Direct development in the lungless salamanders: what are the consequences for developmental biology, evolution and phylogenesis?

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ABSTRACT   Direct development is a widespread alternate reproductive mode in living amphibians that is characterized by evolutionary loss of the free-living, aquatic larval stage. Courtship, mating, and oviposition occur on land, and the terrestrial egg hatches as a fully formed, miniature adult. While it is the most common reproductive mode in urodeles, development outside the reproductive tract of the female that proceeds directly to a terrestrial hatchling occurs in only a single lineage, the lungless salamanders of the family Plethodontidae. Evolution of direct development in plethodontids has contributed importantly to the extraordinary evolutionary success of this speciose, geographically widespread, and morphologically and ecologically diverse taxon. Developmental consequences and correlates include increased egg size and embryonic development time, loss of larval structures and ontogenetic repatterning, and altered pattern formation in organogenesis. Evolutionary and phylogenetic consequences and correlates include the loss of larval constraints and origin of morphological novelty, and frequent homoplasy. Analysis of direct development in an evolutionary context illustrates the complex interplay between processes of phylogenetic divergence and developmental biology, and substantiates the prominent role of developmental processes in both constraining phenotypic variation and promoting phenotypic diversity. Despite the proven suitability of direct-developing plethodontid salamanders for laboratory and field study, knowledge of basic features of their developmental biology remains far below that available for many other urodeles. Examination of such features of these "non-model" organisms is an appropriate and deserving goal of future research.

KEY WORDS: *direct development, Plethodontidae, evolution, ontogeny, constraints*

Introduction

Salamanders have played an indispensable role in the history of developmental biology. The vast majority of studies of urodele development involve species in which embryogenesis culminates in the production of a free-living larva. In most of these taxa, the larva, after a period of weeks or months, metamorphoses to a terrestrial adult. Yet, this complex life history, which most people regard as a characteristic if not defining feature of living amphibians, *is not the predominant reproductive mode in urodeles*. Instead, most living salamanders have direct development: courtship, mating, and oviposition occur on land, and the terrestrial egg hatches as a fully formed, albeit miniature adult; there is no free-living larva (Figs. 1, 2).

Although it is the most common reproductive mode in salamanders and has evolved independently in frogs and caecilians (Wake, M., 1989), development outside the reproductive tract of the female that proceeds directly to a terrestrial hatchling without a free-living larval stage is restricted to a single urodele lineage, the lungless salamanders of the family Plethodontidae. Direct development in plethodontids is believed to underlie, at least in part, the extraordinary evolutionary success of this family, which is manifest both in the large number of living species and in their extensive morphological, functional, and ecological diversity (Wake and Larson, 1987; Wake, 1991). In this paper, we summarize the biology of direct development in plethodontids, focusing on the numerous consequences and correlates of this derived reproductive mode both for developmental biology and for evolution and phylogenesis. Most studies of direct development in plethodontids have focused on ecological and evolutionary aspects; there have been few analyses of development per se. As a means of encouraging more studies of the developmental biology of direct development in

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plethodontids that would redress this imbalance, we conclude this review by identifying a series of promising areas of future research.

The evolution of direct development

Phylogenetic context

Explicit phylogenetic hypotheses are a prerequisite for rigorous examination of evolutionary patterns. We begin, therefore, by summarizing the current understanding of plethodontid relationships, both within the family and with respect to other groups of living urodeles.

The Plethodontidae is one of ten extant families of salamanders (Duellman, 1993). It includes more than 245 species, or about two-thirds of all recognized species of urodeles. Phylogenetic relationships among these ten families are the subject of ongoing debate, and several alternative hypotheses exist; different results are obtained from separate analyses of morphological and molecular data sets, and from analyses of combined data sets (Hedges and Maxson, 1993; Larson and Chippindale, 1993). Traditional views have regarded plethodontids as among the most highly derived of extant families ("deeply nested," in phylogenetic terminology), based on prominent morphological features such as the absence of lungs and several cranial bones, and the presence of nasolabial grooves and specialized feeding mechanisms (e.g., Duellman and Trueb, 1985). Molecular analysis, however, identifies them as one of the most basal taxa (Larson, 1991). The most recent comprehensive analysis, which combines morphological and molecular data sets ("total evidence;" Larson and Dimmick, 1993), places them in an intermediate phylogenetic position – derived with respect to families with apparently ancestral life history features (e.g., external fertilization), but basal with respect to other taxa, including such large families as the Salamandridae and Ambystomatidae (Fig. 3).

