

What Does the Comparative Study of Development Tell Us About Evolution?

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Abstract. During the time period when the metazoan phyla were originating, these animals probably had a life cycle which included larval and adult phases with a short metamorphic period separating each phase. Because of adaptive pressures which function to minimize the metamorphic transition by having adult organs begin their development during the larval period, this phase of the life cycle became the period in which most of the changes occurred that were responsible for the origin of new phyla. One consequence of adultation was the juxtaposition of adult organs in new patterns, in different stages of development in larvae, creating the potential for the formation of new cell types, tissues, and organs. Extreme adultation leads to direct development and lecithotrophy. These developmental trends have caused changes in the way in which determinative events occur during early embryogenesis and provided the basis for a radical reorganization of early embryogenesis.

INTRODUCTION

The most interesting evolutionary events are those that have led to major changes in the anatomical organization of animals. These changes are associated with the formation of new phyla. The fossil record indicates that virtually all of these evolutionary events occurred in the Precambrian, Cambrian, and Ordovician periods. However, the fossil record contains essentially no evidence which indicates how these events occurred.

Virtually all of the speculations that deal with the issue of how the various metazoan animal phyla originated are ultimately based on embryological considerations (1,6,11,14). Any set of descendants that have an anatomical organization which is different from an ancestral stock must have had their process of development modified in some way; this developmental change presumably reflects some kind of genetic change. In order to reconstruct the developmental change which is responsible for a given structural change, embryologists compare the development of two extant forms which have the same common ancestor (6,11). These comparisons are set up so that the form showing a given change in organization is compared with a related form which resembles the ancestor in that it does not show this change in organization.

When the taxonomic units being compared are closely related (e.g., comparisons between different genera or families), one has the impression that most of the phylogenetic changes that took place can be explained by invoking a change in developmental timing. This involves a change in the time of appearance or rate of development of a feature in the descendant's ontogeny with reference to the same set of features in the ontogeny of the ancestor. These changes in developmental timing usually involve the last stages of development. When one is concerned with higher taxonomic categories such as a class or phylum, changes in developmental timing are still of great importance; however, they generally involve earlier stages of development. In addition one has to explain the formation of new organs, the appearance of new cell types, and in some cases, a basic change in body plan.

As one compares the development of animals in higher taxonomic units, the problems involved in constructing phylogenies which relate these animals to a common ancestor become more formidable. While comparative studies on the morphology of animals have made it clear that certain phylogenetic relationships are more probable than others at the level of phyla or classes within a phylum, there are usually several different

ways a given phylogeny can be set up depending upon the facts that one wants to emphasize. For example, speculative phylogenetic sequences have been used to derive the vertebrates from the Nemertines (21), the Echinoderms (8), and the Tunicates (1). The only approach which appears to have the potential of resolving these phylogenetic questions involves the judicious use of appropriate molecular chronometers based on studies of amino acid substitutions in proteins which have evolved at different rates (22). Information gained from these studies should restrict the number of possible phylogenies which can be constructed.

Many of the speculations that deal with the origin of higher taxa take an organ which has recently evolved in some special context in an ancestor and makes its appearance at the end of ontogeny. The time when this organ first appears is then transferred to an earlier stage of development in the descendant and the function of the organ is modified. The major objection against this view is that one cannot move the time when an organ first begins to form during ontogeny without seriously disrupting the developmental program of the animal. This objection is supported by several kinds of data which indicate that a standard insult administered during early embryogenesis usually has a much more pronounced effect on development, than the same insult does when administered during later stages. As examples, one can cite the effect of a given dose of x-rays, or the effect of creating cells homozygous for zygotic lethals at different stages of development (17,24).

