Evolution of developmental decisions and morphogenesis: the view from two camps

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Summary

Modern developmental biology largely ignores evolution and instead focuses on use of standard model organisms to reveal general mechanisms of development. Evolutionary biologists more widely hold developmental biology to be of major consequence in providing potential insights into evolution. Evolutionary insights can enlighten our views of developmental mechanisms as much as developmental data offer clearer views of mechanisms which underlie evolutionary change. However, insights have been limited by the long-term disengagement of the two fields dating to the rise of experimental embryology in the 1890s. Molecular genetics now provides a powerful tool to probe both gene function and evolutionary relationships, and a greater connection has become possible. The expansion of experimental organisms beyond the standard model animals used in most studies of development allows us to ask deeper questions about the interaction of development and evolution. This paper presents an analysis of the complementary uses of the resulting data in the two fields as they grope for accommodation. Analysis of the radical changes in early develoment seen in closely related sea urchins with alternate modes of development illustrate the complementarity of developmental and evolutionary data. These studies show that what have been thought to be constrained mechanisms of axial determination, cell lineage patterning, and gastrulation in fact evolve readily and provide the means for the rapid evolution of development.

Key words: gene function, evolution, sea urchin.

In the Tower of Babel

In 1981, John Tyler Bonner organized a major cross-disciplinary conference to explore effective ways of recombining the study of evolution and development (Bonner, 1982). I was at that stimulating meeting in Dahlem, and came away with an interesting education in just how difficult it really is to combine two fields that for nearly a century have had different goals, approaches and vocabularies. The feeling that it ought to be done has persisted, and has motivated subsequent meetings which have revealed that the rapprochement is still slow in coming. What is different now from a decade ago is that some experimenters have taken steps to make a practicable fusion and have framed problems that can be addressed with appropriate experimental systems.

The separation of developmental biology from evolutionary biology occurred at about the turn of the century, when a focus on mechanistic controls of development replaced the search for phylogenetic relationships through embryonic resemblances as a central concern of the field. Embryology became an experimental discipline with a very different paradigm for exploring biology from that developed by evolutionary biology during the same period (Mayr, 1982). Table 1 summarizes the distinctions that

presently characterize developmental biology and evolutionary biology as disciplines. For evolutionary biologists, the critical issue is to explain the diversity of life. Genes provide the raw material for the generation of diversity, and thus population genetics has been developed as the major tool for relating the behavior of genes in populations to evolutionary events. Another important focus of evolutionary biology has been the definition of phylogenetic relationships using both morphological and molecular tools. On the other hand a very different set of principles are important to developmental biologists. Questions of phylogenetic relationship are seldom part of mainstream developmental biology. Instead, several organisms have been selected from various taxa purely for their convenience as experimental systems in the study of various developmental processes. Mechanistic universality in developmental processes is considered to exist despite the diversity of organisms: thus common mechanisms for gastrulation are sought. Genes are regarded as important not as sources of variation, but as controllers and executers of developmental processes.

The consequence of these differences in disciplinary histories is that when developmental and evolutionary biologists are brought together there is some difficulty in deciding just what people from that other discipline are talking about. Yet, a boundary discipline exists, and its investiga-

Table 1. *Disciplinary digressions: evolution versus development*

EVOLUTION	DEVELOPMENT
Exploration of diversity	Mechanistic universality
Genes as sources of variation	Functional role of genes
Phylogenetic relationships	Standard model organisms

tion can yield important complementary insights in the two fields. This paper attempts to show how that can be done.

Common ground and experimental systems

The most crucial issue to evolutionary biologists interested in development has been one of how morphology evolves. Both deBeer (1958) and Gould (1977) laid out their considerations of the interface between ontogeny and phylogeny in terms of a unifying developmental mechanism that would explain the relatively easy transformation of form suggested by evolutionary histories. That mechanism was heterochrony, the concept that events in development can shift in timing relative to each other to produce new ontogenies. For example, humans resemble young apes more closely than adult ones, suggesting that human evolution might have involved developmental changes resulting in a more juvenilized morphology in sexually mature adults. [In Aldous Huxley's novel, *After Many a Summer Dies the Swan*, that concept is followed out to a bizzare denouement in which tampering to prolong life has predictable if unseemly consequences.] In the hands of its proponents, heterochrony has continued to provide a major explanatory concept for interpreting evolutionary change in fossil as well as living organisms. The insistence that heterochrony is the dominant mechanism for evolutionary changes is overdone, but the goal of providing a simple unifying mechanism around which data can be ordered is clear.

