

Developmental characters in phylogenetic inference: a test case using amphibians

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Introduction

At the present time there is tremendous interest in the relation between development and evolution. As one might expect from a discipline as broad as that encompassed by these two terms, it comprises a number of problems, approaches, and methods. In this paper I focus on one approach: the use of developmental characters in phylogenetic inference. This is a broad topic in itself and one that has dogged the study of phylogenetic relationships since before Darwin.

I present several generalizations, but I do so in the context of one particular example which concerns the evolutionary origins and relationships of the three orders of Recent amphibians – Anura (frogs), Caudata (salamanders), and Gymnophiona (caecilians). My reasons for choosing this example are twofold (references are provided in HANKEN, 1986). First, this is a problem that has received a great deal of attention from the standpoint of adult characters, which have nevertheless failed to yield a satisfactory resolution. Second, in the last few years developmental biologists have offered a number of characters from early development in support of one or another theory of phylogenetic relationships. In most cases the theory being supported is that Recent amphibians represent a polyphyletic evolution of tetrapods from bony fishes, a conclusion which I consider not justified. Hopefully, the general considerations may be successfully applied to similar problems involving other taxa.

Results and discussion

Generalization 1: Embryonic characters are not necessarily more valuable than later characters

Historically, consideration of the phylogenetic value of developmental features has been tremendously influenced by the doctrine of recapitulationism. This doctrine, which was formalized by Haeckel in the 19th Century as the so-called biogenetic law, holds that phenotypic states characteristic of early development represent adult stages of early ancestors; conversely, states characteristic of later stages represent adults of more recent ancestors. Accordingly, embryonic differences between two taxa that are manifest early in

development indicate a remote divergence of the two lineages. They also are intrinsically more valuable at indicating such a split than differences manifest at later stages.

The recapitulationist doctrine has influenced the phylogenetic interpretation of developmental characters in nearly all groups, invertebrate and vertebrate, including amphibians. Nieuwkoop and Sutasurya, for example, in a review of their extensive studies of amphibian origins state the following: «the more significant and fundamental are the observed differences in the embryonic development of different species, and the earlier they become manifest, the less closely related are the species or the more ancient was the deviation of their phylogenetic history» (1983: 123–124). There are, however, numerous and telling objections to such a strict phylogenetic interpretation of developmental sequence data (see reviews by GOULD, 1977, and ALBERCH, 1985). In the case of amphibians the objections are straightforward and simple: there are just too many exceptions in which the biogenetic law is clearly wrong.

A good example is mesoderm formation. In anurans, mesoderm is frequently described as derived exclusively from the deep layer of the marginal zone of the blastula, whereas in urodeles it is derived from superficial cells (NIEUWKOOP and SUTASURYA 1976). Such a difference between these two orders, if valid, could indicate an ancient split between these lineages. In fact, however, the purported distinction between developmental modes in the two groups is blurred by consideration of the range of developmental patterns within each order, especially the Anura. Thus, derivation of mesoderm exclusively from the deep layer is apparently unique to the genus *Xenopus*; early work by PASTEELS (1942) and VOGT (1929) described derivation of mesoderm from the superficial layer in the genera *Discoglossus* and *Bombina*, respectively. In addition, a novel pattern of gastrulation in the hylid genus *Gastrotheca*, which presumably entails an equally novel mode of mesoderm formation, differs from the pattern seen in other genera in the same family (del PINO and ELINSON, 1983).

These and other examples in which closely related taxa evince dramatically different patterns of early development severely diminish the validity of the recapitulationist doctrine as a guiding principle in interpreting developmental sequences. That is, while, in general, developmental patterns in closely related taxa are more similar than those in distantly related taxa, there are so many exceptions that the doctrine has very little, if any, predictive value, and can't be used to reliably infer phylogenetic relationships.

Generalization 2: Differences alone do not provide unequivocal support for phylogenetic hypotheses

There is often a tendency, following identification of a contrasting pattern of development between two taxa, to consider the observed difference as convincing evidence of at best a remote relationship between the two taxa. (This tendency is equally common when considering adult features as well). Differences between taxa, however, do not provide reliable data on which to base phylogenetic hypotheses without additional data from appropriate outgroups. Such data is needed to evaluate primitive vs. derived character states and to establish whether the taxa under consideration show contrasting patterns of affinity to these outgroups, which is in fact the crux of the issue in most cases of phylogenetic uncertainty.

