

Pattern and process heterochronies in the early development of sea urchins

Gregory A. Wray and Rudolf A. Raff

Heterochrony is a prominent feature of echinoid evolution. This does not, however, reflect the repeated occurrence of just a few kinds of change in developmental mechanism during the course of evolution. Instead, heterochronies in echinoid development are extremely diverse, in terms of both their phenotypic effect and the developmental processes involved. Heterochronies arise throughout the life cycle, including oogenesis and early development, and can affect any biological process with a temporal component. The large base of comparative data on echinoid development, coupled with a relatively good understanding of their phylogenetic history and fossil record, makes sea urchins an excellent model system for the studying heterochrony and other kinds of evolutionary change in development.

Key words: heterochrony / echinoids / morphogenesis / gene expression / cell lineage

MOST STUDIES of heterochrony have focused on changes in morphological parameters late in development.¹⁻³ These heterochronies, which we call deBeerian after their first comprehensive classifier,⁴ are usually recognized as a relative acceleration or retardation between somatic and gonadal maturation. The analysis of deBeerian heterochrony has proven useful for assessing global phenotypic effects such as body size or extent of somatic development at reproductive maturity. These heterochronic effects can have profound ecological significance.^{1,5} However, the importance of heterochrony is not limited to this class of changes. In this review we consider the significance of heterochronies in early development. We also stress the importance of studies of developmental mechanism as an adjunct to understanding morphological heterochronies. To illustrate these points, we draw upon studies of heterochrony in the early development of echinoderms.

The scale of spatial domains within which heterochrony occurs is broad, ranging from global to much more local processes. Along the temporal spectrum, heterochronies occur throughout the life cycle, affecting developmental processes from oogenesis and early development through senescence. Heterochronies also occur in developmental processes operating at various organizational levels, from the molecular through the morphological. Since the majority of studies have sought heterochronies producing global changes in adult morphology, the representation of documented heterochronies is skewed towards one end of each of these spectra.³ Temporal alterations affecting non-morphological processes, early development, or localized processes are sparsely documented.

Over the past several years, it has become apparent that evolutionary changes in timing are common in early development.^{3,6-8} Many heterochronies in early development do not appear to significantly alter adult morphology, and may represent adaptations peculiar to specific phases of the life cycle. The most obvious examples concern larval adaptations for protection, dispersal, and feeding.⁹ Because they affect larval phenotype, many heterochronies in early development profoundly affect life history strategies,^{7,10} and have clear ecological and evolutionary significance.¹¹⁻¹³

Heterochronic pattern and process

It is important to realize that the developmental mechanisms which underlie the vast majority of observed heterochronies are very poorly understood. Recognizing a pattern of change is independent of mechanistic considerations. We therefore distinguish pattern heterochrony, an evolutionary change in the time a feature appears during development, from process heterochrony, an evolutionary alteration in developmental mechanism which involves timing. This is a crucial distinction: pattern heterochronies often result from developmental mechanisms which have nothing to do with timing, and process hete-

From the Institute for Molecular and Cellular Biology, and Department of Biology, Indiana University, Bloomington, IN 47405, USA

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rochronics do not always produce pattern heterochronies.³

The distinction between pattern and process depends upon the hierarchical level of biological organization of interest to the observer, and one investigator's process may be another's pattern. Failure to acknowledge the relative relationship between process and pattern, however, allows almost any evolutionary change to be interpreted as heterochronic. Because of the prevalence of pattern heterochronies, it has become commonplace to regard heterochrony as the most important 'mechanism' for phenotypic change in evolution. The absence of mechanistic information for the overwhelming majority of cases renders such assurance questionable. Even given the fact that pattern heterochronies are common,^{1,14} there is no clear reason for expecting that they result predominantly from mutations in regulatory genes which directly control timing during development.³

Fundamentally, there are only two types of pattern heterochrony: acceleration and retardation.^{1,2} Acceleration is the appearance of a feature earlier in the ontogeny of a descendant than in that of the ancestor, while retardation is the delayed appearance of such a feature. In contrast, there are six types of process heterochronies.² These concern the initiation, termination, and rate of a process. Thus, a developmental process may begin earlier or later in a descendant than in its ancestor. Similarly, a developmental process may end earlier or later in the descendant ontogeny. Finally, a descendant developmental process may progress more rapidly or slowly. In each case, the effects of a process alteration on the resulting pattern may be complex, and are not strictly predictable. For example, changes in the time of initiation or rate may or may not alter the duration of the process, depending upon the timing mechanisms controlling termination.³

Temporal domains

All evolutionary change in development, including heterochrony, requires functional dissociation between developmental processes.¹⁵ Although dissociations can occur throughout development, the kinds of dissociations that are possible change as different processes come to dominate development.³ The kinds of heterochronies that arise at different phases of the life cycle will therefore differ qualitatively.