THE IMPORTANCE OF LARVAE

This objection has to be considered in the context of the life cycle which animals in most phyla have. This life cycle begins with a period of embryogenesis which generates a larva. The larva functions in a given ecological setting for a period of time and then undergoes metamorphosis, which can be viewed as a second period of embryogenesis, to become an adult which will function in another ecological setting. Only two or

three of the twenty or so animal phyla lack classes which fit this life cycle pattern (14). There is no reason to think that this kind of life cycle was not prevalent when the majority of animal phyla first appeared. In most cases larval and adult phenotypes of a given animal have become quite different, presumably because of evolutionary pressures operating in two different adaptive contexts. Metamorphosis, like embryogenesis, is a critical period in the life cycle. The metamorphic transition occurs relatively rapidly. Frequently the animal stops feeding during the transition. It is losing the anatomical features which have allowed it to function in one ecological setting, but it has not yet had time to finish building those anatomical features which will adapt it to its new environment. Since the adult phase of the life cycle is the reproductive phase, any change in either the larval or the adult phase of the cycle as a consequence of evolutionary pressures cannot be so overwhelming that the metamorphic transition is jeopardized. This situation has put evolutionary pressure on the larval stage of the life cycle to accomodate metamorphosis by anticipating this developmental event in some way. From an embryological point of view, the larval period of the life cycle is a time of relative developmental stasis. However, the tissues that make up larvae are clearly developmentally competent; many kinds of larvae have a remarkable capacity to regenerate parts (12). Therefore this stage is ideally suited for developmental programming which facilitates and anticipates metamorphosis.

COMPONENTS OF METAMORPHOSIS

There are a number of questions that come to mind when one considers the mechanics of metamorphosis. One wants to define the changes in the cells that make up the larva. As a consequence of metamorphosis, some cells may become functionally differentiated for the first time, other cells may change their differentiated state exhibiting different degrees of polymorphism. There may not be any change in the differentiation of some cells, while some cells may die. Not all of the

cells in some larvae will make a contribution to the adult; in these cases a distinction is made between larval and adult action systems. The amount of development a given adult action system undergoes in a larva is variable. Just prior to metamorphosis, some adult action systems may be a disc of embryonic cells, other action systems may consist of cells which have undergone variable amounts of morphogenesis and differentiation, while others are represented by fully differentiated organs. In the larva of an insect such as *Drosophila*, the imaginal discs represent components of the adult action system. Most prosobranch molluscs have a veliger larva. The veliger is a modified trochophore larva in which many of the adult action systems such as the foot and shell have appeared.

One also wants to identify the developmental mechanisms which are responsible for specifying the way a given population of cells in the larva will behave at metamorphosis. At one extreme one could envisage a situation in which the entire larva has the properties of an embryonic field. When the appropriate metamorphic stimulus is conveyed to the larva, the axial system that was set up in the larva during embryogenesis is used as a source of positional information for integrating the morphogenetic and differentiative changes that constitute metamorphosis. This kind of mechanism can also operate in larvae with adult action systems; here a given action system constitutes the field. At the other end of the spectrum, one could envisage a situation in which all of the cells of the larva are already specified with respect to their developmental state in the adult. Presumably these determinative events occurred at an earlier time in the larval period. The physiological mechanism which mediates metamorphosis causes these cells to express their adult phenotype. Each cell is part of an adult mosaic and there is essentially no developmental interaction between cells. In different groups of animals, each of these developmental strategies is combined in different ways in different parts of the larva to effect metamorphosis. The metamorphosis of the hydrozoan planula

provides an example of a situation in which the operation of an embryonic field is emphasized (15) while the metamorphosis of the tunicate tadpole provides an example of a situation in which the operation of a stimulus on a predetermined substrate is emphasized (3).

ADULT ACTION SYSTEMS IN LARVAE

At some point in their development, virtually all larvae form one or more adult action systems. This phenomenon is referred to as adulthood (14). When one examines representative larvae from each class in a phylum, one sees that the number and kinds of elements from the adult body plan that make their appearance in these larvae are usually quite variable. The temporal order in which two or more unrelated adult elements first appear in these larvae can also be different. The site of formation of a given adult action system and the position of this action system relative to another adult system may be different in different larvae. This set of generalizations can be easily tested by taking a large phylum such as the molluscs, picking a set of adult characters such as the mantle, the foot, the radula, the gills, the metanephridia, etc., and following the development of these characters in representative larvae of each class.