With respect to amphibians, contrasting patterns of development between at least two of the three amphibian orders, typically frogs and salamanders, have been cited as evidence of an independent derivation of these groups from bony fishes. One of the most conspicuous differences concerns the presence or absence of a block to polyspermy (ELINSON, 1986). In anurans, a complex series of modifications of the unfertilized egg immedi-

ately follow sperm penetration. These modifications, or fertilization response, prevent additional sperm from penetrating the egg, thereby constituting a block to polyspermy. Urodeles lack this response and, not surprisingly, polyspermy is common.

The problem with applying conspicuous differences such as this to the question of Recent amphibian relationships is that the necessary information concerning the presence or absence of a block to polyspermy among the putative ancestral taxa are not available (reviewed in HANKEN, 1986). That frogs, salamanders, and caecilians diverged from a common ancestor a long time ago is no longer questioned. The important question at this time is whether this remote divergence involved direct piscine ancestors as well, a question that is not answered by simply tabulating additional, embryonic differences among only these taxa.

Generalization 3: Discard outmoded, inappropriate interpretations

Unlike some branches of biology which were founded only recently, developmental biology (and its antecedent, descriptive embryology) has a history dating back several centuries. One of the hallmarks of progress in the field has been the successive replacement of interpretations, controversies, and paradigms as a consequence of the accumulation of greater and more accurate knowledge of the workings of development. Regrettably, although not surprisingly, individual interpretations, controversies, or paradigms may outlast their usefulness and serve to delay further understanding. One such interpretation, which although outmoded continues to impose a strong influence on at least some branches of the field, is the dichotomy between epigenetic and preformationist development. It is exemplified by the problem of the origin of primordial germ cells (PGCs) in amphibians.

In anurans, PGCs are derived from endodermal cells of the vegetative yolk mass. A characteristic and unique feature of these cells is the germinal cytoplasm, an aggregation of mitochondria, polyribosomes, and electron-dense germinal granules which is visible as early as the mature oocyte (SMITH and WILLIAMS, 1975). Because the germinal plasm is visible at such an early stage of development, and because the endodermal cells that retain it have been believed to differentiate solely as gametes, PGC derivation in anurans has been considered «preformationist» (SUTASURYA and NIEUWKOOP, 1974). Urodeles, however, lack a distinct germinal cytoplasm visible at early embryonic stages and PGCs are derived from mesodermal cells of the blastula animal cap following an inductive interaction with the vegetative yolk mass (MICHAEL, 1984; NIEUWKOOP and SUTASURYA 1976, 1979). Because PGCs are derived from what have been considered indeterminate somatic cells of the animal cap, PGC derivation in urodeles has thus been described as «epigenetic».

This characteristic difference in PGC origin (induced vs. preformed) has been considered fundamental, «understood [only] if the bifurcation in the evolution of the two groups is placed as far back as possible in the phylogenetic history of the vertebrates» (NIEUWKOOP and SUTASURYA, 1976: 164). The results of several recent studies, however, seriously challenge the validity of the epigenetic-preformationist dichotomy as applied to PGC derivation, and in doing so diminish its phylogenetic significance. First, germinal granules, one of the components of germinal cytoplasm in anurans, is present in the axolotl within the marginal zone where PGCs will form (SMITH and WILLIAMS, 1975; WILLIAMS and SMITH, 1971). Second, in urodeles the ability of cells of the animal cap to form PGCs is actually restricted to the marginal zone, the same general region which contains germinal granules, suggesting that PGCs may in fact form from predetermined cells in the apical cap following a permissive inductive interaction with the vegetative yolk mass (MICHAEL 1984). Third, in *Xenopus* endodermal cells with germinal plasm are not,

at least following experimental manipulation, irrevocably committed to form PGCs and can instead form a wide range of alternate tissue types (WYLIE et al., 1985).

These and other recent studies preclude further interpretation and discussion of modes of PGC derivation in amphibians in terms of a strict epigenetic vs. preformationist dichotomy. Instead, developmental patterns must be interpreted using the current dialogue reflecting primary attention to actual developmental mechanics and physiology. Only with such descriptions of the full range of developmental processes that underlie such complex phenomena as PGC derivation, can a solid data base be built on which to base phylogenetic inference.