The study of the role of development in evolution must shift from a focus on theoretical considerations to the framing of experimental questions that can reveal mechanisms by which developmental processes influence evolutionary change. Subsidiary questions about the evolution of development grow from other themes in evolutionary biology. Thus, the demonstration of developmental innovations becomes a major part of understanding the origins of novel features that have led to origins of new groups of animals. Comparative studies become important to trace the directions of evolutionary changes in development (Wray and Raff, 1991; Raff, 1992a), the number of times they might have occurred independently (Emlet, 1990), and even their potential reversibility (Raff et al., 1993). Evolutionary data also reveal that some aspects or stages of development evolve readily and others do not, providing clues that different mechanisms might govern various stages of development (Raff et al., 1991; Raff, 1992b). The basic issue is whether natural selection can elicit responses in any direction from the mutations that appear in organisms, or if evolution is somehow constrained by existing genetic and developmental systems. If so, a major principle of evolution exists beyond an all-powerful selection working on randomly generated variation, and the inner workings of

genetic regulatory systems and developmental processes as well as their histories must be considered as key elements of evolution (Alberch, 1982; Jacob, 1977; Müller, 1990; Thomson, 1991).

Because of the ability to clone regulatory genes, purely developmental questions have quickly become issues in evolutionary biology. The finding that homeobox-containing genes are major regulators of axial specification in both insects and vertebrates suggests that very ancient developmental-genetic regulatory mechanisms are shared by widely diverged animal groups. These genes have been detected even in the most primitive metazoans, the cnidarians (jelly fish, anemones, etc.), as well as many other phyla (Holland, 1991; Murtha et al., 1991; Schiewater et al., 1991). The phylogenetic distribution of homeobox genes, and the additional roles that can be shown for them in aspects of vertebrate development indicates a pattern of gene duplication, divergence, and co-option for new functions (eg. Holland 1991; Hunt et al., 1991). Other regulatory gene families, such as the steroid receptor family (Evans, 1988; Amero et al., 1992), show analogous patterns of evolutionary expansion and co-option to provide genetic raw material for regulatory innovations in the evolution of development.

Old pathways and evolutionary innovations

It is perhaps not so easy to define precisely what constitutes an evolutionary innovation. Certainly a novel feature must differ in some important qualitative way from the ancestral feature. Discussions of innovation (Mayr, 1960; Cracraft, 1990; Liem, 1990; Müller, 1990; Raff et al., 1990) visualize novelty in two ways. First, novel features are considered to be significant if they permit assumption of a new function or provide a key element upon which new evolutionary directions can be taken by an evolving lineage; key innovations are thus recognized in evolutionary retrospect. Because multiple features emerge during an evolutionary history, there is considerable disagreement on the existence and phylogenetic role of key innovations (eg. Cracraft, 1990 versus Liem, 1990). The second aspect of novelty is that it represents a departure from an existing pattern of development; thus, a new feature can be novel even if no new group arises as a result (Raff et al., 1990).

Responses to selection may be limited by the properties of the ancestral developmental program: hypothetically adaptive responses to selection may not be attainable because the starting genetic control networks cannot reach certain states; interactions between developmental processes may be too complex to modify; developmental programs may follow epigenetic rules that exclude certain outcomes. Some descendant states indeed may be favored by features of the existing developmental program that bias the response to selection in particular directions. On the other hand, some selective pressures might push developing systems over a threshold to yield a new state or interactions (Müller, 1990). The descendant states ultimately achieved only can be those which are both adaptive and attainable by some evolutionary trajectory not blocked by developmental constraints.