THE CONSEQUENCES OF ADULTATION IN LARVAE

One consequence of the adulthood of larvae is that this developmental stage becomes a setting for creating Richard Goldschmidt's hopeful monster (10). By creating different topological and temporal juxtapositions of various adult organs at different stages of development in a larva, one provides the conditions in which one has the potential for initiating significant new cellular or anatomical changes by virtue of developmental interactions between these parts. One way to envisage such an interaction at a cellular level is to have an ancestor in which the larval cells in a given region produce a unique group of metabolic products, "A and B." After the larva undergoes metamorphosis, these same cells or their progeny may produce another unique group of metabolic

products "C and D." In a descendant of this animal, the adult tissue produced by these cells may change its time of appearance to the larval phase of the life cycle. This could conceivably create a condition in which these cells simultaneously produce larval products "A and B" and adult products "C and D." These four products may interact in a fortuitous manner giving these cells a new set of properties, thereby providing the basis for the origin of a new cell type. Similar scenarios can be worked out for the origin of new organs.

Another consequence of adulthood is that it frequently creates conditions in which selective pressures can operate to cause a change in the relative length of the larval period of the life cycle. Many larvae feed in the plankton. Under certain ecological conditions in cases where adulthood has been extensive, adaptations have occurred which are designed to minimize the risks involved in larval feeding. One of these adaptations has involved increasing the store of yolk in the egg so that the larvae do not have to feed (lecithotrophy) and can metamorphose to form adults in a protective environment such as an egg capsule or brood pouch. This kind of adaptive pressure frequently leads to a progressive compression of the larval period of the life cycle and finally to direct development in which one goes from egg to adult.

Direct development has evolved in a large number of lineages; the phenomenon is seen at every taxonomic level from species to phylum. For example, if one considers the opisthobranch molluscs in the order sacoglossa, one can classify the veliger larvae according to their developmental patterns which range from a feeding free swimming larval stage (planktotrophy), to lecithotrophy with a shortened feeding period, to lecithotrophy with the larva in a capsule which protects it and prevents feeding, to direct development. Sometimes two or three developmental patterns exist in one species (2). The entire cephalopod class within the molluscs shows direct development. Direct development appears to have effected

early embryogenesis in a number of ways which has the potential of creating new developmental patterns.

The cleavage pattern, the mode of gastrulation, and the correspondence between the main features of the body plan and certain features of the early embryo such as the fate of the blastopore are generally very similar within an assemblage of related phyla such as protostomes and deuterostomes. Usually evolutionary pressures which have compressed the larval period and lead to direct development do not change this conservative developmental pattern. However, they appear to be able to alter the time when basic determinative events such as the formation of planes of symmetry occur and the way in which symmetry properties function in integrating development. Presumably these changes take place because of adaptive pressures to shorten the developmental period. The amount of solid information that bears on this point is limited because states of determination are defined by experimental procedures and very little comparative work has been done. As an example I want to consider the determination of the median plane in a series of representative deuterostomes.

All of the deuterostomes begin developing as radially symmetrical embryos; at some point in each group bilateral symmetry first becomes evident. A set of determinative events will precede these overt events. These determinative events may occur over a relatively long period of time, or they may occur quickly. The amount of time which elapses between the determination of the median plane and the overt establishment of bilateral symmetry may be variable. One approach to this problem is to examine the relationship between the plane of the first cleavage and the median plane of the embryo. This can be done by marking the plane of the first cleavage and by separating the first two blastomeres and raising them in isolation.

In echinoids with a pluteus larva, the first overt indication of bilateral symmetry is at mid-gastrulation. Each blastomere isolated at the two-cell stage of an echinoid almost always develops into a normal pluteus larva. When one of the first two blastomeres is stained, the stain corresponds to the right or left, dorsal or ventral or oblique meridional part of the egg (13). This work shows that the median plane is not related to the first cleavage plane and suggests that this plane is not determined until after the two-cell stage. No attempt has been made to do these experiments on the hemichordate pterobranchs which have a larval stage; however, these experiments have been done on the enteropneust, *Saccoglossus*, which shows direct development. *Saccoglossus* first shows overt bilateral symmetry at post-gastrula stages of development. Each isolated two-cell stage blastomere develops into a normal larva. When one blastomere is stained, a lateral half of the larva is always stained, indicating that the median plane is set up by the two-cell stage (4,5).