During early development, the egg cytoplasm is partitioned into a large number of cells which become committed to specific and regionalized pathways of differentiation within the embryo. As these regional identities become established, short and long range interactions such as induction and hormones come to dominate development. These interactions are initially broad in scope, and include global events such as primary induction. Later, the majority of interactions are much more local in scope, as in limb development, where interactions are spatially restricted. The degree of interaction between individual developmental processes is thus relatively low early in ontogeny, increases as the definitive adult body plan is established, and finally decreases as interactions become more localized.

These generalizations regarding changes in the degree of dissociability among developmental processes are borne out by empirical data. Heterochronies are in fact common during the early development of numerous animal groups.^{3,6,8} In most phyla there is substantially less variation, however, as the adult body plan is established.¹⁶ Thus all vertebrates pass through a morphologically conservative 'pharyngula' stage, but during both earlier and later development there is considerably more variation.^{6,16} In echinoderms the juvenile rudiment is the comparable conservative stage, and once again morphological variation in earlier and later development is much broader.⁷

Heterochrony in echinoid evolution

The Echinoidea, the echinoderm class which includes sea urchins and sand dollars, constitutes an excellent group for the study of heterochrony. This is due to a good understanding of the phylogenetic history of the group, as well as a relatively large base of comparative information on their development. Because echinoids have a complex adult morphology which fossilizes reasonably well, it is possible to trace deBeerian heterochronies in late (postmetamorphic) development in both extant and fossil species (reviewed in ref 14). Some of these heterochronies have resulted in global changes in adult body size and shape,^{17,18} while others have produced more local effects on specific structures such as test plates and teeth.¹⁴

Because their embryos are so amenable to manipulation, echinoids also provide an excellent experimental system for studying evolutionary change in

the cellular and molecular processes of early development. It is on changes in these processes that the remainder of this discussion will focus. The ancestral mode of development for echinoids as a group is by means of an elaborate feeding larva called the pluteus.^{7,11,19} This indirect mode of development has been conserved through hundreds of millions of years of echinoid evolution, even in groups such as sand dollars (*Scutellina*) and heart urchins (*Spatangoida*) where adult morphology is highly modified. Nevertheless, heterochronies in cellular and molecular processes have arisen within the confines of this morphologically conservative developmental mode. Heterochronies are also common in echinoids which have evolved radically different developmental modes. These species undergo direct, or lecithotrophic, development; they do not produce a feeding larva but instead rapidly develop into a juvenile sea urchin.

Although heterochronies occur in both indirect and direct developing echinoids, to some extent they differ qualitatively according to developmental mode. The heterochronies that have been identified in indirect development occur predominantly in gene expression and some aspects of morphogenesis. The evolution of direct development has entailed heterochronies in a much wider diversity of processes during early development, including in addition to the above cell lineage, cell determination, and morphogenesis. Examples of heterochronies are discussed below in turn for indirect and direct developmental modes.

Heterochrony within a conserved developmental mode

As mentioned earlier, heterochronies occur throughout development. Studies with echinoids demonstrate that heterochronies can occur as early as oogenesis. Variations in egg size among closely related echinoid species are likely the results of changes in the duration of oogenesis. Such variations are quite common.^{13,20} The eggs of *Strongylocentrotus* species, for example, range from 80 μm diameter (*S. purpuratus*) to 160 μm (*S. droebachiensis*), an 8-fold difference in cytoplasmic volume. Although differences in egg volume do not significantly affect the size or morphology of adults, they do influence larval form and rates of early development.²¹

Oogenesis is a time of intensive transcription as maternal mRNA crucial for early development accu-

mulates within the oocyte. A heterochrony in transcription during oogenesis of the gene encoding α -subtype histone has occurred within the echinoids.²² In several 'advanced' sea urchin species (Euechinoidea) transcription takes place during oogenesis, while it does not begin until midblastula in the pencil urchin *Eucidaris tribuloides* (Cidarioidea). Maternal α -subtype histone mRNA was also observed to be absent from eggs of a starfish and a sea cucumber, both members of non-echinoid echinoderm classes. Since expression in the starfish also begins during embryogenesis, the pattern of later expression observed in the cidaroid is likely the ancestral condition. Maternal expression of the α -subtype histone gene in euechinoids probably represents a pre-displacement heterochrony at the level of gene expression.

