.
- Smith, A. B.** (1984). *Echinoid Palaeobiology*. London: Allen & Unwin.
- Smith, A. B.** (1988). Fossil evidence for the relationship of extant echinoderm classes and their times of divergence. In *Echinoderm Phylogeny and Evolutionary Biology* (ed. C. R. C. Paul and A. B. Smith), pp. 85-97. New York: Oxford University Press.
- Smith, A. B.** (1992). Echinoderm phylogeny: morphology and molecules reach accord. *Trends Ecol. Evol.* **7**, 224-229.
- Smith, A. B., Lafay, B. and Christen, R.** (1993). Comparative variation of morphological and molecular evolution through geological time: 28S ribosomal RNA versus morphology in echinoids. *Phil. Trans. Roy. Soc. Lond. B* **338**, 365-382.
- Strathmann, R. R.** (1978). The evolution and loss of feeding larval stages of marine invertebrates. *Evolution* **32**, 894-906.
- Strathmann, R. R.** (1985). Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. *Ann. Rev. Ecol. Syst.* **16**, 339-361.
- Thomson, K. S.** (1988). *Morphogenesis and Evolution*. Cambridge, UK: Cambridge University Press.
- Williams, D. H. C. and Anderson, D. T.** (1975). The reproductive system, embryonic development, larval development and metamorphosis of the sea urchin *Heliocidaris erythrogramma* (Val.) (Echinoidea: Echinometridae). *Australian J. Zool.* **23**, 371-403.
- Wilt, F. H.** (1987). Determination and morphogenesis in the sea urchin embryo. *Development* **100**, 559-575.
- Wray, G. A.** (1992). The evolution of larval morphology during the post-Paleozoic radiation of echinoids. *Paleobiol.* **18**, 258-287.
- Wray, G. A.** (1994). The evolution of cell lineage in echinoderms. *Amer. Zool.* (in press).
- Wray, G. A. and McClay, D. R.** (1988). The origin of spicule-forming cells in a 'primitive' sea urchin (*Eucidaris tribuloides*) which appears to lack primary mesenchyme cells. *Development* **103**, 305-315.
- Wray, G. A. and McClay, D. R.** (1989). Molecular heterochronies and heterotopies in early echinoid development. *Evolution* **43**, 803-813.
- Wray, G. A. and Raff, R. A.** (1989). Evolutionary modification of cell lineage in the direct-developing sea urchin *Heliocidaris erythrogramma*. *Dev. Biol.* **132**, 458-470.
- Wray, G. A. and Raff, R. A.** (1990). Novel origins of lineage founder cells in the direct-developing sea urchin *Heliocidaris erythrogramma*. *Dev. Biol.* **141**, 41-54.
- Wray, G. A. and Raff, R. A.** (1991a). The evolution of developmental strategy in marine invertebrates. *Trends Ecol. Evol.* **6**, 45-50.
- Wray, G. A. and Raff, R. A.** (1991b). Rapid evolution of gastrulation mechanisms in a sea urchin with lecithotrophic larvae. *Evolution* **45**, 1741-1750.