These considerations suggest that we might need to look
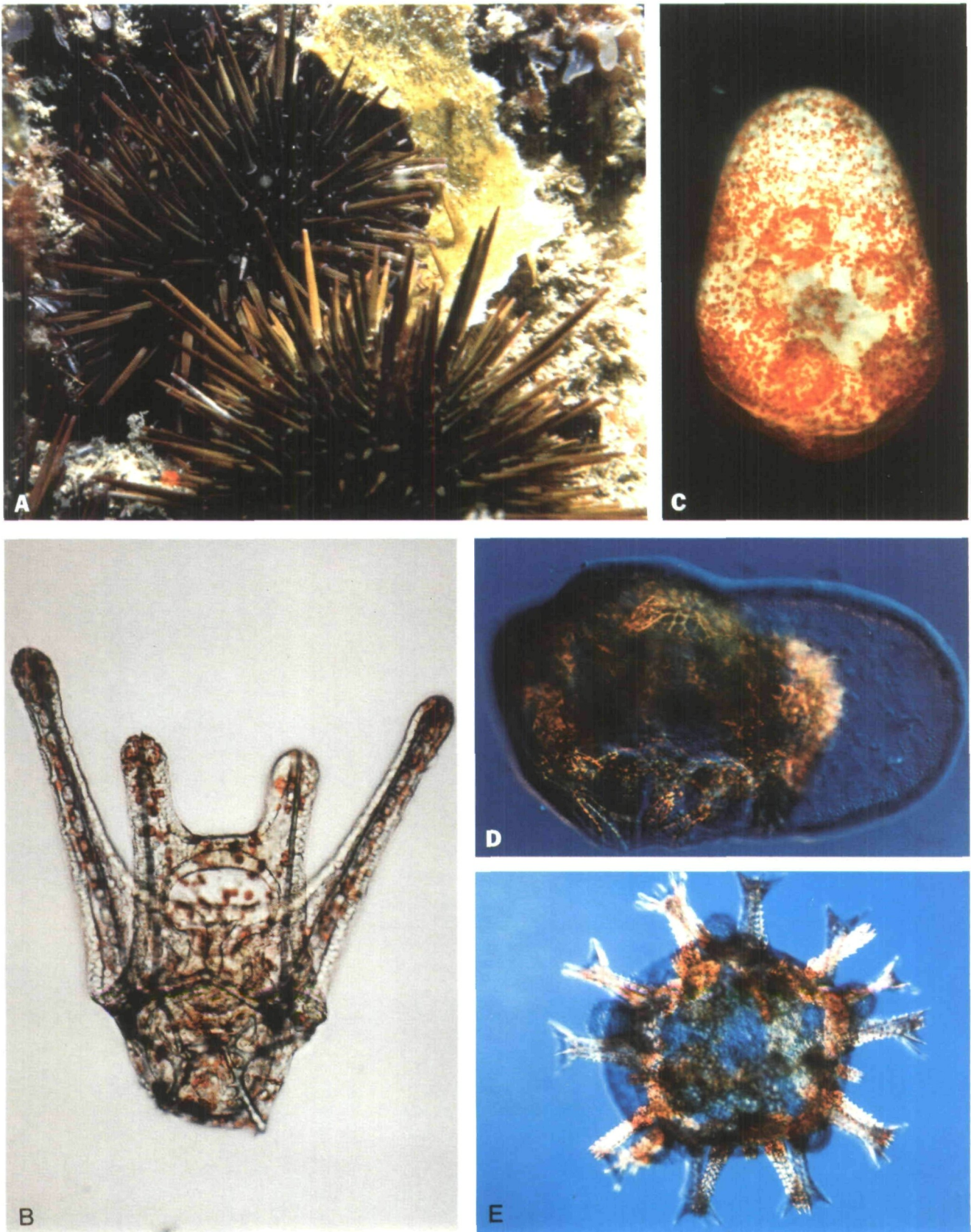
Fig. 1. Divergent development in two closely related sea urchins. A, foreground, adult of *H. tuberculata*. Note the longer, spatulate spins as compared to *H. erythrogramma* in the background. The two species live in the same shallow intertidal habitats on the East coast of Australia. B, pluteus larva of *H. tuberculata*. Typical structures of the 4-arm pluteus include pigment cells, spicular skeleton, ciliated larval arms, and functioning gut. Length from vertex to arm tips 300 μm. C, oral side of *H. erythrogramma* "larva" about half way through development. Note that the only larval features are the pigment cells and the ciliary band at one end; the five primary adult tube feet show prominantly. There is no spicular skeleton or larval gut. Length 550 μm. D, polarizing optic view of cleared *H. erythrogramma* larva from the side. Tube feet are visible on the oral side. Two kinds of adult skeletal structures are visible in polarizing light; juvenile adult spines facing orally, and fenestrated juvenile adult test plates. E, cleared newly metamorphosed juvenile *H. erythrogramma* under polarized light.