The ascidians will be used as a representative urochordate. These animals have tadpole larvae; but the larval stage has a very short duration. Some zoologists think that the tadpole is a secondary larva derived from an ancestral adult (14). In ascidians the median plane of the embryo is defined prior to the first cleavage by the formation of a cytoplasmic localization of developmental potential- the yellow crescent. The first cleavage invariably passes through the middle of this localized material. Each isolated blastomere at the two-cell stage develops into a form which approximates the lateral half of a normal larva (16). The cephalochordate *Amphioxus* also has a larva. This larva is essentially in adult form; in *Amphioxus* the median plane is also defined prior to the first cleavage by a cytoplasmic localization of developmental potential. The first cleavage plane does not always pass through this region. When it does each isolated blastomere develops into a normal larva. On those occasions when the first cleavage does not pass through this localization, the

whole embryo develops into a normal larva; however, blastomeres isolated at the two-cell stage develop abnormally (10).

In each of the groups with direct development, the time when bilateral symmetry is determined is shifted to an early stage of development. In echinoids, the determination of bilateral symmetry occurs over a relatively long period of time and probably involves a number of inductive interactions which are occurring as bilateral symmetry becomes manifest. In the forms with direct development, the determination of bilateral symmetry has become associated with local cytoplasmic regions which certain blastomeres inherit; in some of these groups this has been coupled to the cleavage pattern. There are other examples like this for the coelenterates and certain protostome phyla.

Another consequence of lecithotrophy is that in some cases the size of the egg becomes so large that there is a reorganization of the cleavage stages of development so that the embryo shows some form of partial or superficial cleavage. This has occurred in several phyla including the cnidarians, the molluscs, the arthropods, the echinoderms, and the chordates. In the forms in which the cleavage pattern has changed dramatically, it is frequently apparent that it would be difficult to make the developmental mechanisms, which have been shown to exist in related groups in which cleavage has not been modified, function after the cleavage pattern has been altered. As an example of this sort of problem, the determination of the anterior-posterior axis in the cnidarian planula larva will be considered.

In cnidarians with small eggs cleavage is unipolar; the furrow begins at one point on the egg surface and slowly moves through to the other side. Marking experiments have demonstrated that the site where the first cleavage is initiated becomes the posterior pole of the planula larva (18). Experiments in which the site of the first cleavage is altered so

that it is not initiated at the site where it would normally occur have made it clear that the anterior-posterior axis is set up at the time of the first cleavage and that the cleavage initiation site specifies the posterior pole of this axis (7).

This axial system plays a major role in integrating development. In some hydrozoans and anthozoans, nuclear divisions occur after the egg has been fertilized, but cell divisions do not. After a number of nuclei have formed, cellulation occurs more or less simultaneously throughout the embryo (18). In these embryos it becomes difficult to argue that cytokinesis is setting up polarity. One either has to assume that there is some common element operating in both cases that has not been identified, or that another developmental mechanism for handling the establishment of polarity has originated.

The same kind of problems come up when one attempts to visualize the transitions that lead to the origin of the cephalopods or most arthropod lineages from a set of ancestors that had a spiral pattern of cleavage during embryogenesis (23). While it is difficult to imagine how one of these transitions took place, it is probable that the change in egg size created physical stresses that led to a change in the pattern of morphogenetic movements during early embryogenesis. In some cases this may have facilitated new modes of germ layer formation and provided the basis for new kinds of inductive interactions during early embryogenesis.

This short discussion has argued that acceleration has played a more important role than retardation in the origin of new classes and phyla. Acceleration has been assigned this role because of the presence of a larval phase in the life cycle, and because of adaptive pressures relating to metamorphosis which have caused varying degrees of adultation to occur at this stage.

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