Several heterochronies in embryonic gene expression have arisen during the radiation of echinoids. Temporal patterns of expression for three proteins (*msp130*, *Meso1*, and *SP-12* antigen) have been compared in species representing a broad taxonomic range within this group.⁸ The expression of all three proteins differed among the species examined, the most common changes being heterochronies in the time of initial synthesis. The protein *msp130*, for example, appeared coincident with primary mesenchyme cell ingress, just after ingress, or following migration in three species (Figure 1). A minimum of eight initiation heterochronies are required to generate the diversity of expression patterns among the seven species examined. In addition, two of the proteins were shown to have undergone heterotopies, or changes in spatial expression within the embryo. Heterochronies have also been reported in the transcription of actin genes in euechinoids,²³ although the nature of these changes are less clear.

Despite the growing list of molecular heterochronies, morphogenetic processes are highly conserved among indirect developing echinoids. The few heterochronies in morphogenesis that have been noted primarily affect the timing of appearance of specific mesenchyme cell populations. In echinoids, mesenchyme cells ingress into the blastocoel and give rise to several differentiated cell types, including spicule-forming cells and pigment cells. A heterochrony in spicule-forming cell ingress has accompanied the divergence of the two main lines of echinoid evolution. These cells ingress prior to gastrulation in euechinoid species,²⁴ but not until gastrulation is underway in cidaroids.²⁵ Similarly, pigmented mesenchyme cells ingress during

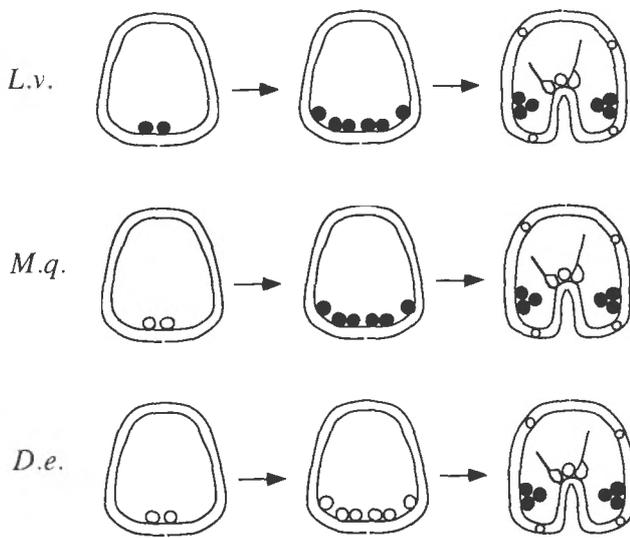


Figure 1. Initiation heterochronies in *msp130* expression. The primary mesenchyme cell-specific glycoprotein *msp130* is first synthesized at different times among echinoid species. This figure summarizes the presence of *msp130* (indicated in black) in three species: *Dendraster excentricus* (Clypeasteroidea), *Mellita quinquesperforata* (Clypeasteroidea), and *Lytechinus variegatus* (Temnoplueroida). Adapted from ref 8.

gastrulation in most echinoids^{24,26} but prior to gastrulation in a heart urchin (Spatangoida).²⁷ These heterochronies are significant in that gastrulation and mesenchyme cell ingressions are among the earliest morphogenetic events during echinoid development.

Heterochrony and switches in developmental mode

Changes in developmental mode almost invariably involve a general shortening of the period between fertilization and metamorphosis, and thus constitute global heterochronies in early development.⁷ The pluteus larvae of planktotrophic echinoids remain in the water column for weeks or months, but lecithotrophic larvae typically settle and undergo metamorphosis in a few days.¹⁰ Larvae of the Australian species *Heliocidaris erythrogramma* undergo metamorphosis just 3.5 days following fertilization.²⁸ Our recent work with *H. erythrogramma* suggests that this global predisplacement and acceleration is actually the product of numerous and diverse changes in underlying developmental mechanism.

The larval morphology of *H. erythrogramma* is highly modified, and the duration and order of morphogenetic processes reflects this change (Figure 2).⁷ Some processes, such as secretion of the adult calcareous skeleton of test plates and formation of the rudiment are accelerated; other processes, such as secretion of the larval skeleton and formation of a functional larval gut and mouth, have been eliminated.^{7,28} Some of these overt morphological changes are accompanied by heterochronies in gene expression. For example, *msp130* synthesis, which occurs exclusively in spicule-forming cells, parallels the change in timing of spicule production.²⁹

Changes in the development of *H. erythrogramma* also include extensive alterations in the mechanisms which control cell lineage and fate.^{30,31} The founder cells for particular embryonic and adult cell types appear at different times during development and in an altered sequence (Table 1). Ectodermal cell lineages are often founded earlier in *H. erythrogramma*, while internal cell types such as spicule-forming cells generally appear later. Heterochronies in the segregation of various cell lineages produce changes in proportions of specific cell types within the embryo. In *H. erythrogramma*, changes in the time of cell lineage segregation have resulted in a greater proportion of ectoderm at the expense of internal cell types (Figure 3).