for key innovations as initial genetic events that make possible a series of subsequent evolutionary modifications in ontogeny. One's first reaction would be to seek initial additions to, striking modifications of, or duplications and subsequent divergence of pre-existing features. However, many developmental regulatory genes act by repressing the function of other genes. The evolutionary result of the insertion of such repressors would be to remove some aspect of expression of a primitive feature. For example, a major aspect of the function of homoeotic genes in insect development is to prevent features of more anterior or more posterior segments from being expressed in the "wrong" segments. The result is tagmosis (Raff and Kaufman, 1983). The unitary head has been evolved from a primitive condition in arthropods in which there were several separate limb-bearing anterior segments (Della Cave and Simonetta, 1991). Mutations in the *Drosophila* homoeotic gene *fork head* transform the head into separate segments that express features of limb-bearing segments. The gene thus normally acts in development to prevent segmentation in the head (Jürgens and Weigel, 1988; Finkelstein and Perrimon, 1991). The initial crucial step in an evolutionary innovation thus may be a suppression of part of an older program. The suppression event then resets the stage for additions to the modified feature, such as addition of head specializations to the .fused segments. The suppression of older genetic controls can be a key innovation, because it provides the basis for subsequent genetic modifications and additions.

Direct development and alternative ontogenies

In many cases larval development is extraordinarily conservative in evolution over long periods, with similar larvae conserved over 500 Myr of evolutionary time (Müller, K. J. and Walossek, D., 1986). The sea urchin pluteus has been conserved for at least 250 Myr (Wray, 1992). Yet, radically different modes of early development exist among direct developing species in many taxa. These forms offer important experimental systems. They represent alternate ways to achieve the same developmental end as achieved by their relatives with feeding larvae, and provide natural experiments in developmental genetics. Although indirect development is widely conserved, it has been often replaced by direct development in such diverse groups as corals, polychaete annelids, starfish, sea urchins, ascidians, and frogs (Raff and Kaufman 1983; del Pino, 1989; Emlet, 1990; Jeffery and Swalla, 1992). In many instances, indirect- and direct-developing species occur within the same genus, and can even produce hybrids, indicating rapid evolutionary changes in developmental mode (Levin et al., 1991; Jeffery and Swalla, 1992; Raff, 1992a). The replacement of complex patterns of larval development by direct-development offers an instance of creation of an evolutionary novelty by an initial suppression of an old pattern.

This proposition is illustrated by two congeneric sea urchin species, *Heliocidaris tuberculata* and *H. erythrogramma* (Fig. 1). The two species of *Heliocidaris* are similar as adults but differ greatly in development. *H. tuberculata* develops from a small egg via a typical feeding

pluteus, whereas *H. erythrogramma* has omitted the pluteus larva and undergoes direct development in which a large egg develops rapidly into a small sea urchin without feeding. The two species diverged about 10 million years ago, as estimated from single copy and mitochondrial DNA distances (Smith et al., 1990; McMillan et al., 1992). The initial innovation setting the stage for subsequent remodeling of early development in direct-developing species probably lies in oogenesis. Egg sizes in sea urchins fall into two classes. Indirect developers which produce feeding larvae generally have small eggs of about 100 μm diameter. Direct developers feature eggs of 300 μm to 1500 μm, and produce non-feeding larvae (Wray and Raff, 1991). In between are a very few species with eggs of about 300 μm that produce feeding plutei. In one case, it has been demonstrated that the large pluteus can metamorphose even if not fed; it is facultative (Emlet, 1986). Thus, there is a threshold. Species that pass over it via a facultative feeding larva can afford to suppress pluteus features and assemble a new complex of features that result in rapid, direct development of the juvenile.