There are 28 plethodontid genera and more than 245 species, with more being described almost every year (e.g., Good and Wake, 1993; Hanken and Wake, 1994). The geographic range of the family spans North America and Central America, and extends to much of South America as well as southern Europe. The family includes all tropical salamanders, which account for nearly one-half of all salamander species. Widespread homoplasy has complicated attempts to resolve phylogenetic relationships within the family (Wake and Larson, 1987; Wake, 1991). Yet, identities of and relationships among major lineages (clades) are for the most part well resolved (Wake, 1992, 1993a; Fig. 4; Table 1) as a result of a series of morphological (Lombard and Wake, 1986) and molecular
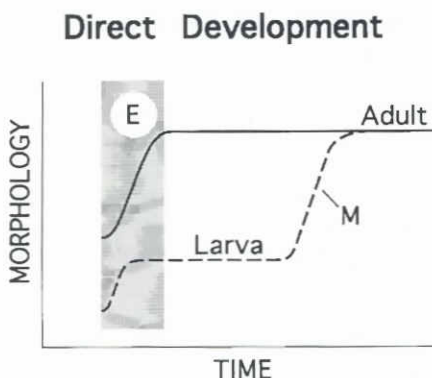
Fig. 2. Direct development, the most common reproductive mode in urodeles. (A) *Ovipositing female Bolitoglossa compacta, a plethodontid salamander from western Panama. The large, unpigmented eggs are characteristic of many direct-developing amphibians.* **(B)** *Hatchling soon after emerging from an egg following an incubation period of 249 days (8 months) at 13°C.*

studies (references in Larson and Chippindale, 1993). There are two subfamilies. One, the Desmognathinae, includes 3 genera and 14 species confined to eastern North America. The other subfamily, Plethodontinae, comprises three tribes. Plethodontini (3 genera and 48 species) and Hemidactyliini (8 genera and 24 species) are both restricted to North America. Bolitoglossini, with 14 genera and 160 species, is further subdivided into three supergenera – *Hydromantes* (one genus in Europe and California), *Batrachoseps* (one genus in western North America), and *Bolitoglossa* (the 12 tropical genera).

How many times has direct development evolved?

The presumed ancestral, biphasic life history, comprising aquatic eggs and larvae and terrestrial adults, is retained in many plethodontids, viz., most species of desmognathines and all hemidactyliines (Tilley and Bernardo, 1993; Collazo and Marks, 1994). Obligate loss of the adult stage and consequent larval reproduction characterizes several hemidactyliines, mainly cave-dwelling species presently placed in four genera (*Eurycea, Gyrinophilus, Haideotriton, Typhlomolge*). Direct development characterizes all species of Plethodontini and Bolitoglossini and three species of Desmognathinae – *Phaeognathus hubrichti,*

Direct Development

Fig. 1. Two alternate reproductive modes in amphibians. *The ancestral life history (dashed line) involves aquatic larval and terrestrial adult stages separated by a discrete metamorphosis (M). In direct development (solid line), the free-living larval stage is lost, and a miniature, terrestrial adult hatches from the egg at the end of embryogenesis (E).*
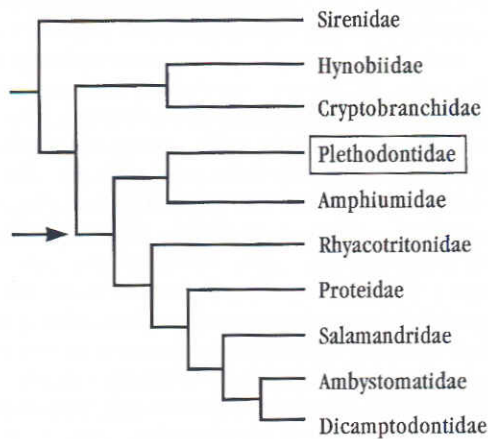
Fig. 3. Phylogenetic relationships among extant urodeles, based on analysis of "total evidence" *(Larson and Dimmick, 1993). This scheme unites all salamander families with internal fertilization into a single clade (arrow), which is distinct from those with external fertilization (Sirenidae, Hynobiidae, and Cryptobranchidae). Redrawn from Larson and Chippindale (1993; Fig. 3B).*

Desmognathus aeneus, and *D. wrighti* – but no hemidactyliines.