Another kind of cell lineage heterochrony has also occurred during the evolution of direct development in *H. erythrogramma*: dorsoventral differences in cell fate arise earlier than during indirect development.³¹ This change in cell lineage is mirrored by a heterochrony in cell fate determination, such that dorsoventral axis determination occurs by the 2-cell stage.³² In contrast, the dorsoventral axis is labile beyond the 4-cell stage during indirect development.³³

Table 1. Heterochronies in cell lineage segregation*

Cell type	Cleavage stage	
	<i>H. erythrogramma</i>	Indirect development
Spicule-forming cells	After 64-cell *	32-cell
Vegetal ectoderm	16- and 32-cell†	64-cell
Coelomic cells	64-cell	32- and after 64-cell†
Vestibular ectoderm	8-cell	After 128-cell

*Adapted from ref 31.

†Cells arise from separate lineages which segregate at different times.

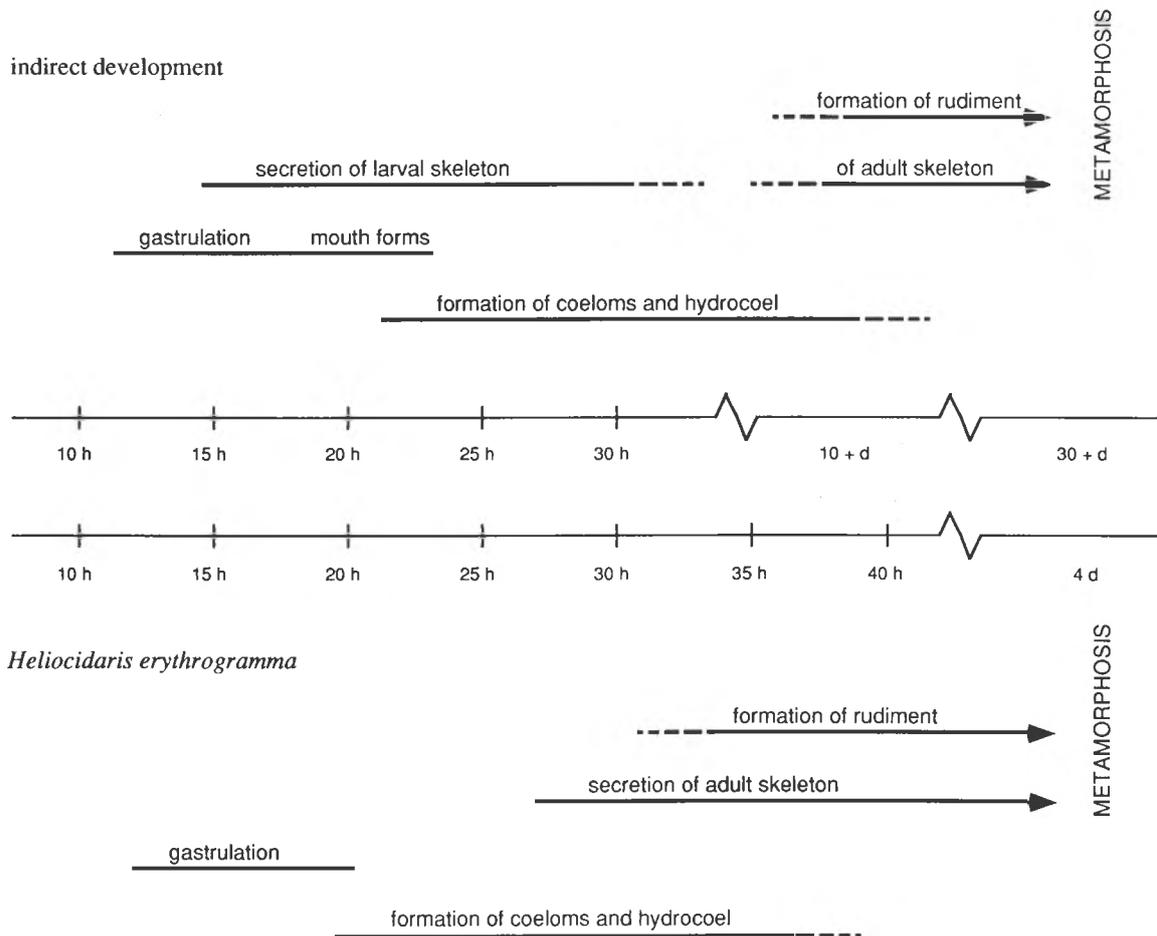


Figure 2. Heterochronies in direct development. Numerous temporal changes have arisen during the evolution of direct development in *H. erythrogramma*. Some developmental processes, such as synthesis of the larval skeleton and formation of the larval mouth, have been lost. Other processes, such as formation of the adult rudiment and skeleton begin earlier or occur at a faster rate. These changes result in a general acceleration of premetamorphic development (note that the time scales are no longer colinear after 30 h). Several other developmental processes not shown here, such as cell lineage segregations and expression of *msp130*, have also undergone heterochronies (see text). Based on refs 7, 28, 29.

Although the evolutionary changes in cell lineage and cell fate which have been documented in *H. erythrogramma* are pattern heterochronies, they may be due to alterations in developmental mechanism which do not involve timing. The earlier time of cell commitment, for example, is most likely the result of a shift in spatial localization within the egg, and is thus a heterotopy in terms of mechanism. Similarly, the changes in cell lineage are the result of changes in cell cleavage geometry and may also involve altered patterns of cell-cell interaction. Again, the mechanistic changes are spatial in nature, yet they have temporal consequences.

Are heterochronies in early development meaningful?