Evolution of gastrulation and origins of a novel morphogenesis

The larva of *H. erythrogramma* looks quite different from the pluteus of *H. tuberculata* (Fig. 1). A superficial view suggests that the main evolutionary change has been a loss of feeding structures in the larva and an acceleration of adult development, relatively simple heterochronies. In fact, the underlying changes are pervasive and have reorganized gametogenesis and development. In *H. erythrogramma*, the eggs are 100 times the volume of *H. tuberculata* eggs, the sperm heads are longer and narrower, the nuclear genome is about 30 percent larger, storage proteins are different (Raff, 1992a). Maternal localization and important aspects of axial determination have been modified (Henry and Raff, 1990; Henry et al., 1990). As development begins, cleavage is radial, but no micromeres are produced as in indirect developing sea urchins. The result is that the cellular precursors and cell fates have been changed from those of indirect development (Wray and Raff, 1990). Because no larval skeleton is produced, and because adult structures start to form at the end of gastrulation, the morphogenetic processes that shape the post-gastrula larva have been highly modified. No pluteus with its elaborate feeding structures is made; all morphogenetic processes are directed at rapid production of the juvenile sea urchin. The overarching changes in early development of *H. erythrogramma* have been recently reviewed (Raff, 1992a; Raff et al., 1992), and do not need to be detailed here. I instead focus on gastrulation to show how its evolutionary modification makes possible the accelerated development of the adult.

Gastrulation is the most fundamental morphogenetic movement in animal development, and establishes the primary germ layers and their topological relationships for subsequent inductive interactions. Gastrulation varies between classes, but would be expected to be conserved between closely related species, because these would share many inductive interactions and downstream morpho-

genetic processes that would constrain evolutionary changes in gastrulation. It is evident from experimental manipulations of indirect-developing sea urchins that abundant inductive interactions occur prior to and during gastrulation (Ettensohn and McClay, 1988; Henry et al., 1989; Hardin and McClay, 1991). However, the timing and topological differences in morphogenesis in *H. erythrogramma* versus *H. tuberculata* indicate that cell-cell communication has been substantially modified despite the clear role for such inductive interactions. Thus, the expected constraints on gastrulation might not actually exist.

In the indirect-developing sea urchins that have been studied, gastrulation has two phases. In the first, there is an active and symmetrical movement of cells from the vegetal region of the blastula to form a short, wide tube (Burke et al., 1991). This initial archenteron precursor is transformed during the second phase of gastrulation into a long slender thin walled tube (Ettensohn, 1985; Hardin and Cheng, 1986). No involution occurs during the second phase; instead, the extension is driven by changes in cell shape and position (Ettensohn, 1984; Ettensohn, 1985; Hardin and Cheng, 1986; Hardin, 1989). It is important to note that no addition of cells into the archenteron occurs during the second phase of archenteron elongation in this ancestral mode of sea urchin gastrulation.

As in typical sea urchin gastrulation, in *H. erythrogramma*, there is an initial symmetric phase of invagination (Fig. 2). However, the cell rearrangements characteristic of the second phase of the ancestral archenteron elongation process have been replaced by a novel asymmetric involution. This change meets both definitions of evolutionary innovations in that the ancestral elongation process is not merely modified but is replaced by a new process that has novel consequences. In *H. erythrogramma* (Fig. 3), gastrulation is immediately followed by generation from the tip of the archenteron of a very large coelom that provides the extensive hydrocoel mesoderm which interacts with the ingressing ectodermal vestibule. Together these cell layers form much of the developing juvenile sea urchin. These same coelomic-vestibular interactions occur in indirect development as well. However, they occur much later and involve a different strategy of cellular recruitment. Only a few (10-12 cells) from the archenteron contribute to each left and right coelomic pouch precursor (Cameron et al., 1991). Subsequent increases in coelomic cells are produced by cell growth and division in the feeding and growing pluteus larva (Pehrson and Cohen, 1986). In *H. erythrogramma*, the origins of coelomic cells are different. There is no growth. The juvenile adult is formed directly from cells of the embryo. Thus, whereas in the fate map of an indirect developer only a part of two cells of the 32-cell embryo give rise to vestibule, a full eight cells of the 32-cell *H. erythrogramma* embryo produce the vestibule (Wray and Raff, 1990).