Generalization 4: Evaluate variation – diversity is the rule

With their primary focus on developmental mechanics, and the common choice of a single species to be used as a «model system» for examining general aspects of vertebrate development, developmental biologists have increasingly limited their consideration to relatively few amphibian taxa, especially those that are amenable to experimental manipulation. At the same time, developmental patterns observed in one or a few species of frog or salamander are often routinely extrapolated to the entire order. This tendency has been especially common when differences in pattern have been described between a given pair of taxa, one urodele and one anuran. Such extrapolation, however, assumes that variation at the level of the genus and family is insignificant relative to that seen in interordinal comparisons, an assumption that is not justified for many aspects of development under consideration. Indeed, consideration of a wider variety of taxa within each order frequently reveals substantial diversity in developmental pattern within one or another amphibian order, diversity that often serves to obscure the supposedly characteristic differences among orders.

Notochord formation is a case in point (reviewed by BRUN and GARSON, 1984, and KELLER, 1985). In the axolotl, notochordal cells are derived from both deep and superficial regions of the blastula marginal zone. These cells for a time form the roof of the archenteron ventral to the neural plate, but eventually they ingress dorsally away from the surface to form the notochord as endoderm moves in to form the definitive archenteron roof. In contrast, notochord formation in *Xenopus* is very different: notochordal cells are derived exclusively from the deep region of the marginal zone, and at no time do the cells form the archenteron roof. The basic pattern seen in the axolotl is shared by the few other urodele species that have been examined. The pattern seen in *Xenopus*, however, while apparently also characteristic of *Rana pipiens* (RUFFINI 1925; cited in BRUN and GARSON, 1984), is not typical of all anurans; at least two additional species, *Rana palustris* and *Bufo terrestris*, combine features of both patterns (KING, 1903).

The apparent diversity of patterns of notochord formation in amphibians, especially anurans, while recognized more than three-quarters of a century ago (KING, 1903), has not been appreciated by subsequent workers. This diversity severely restricts the use of notochord formation as a character in resolving the problem concerning the relationships of frogs to salamanders. To the extent that such diversity is typical of other characters, their utility is similarly restricted.

Generalization 5: Consider adaptive (vs. phylogenetic) explanations of developmental characters

Perhaps the greatest potential limitation to the use of developmental characters in phylogenetic inference is posed by the process of adaptation. That is, if development is

sufficiently malleable with respect to natural selection that a given developmental pattern is more likely to represent accommodation to present demand than a chronicle of phylogenetic descent, then the task of inferring phylogenetic relations from developmental data is made much more difficult. The task, however, is not necessarily made impossible. After all, the «problem» of adaptation in the context of phylogenetic inference has not precluded the use of adult morphological characters. Yet, the adaptive value of developmental patterns – and particularly differences in developmental patterns – is addressed much less frequently than are descriptions of the patterns themselves or claims of their phylogenetic import.

An example in which developmental pattern seemingly more reflects adaptation than phylogeny is the ontogeny of cranial ossification in caecilians (WAKE and HANKEN, 1982). The particular complement of bones that comprise the gymnophionan skull as well as their development have been used to support the view that caecilians are the direct descendants, separate from frogs and salamanders, from archaic amphibians, particularly the microsaur. This argument stems from the claim made earlier this century that caecilians are embryos recapitulate the skull of microsaur in the course of developing their characteristic adult cranium. Recent reexamination of this claim in the viviparous species *Dermophis mexicanus*, however, has concluded that the early descriptions of cranial ossification were largely erroneous, and that skull development, at least in *Dermophis*, reflects adaptation to specialized embryonic and fetal processes (such as fetal nutrition via oviducal secretions) more than it provides evidence in support of any particular phylogenetic hypothesis concerning higher-order relationships among amphibians.

Conclusions

The above generalizations constitute a set of guidelines for employing developmental characters in phylogenetic inference. When applied to the example at hand – the origins of Recent amphibians – the available developmental characters sort into two groups. The first group comprises those characters that fail to reveal consistent and unequivocal differences between modern orders. The second group comprises those characters that do show consistent differences between at least two of the orders. In the absence, however, of relevant, comparable data from putative ancestral taxa, as well as the identification of shared-derived characters that would ally individual orders with different descendant taxa, these differences do not provide the evidence required to accept or reject alternate phylogenetic hypotheses; they are equally compatible with views of monophyly and polyphyly.

This protocol may appear pessimistic, or too confining, but this need not be the case. Developmental data is an appropriate substrate for phylogenetic analysis, i.e., there is phylogeny in ontogeny. The trick is to identify how much, but finding out is not as simple as reading an ontogenetic sequence or tabulating differences. Instead, the analysis requires careful, deliberate choice of the right questions and of the appropriate taxa. Such analysis will bear fruit in the form of insights into both the relationships among organisms and the processes that underlie their diversity.

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