The exact number of times that direct development has evolved among plethodontids remains unknown (Collazo and Marks, 1994). It almost certainly evolved more than once or even twice, and may have evolved as many as five times (separately in all three desmognathines, plus once each in Plethodontini and Bolitoglossini; Wake, 1966). Other possibilities include four times (assuming that it arose in the common ancestor of Plethodontini + Bolitoglossini and was retained), or three times (assuming that it also arose in the common ancestor of *D. wrighti* and *D. aeneus*). If direct development evolved only once, then a free-living, aquatic larval stage must have reappeared at least twice (within *Desmognathus*, and at least once within Hemidactyliini). Re-evolution of the larval stage – a major evolutionary reversal – would appear to be more complicated developmentally than its evolutionary loss. However, re-evolution of larvae has been hypothesized for other amphibian taxa, e.g., marsupial frogs in the South American genus *Gastrotheca*, in which direct development involves a simple truncation of the development of larval features before the formation of adult components (Duellman *et al.*, 1988). Eggs of these frogs develop in a dorsal skin pouch on the female parent; after hatching the tadpole remains in the pouch until metamorphosis is complete (Wassersug and Duellman, 1984). Resolution of this problem in plethodontids awaits more robust phylogenetic hypotheses than are presently available. Developmental data may also make an important contribution if it can be shown that "direct development" is not a uniform developmental mode in all species, but instead comprises two or more distinct ontogenetic patterns within the family. For example, preliminary evidence that direct development has evolved at least two or even three times among plethodontids is seen in the contrasting patterns of embryonic development of several organ systems in plethodontines, bolitoglossines, and desmognathines (see below).

Functional and ecological context

Primitive features of plethodontid larvae give insight into the likely functional and ecological context in which direct development evolved.

Plethodontids are generally regarded as derived from an ancestral stock associated with stream dwelling, and it is within this ancestral ecology that both lunglessness and stream-adapted larvae arose (Beachy and Bruce, 1993). Stream larvae must be able to cope with the dynamic nature of the microenvironments in which they live, and this requires that they have well-developed limbs upon hatching. An advanced state of development upon hatching in turn demands a well-provisioned egg. The large, yolky eggs develop slowly but fully, so that at hatching the head and jaws, like the limbs, are at an advanced stage of development and have essentially the "mature" larval form. Because they retain yolk, hatchlings may not need to feed for up to a week or more, and by the time feeding is initiated the trophic apparatus is fully functional. Feeding in larvae, whether pond- or stream-adapted, involves electroreception and mechanoreception – both localized to the lateral line system – rather than vision. Accordingly, vision develops slowly in larval salamanders; only by the time of metamorphosis, when vision becomes critical for prey detection, is the characteristic amphibian visual system in place, dominated by strong contralateral retinotectal projections (Roth, 1987). Indeed, the eyes of hatchlings are little more than light-dark predator detection organs.

The large eggs and slow development of plethodontids with stream larvae (Collazo and Marks, 1994) also are characteristic of all direct-developing species (see below); they represent the ancestral point of departure for the evolution of direct development in this family. Indeed, they may be necessary initial conditions for the evolution of direct development in all urodeles. That they alone are not sufficient conditions is illustrated by the Dicamptodontidae and the Rhyacotritonidae. Whereas members of both families have large, slowly developing eggs and stream larvae, neither family has evolved direct development, despite their great phylogenetic age.