In order to allow large scale elaboration of the coelom by *H. erythrogramma*, cells must be added to the archenteron. Isovolumetric cell-cell rearrangements alone would be inadequate. Thus, the second phase of the ancestral mode of gastrulation has been abandoned and involution continues instead of terminating early. There are two phases of involution in *H. erythrogramma*. The first is symmetric, and

indirect development
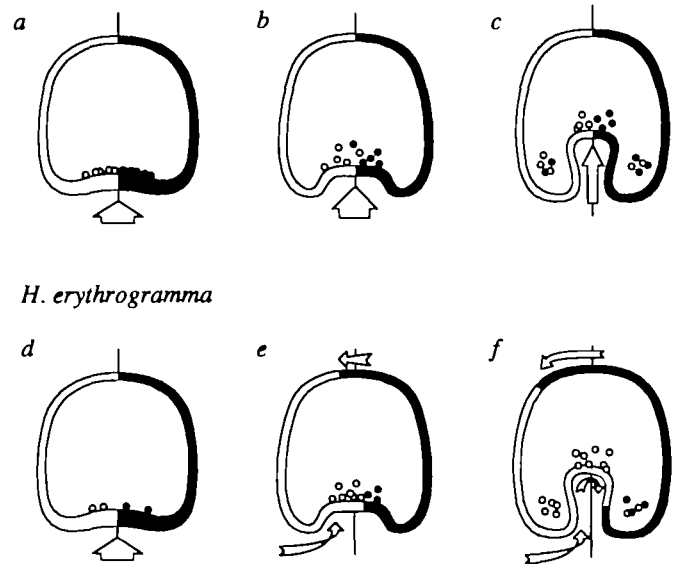
H. erythrogramma

Fig. 2. Cell movements in the two modes of sea urchin gastrulation. A, In indirect development, primary mesenchyme cells enter the blastocoel prior to the start of archenteron ingression. B, Initial ingression of cells of the vegetal plate. C, Elongation of the archenteron involves only cell rearrangements and cell shape without involution. In *H. erythrogramma*, D and E, primary mesenchyme cells enter the blastocoel as ingression progresses. Ingression begins similarly to that of indirect development, but rapidly shows a ventral bias in the involution of the vegetal plate. F, As gastrulation continues, archenteron extension is by asymmetric involution of cells from the ventral side. Reproduced from Wray and Raff (1991), with permission of Evolution.