The evolutionarily conservative nature of early development is a common theme in discussions of the relationships between ontogeny and phylogeny. It is often argued that because late events during development depend upon preceding processes, early development is highly burdened or constrained from evolutionary change.¹ Neither the number nor the diversity of heterochronies now known from the early development of echinoids are predicted by concepts of developmental constraint. Indeed, the influence of

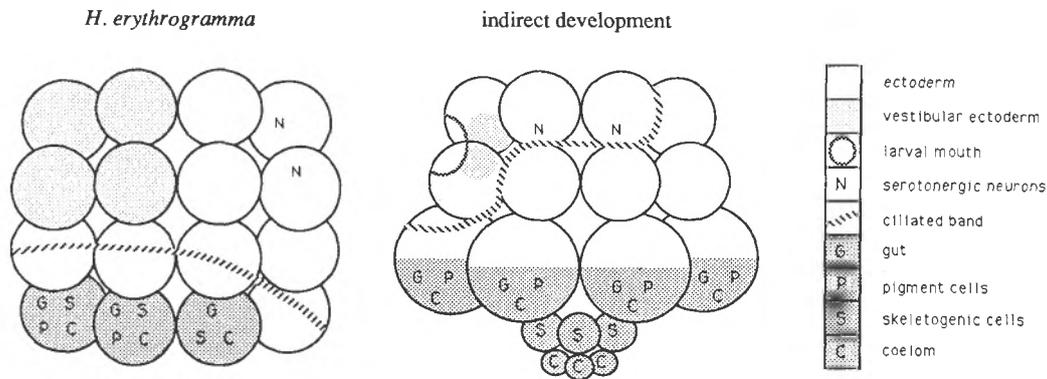


Figure 3. Fate maps of 32-cell embryos. The embryos of *Heliocidaris erythrogramma* and an indirect developer differ markedly in terms of cell fate distributions. Some of these differences, such as the greater proportion of cytoplasmic volume fated to become ectoderm in *H. erythrogramma*, are due to heterochronies in the time of cell lineage segregation (see Table 1). Dorsoventral asymmetry in cell fates is accelerated in *H. erythrogramma* relative to indirect development, and is already pronounced at this time. From ref 31.

developmental constraints seems weak in a group where changes are common in fundamental early developmental processes such as morphogenesis, differential gene expression, cell fate determination, and cell lineage segregation.

A salient, though not exclusive, feature of direct development in echinoids is the loss of characteristic larval features. It might be argued that because only dispensable larval features have been lost, the heterochronies involved in direct development do not violate notions of evolutionary constraint in early development. This would be true if cells and processes leading to the adult were unaffected by heterochronies in early development: the evolution of direct development could then be relegated to an evolutionary sideshow involving changes in purely larval features.

This is not, however, the case for *H. erythrogramma*. We have shown that evolutionary changes in cell lineage^{30,31} and in cell fate determination³² have arisen prior to the divergence between larval and adult developmental programs. Figure 3 illustrates some of the differences in cell fate already present at the 32-cell stage, when segregation of cell lineages leading to exclusively adult fates has not yet begun. The organization of development in *H. erythrogramma* is thus not merely a condensation of the indirect developmental program brought about by the excision of dispensable larval features, but a profoundly novel pathway to the adult.

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