Developmental consequences and correlates

Egg size and development time

Eggs of all plethodontids are large and yolky, but this is especially true of species with direct development (Duellman and Trueb, 1985; Collazo and Marks, 1994; Collazo, 1996). Egg diameter among desmognathines varies from 1.4 mm in *Desmognathus ochrophaeus*, a metamorphosing species, to 7.2 mm in direct-developing *Phaeognathus hubrichti* (Tilley and Bernardo, 1993). Studies of two Californian species have examined some of the consequences of increased egg size in direct-developing forms (Collazo, 1988, 1990). In the slender salamander, *Batrachoseps attenuatus* (Bolitoglossini), the egg is relatively small, cleavage is holoblastic, and morphogenetic movements associated with gastrulation and neurulation are typical for salamanders generally, including those with free-living larvae. In Ensatina (*Ensatina eschscholtzii*; Plethodontini), the egg is very large (to 6.9 mm dia.), cleavage is meroblastic, and the early embryo forms an embryonic disk. These derived features of Ensatina may be functionally related; the unusual pattern of cleavage and morphogenetic movements likely are consequences of the increased egg size and amount of yolk (Collazo, 1988, 1990). Indeed, they are analogous to those typically found in the yolk-rich eggs of most birds and reptiles, as well as in the large-egged marsupial frogs *(Gastrotheca)* discussed above (del Pino and Elinson, 1983; Elinson and del Pino, 1985; Elinson, 1987; del Pino, 1989). Interestingly, the contrasting patterns of early development represented by *Batrachoseps* and Ensatina may not be characteristic of their

respective lineages, which instead are highly variable. For example, egg size, cleavage, and morphogenetic movements in *Plethodon cinereus*, a member of a sister taxon of Ensatina, are more like those of *Batrachoseps* (Piersol, 1908-09; Dent, 1942). *Aneides*, an even closer relative of *Plethodon* than is Ensatina, has the largest egg reported for plethodontids (7.4 mm dia.; Stebbins, 1951) and an embryonic disk, as in Ensatina (Collazo, 1990).

Another feature associated with direct development in many plethodontids is increased duration of embryonic development (Collazo and Marks, 1994). In some species of bolitoglossines, the period of intracapsular development is extraordinarily long – as many as 251 days in *Bolitoglossa compacta* maintained at 13°C (Hanken, 1979; Fig. 2), and more than 270 days in *Hydromantes italicus* reared at 11°C (Durand, 1970). The relation between reproductive mode and development time is complicated, however, by the effects of egg size and especially genome size (Jockusch, 1994), which is large in plethodontids generally but especially in bolitoglossines (*Bolitoglossa* and *Hydromantes* have the largest genomes among plethodontid genera; Sessions and Kezer, 1991). Indeed, even eggs of some species that produce larvae normally require 70 or more days to hatch (Collazo and Marks, 1994). There are some relatively small-genomed, small-egged plethodontids that have direct development (e.g., *Desmognathus wrighti*; Collazo and Marks, 1989) and although these are the most rapidly developing of the direct-developing species, hatching still requires at least 79 days (Collazo, 1990).

Loss of larval structures and ontogenetic repatterning

A defining feature of direct development is the precocious (embryonic) formation of adult features, which in the ancestral biphasic ontogeny typically do not appear until metamorphosis. A related and recurring problem in the study of the ontogeny of direct developing taxa is the extent to which ancestral larval features are recapitulated during early development (Hanken, 1992; Hanken *et al.*, 1992). Direct-developing plethodontids exhibit considerable interspecific variation in this regard. To some degree, this variation may reflect differences in the age of the taxa involved or in the recent evolutionary shift in reproductive mode. For example, there appears to be more embryonic recapitulation of larval features in the direct-developing desmognathines, where one finds both direct-developing and metamorphosing species in a single genus (*Desmognathus;* Collazo and Marks, 1989), than in the Bolitoglossini, a large, well-defined clade that contains exclusively direct-developing taxa (Alberch, 1987, 1989; Marks, 1994).

The trend towards loss of larval components in direct-developing taxa is well illustrated by the hyobranchial, or gill-arch skeleton. The larval hyobranchial skeleton of ancestral plethodontids contained four pairs of arches. Larvae of all metamorphosing desmognathines retain this pattern, as do embryos of one species with direct development (Marks, 1994); embryos of another direct-developing species have only three pairs (Alberch, 1987). Larval hemidactyliines have three or four pairs of arches (Rose, 1995a). The remaining taxa, all direct developers, have as embryos either three pairs (Plethodontini; Dent, 1942) or one pair of arches (Bolitoglossini; Wake, 1966; Alberch, 1987). Thus, the trend towards at least partial loss of recapitulation of the larval hyobranchial skeleton has occurred independently as many as three times. In one lineage – the bolitoglossines – the trend is virtually complete; there is only rudimentary evidence of larval structures at any stage.