evidently corresponds to the initial involution of indirect development. The second phase of involution is asymmetric, and involves the ingression of ventral ectoderm. This asymmetric ingression combined with the involution of a full quarter of the cellular volume of the embryo to form the vestibule, results in the apposition of vestibule and coelomic cells sufficient for morphogenesis of the ventral portion of the juvenile adult sea urchin within a few hours after gastrulation (Fig. 3). Finally, since gastrulation is the topic of this symposium it is important to note that evolution shows that parts of the gastrulation process are dissociable from each other. Some features of *H. erythrogramma* gastrulation have been conserved, and others have changed in substantial ways (Wray and Raff, 1991). Thus, the position of gastrulation initiation, invagination by involution, and timing of primary mesenchyme cell ingression have been conserved. The number of primary mesenchyme cells, the origins of cells contributing to the archenteron, the mechanism of archenteron elongation, the symmetry of cellular movements, and the origin of coelomic cells have all been substantially changed. In total the evolution of the novel ontogeny of *H. erythrogramma* has resulted from a suite of changes encompassing changes in timing of developmental events, cell fates, cell-cell interactions, and gastrulation movements.
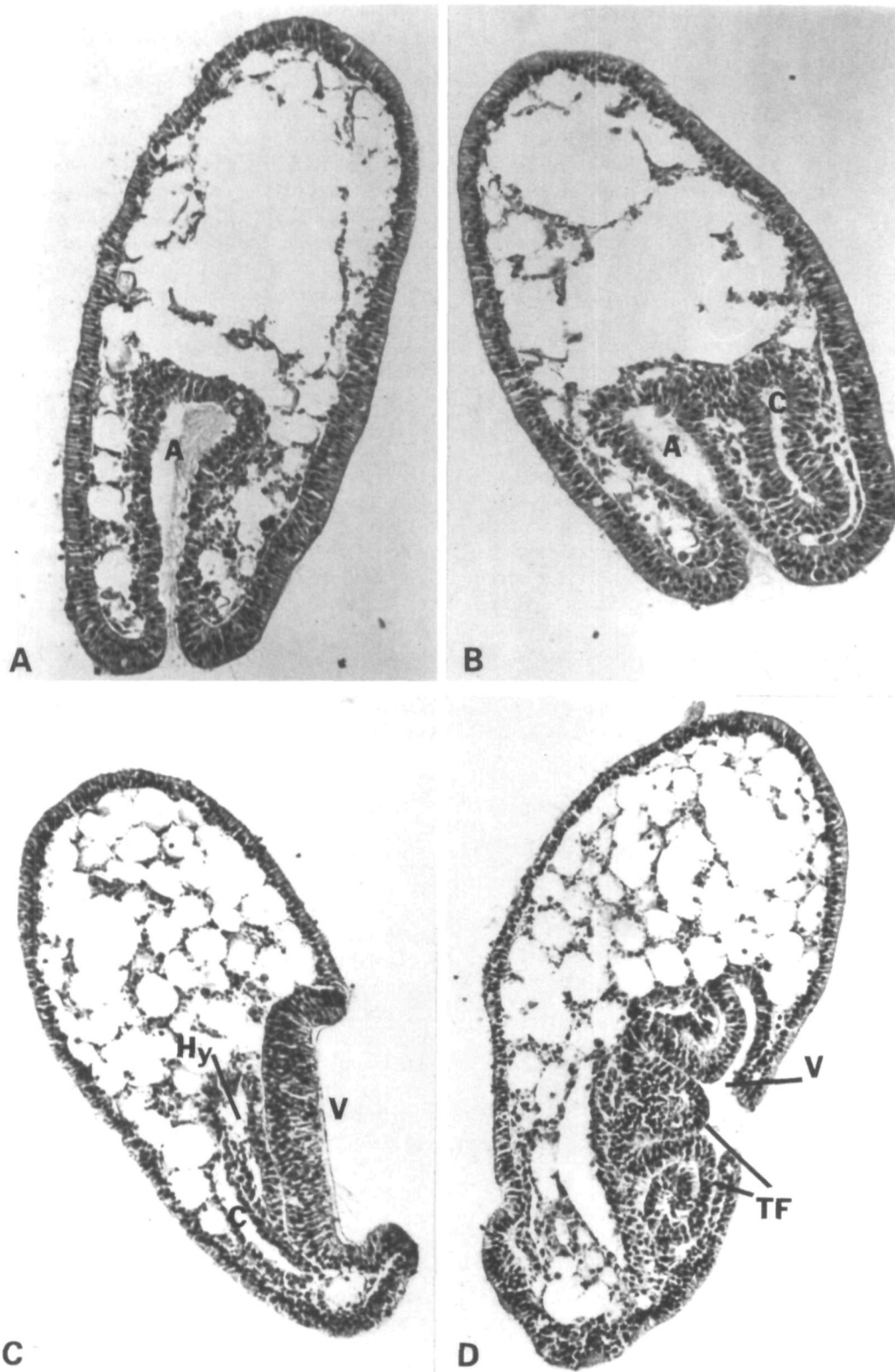
Fig. 3. Gastrulation and subsequent coelom formation in *H. erythrogramma*. Embryos are oriented with animal poles up and ventral side to the right. A, Full extension of the archenteron (A) in the 18 hour mid-gastrula. Note the initial bulging of the archenteron tip as coelomic pouches begin to form. B, Late (22 hour) gastrula with a large coelomic sac (C). The ectoderm overlying the coelom has begun to thicken. C, Early (30 hour) larva with vestibular (V) invagination of ventral ectoderm. The hydrocoel (Hy), which is a derivative of the coelom has formed and is closely associated with the vestibule. D, Mid larval development (34 hour) showing rapid development of tube feet (TF) from the interaction of vestibular ectoderm and hydrocoel mesoderm.

How two disciplines read the story

The usual ending to chapters on development/evolution is to show how the research under discussion advances the fusion of the two fields or provides data on one of the questions posed in the boundary field. That is obviously the objective motivating our research as well. However, at this point in history it is also of interest to look at features important in an evolutionary embryology context and dissect out what is significant about each separately to developmental and evolutionary biology. Table 2 lists several such features, each of which has quite different meanings in the currency of the respective fields.

The most notable aspect of *H. erythrogramma* is its radical reorganization of early development. From a developmental viewpoint, *H. erythrogramma* reveals that early development is governed by independent mechanisms which function in an integrated manner, but are dissociable from each other in evolution. Relationships between mechanisms can be changed in ways that produce other viable ontogenies. That this is so may seem surprising. It is commonly expected that early development must be rigidly constrained because early events must serve to specify the broad foundations of later development. Indeed, much current work in developmental genetics supports a view that early development includes the refinement of maternal information by zygotic gene action to produce the initial patterning of the embryo (Nüsslein-Volhard, 1991). The dissociability revealed by comparative studies is a manifestation of what developmental biologists have long recognized as the regulative abilities of embryos subjected to experimental perturbations. The phenomenon of regulation

is still not understood mechanistically, but evolution gives us an insight into its potentially great importance.

For evolutionary biology, the finding that early development can be reorganized means that development may respond more flexibly to selection than generally appreciated. Just how this occurs, and under what developmental constraints is clearly an important matter for understanding the origins of evolutionary novelties, evolutionary trends, and evolution of life histories. Evolutionary discussions of ontogeny are generally about later stages of development. Yet early development is as able to respond to selection as are later stages, and evolutionary innovations can result from changes in early development. Tropical frogs, for example, exhibit nearly thirty distinct adaptations in early development ranging from gastric brooding of tadpoles to direct development on land (del Pino, 1989; Tyler, 1983). Such experiments in early development should remind us that the key innovation to the vertebrate conquest of the land was probably the evolution of the amniotic egg.

Evolution of specific processes is also highly revealing in having quite different implications for the two disciplines. One of the most striking features of *H. erythrogramma* development is the reorganization of cell fates and cell lineages. The mechanistic consequences to one subsequent developmental process, gastrulation, were mentioned above, but there are more profound implications as well. The focus of developmental biologists on model organisms is usually done so that the species that is most experimentally accessible for the study of a particular process is employed. Without an evolutionary dimension to such studies, a pattern of development that is consistent within a species may be interpreted as mechanistically required. However, such a conclusion is weak. For example, in the well studied sea urchin *Strongylocentrotus purpuratus* there is a constant relationship between the first cleavage plane and the dorso-ventral axis (Cameron et al., 1987). This relationship is reflected in cell placement, cell lineage precursors, and cell fates in morphogenesis. When seen from an evolutionary perspective, constancy breaks down (Henry et al., 1992). Different species do exhibit constant relationships between first cleavage plane and dorso-ventral axis, but several different relationships exist between the orientation of first cleavage and the dorso-ventral axis among closely related species. This is a lesson in what evolution can provide to development; it lets us sort out real mechanisms from the idiosyncrasies of any one model organism. The message for evolutionary biology is a quite different one. Cell types and structures that appear to be homologous arise from quite different cell lineage precursors and different developmental processes. The temptation to seek a developmental basis for homology is always strong. However, early as well as late development proves to be an unreliable guide to homology, a point well realized by earlier embryologists (Wilson, 1895; deBeer, 1971). If a developmental basis for homology is to be defined, it must lie in the genetic regulatory systems that underly particular developmental features.

Table 2. *What we learn from Development/Evolution*

FEATURE	MEANING TO DEVELOPMENT	MEANING TO EVOLUTION
Stages of Development Exhibit Different Amounts of Evolution	Different Mechanisms or Degrees of Integration Between Stages	Existence of Phylotypic Stages and Stable Bauplans
Convergent Evolution of Development	"Standard" Morphogenetic Processes	Convergent Features Confound Phylogenetic Information
Radical Reorganization of Early Development	Multiple Genetic Mechanisms Determine Early Development	Flexible Responsiveness of Development to Selection
Cell Lineage Reorganization	Readily Recognizable Common Features may not be Regulatory Controls	Developmental Basis of Homology Unreliable
Common Regulatory Genes	Homologous Genes may not Play same Roles	Gene Co-option Frequent
Genes that Repress Pathway	Prevent Incorrect Program in Portion of Body	Create Novel Features that Provide Basis for Further Evolution

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