In some direct-developing plethodontids, loss of larval structures is accompanied by extensive change in the initial embryonic patterning of remaining components and the consequent evolution of novel adult morphologies. This phenomenon, in which apparently new sets of morphogenetic processes accompany changes in developmental timing (heterochrony), has been termed "ontogenetic repatterning" (Roth and Wake, 1985; Wake and Roth, 1989). Again, the hyobranchial skeleton is illustrative. In all adult plethodontids, the hyobranchial skeleton includes a prominent pair of elongate epibranchial cartilages. In metamorphosing species (e.g., desmognathines), the adult epibranchials are remodeled from larval counterparts within the first (largest) gill arch and the more posterior arch elements in larvae are lost, although in at least one hemidactyliine genus *(Eurycea)* the adult epibranchial forms as a *de novo* element derived from progenitor cells compartmentalized early in ontogeny (Alberch and Gale, 1986). In direct-developing bolitoglossines, however, development of all epibranchials is repressed save for the definitive adult structure, which is of questionable homology to the different elements in either *Desmognathus* or *Eurycea*. This structure, indeed the entire functional complex of the tongue, is fully developed well before hatching (Fig. 5). Ontogenetic repatterning of the hyobranchial skeleton in bolitoglossines is correlated with the evolution of freely projectile tongues in many taxa, including novel, complex morphologies not present in any metamorphosing species (Lombard and Wake, 1977; Wake, 1982).

Ontogenetic repatterning extends to other parts of the integrated feeding system, including reorganization of the visual system at the level of the tectum opticum and tegmentum
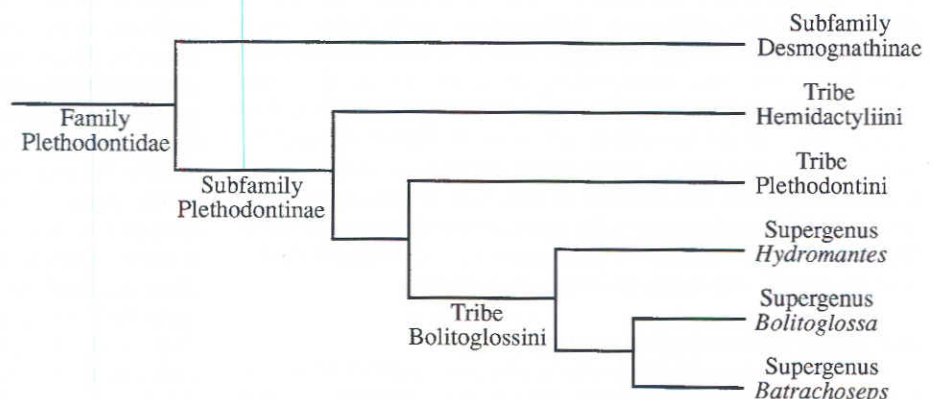

Fig. 4. Phylogenetic relationships among the major lineages of Plethodontidae. *See Table 1 for a list of component genera and numbers of species. Redrawn from Lombard and Wake (1986).*

TABLE 1

CLASSIFICATION OF PLETHODONTID GENERA

Subfamily Desmognathinae	*Phaeognathus* (1), *Leurognathus* (1), *Desmognathus* (12)
Subfamily Plethodontinae	
Tribe Plethodontini	*Plethodon* (42), *Ensatina* (1), *Aneides* (5)
Tribe Hemidactyliini	*Hemidactylium* (1), *Eurycea* (13), *Haideotriton* (1), *Typhlomolge* (2), *Typhlotriton* (1), *Gyrinophilus* (3), *Pseudotriton* (2), *Stereochilus* (1)
Tribe Bolitoglossini	supergenus *Hydromantes: Hydromantes* (6)
	supergenus *Batrachoseps: Batrachoseps* (8)
	supergenus *Bolitoglossa: Bolitoglossa* (67), *Thorius* (9), *Pseudoeurycea* (27), *Chiropterotriton* (9), *Ixalotriton* (1), *Oedipina* (16), *Lineatriton* (1), *Bradytriton* (1), *Dendrotriton* (5), *Nototriton* (8), *Nyctanolis* (1), *Parvimolge* (1)

Numbers of species (in parentheses) are taken from Duellman (1993) and do not include several additional species described since 1993.

mesencephali (Roth *et al.,* 1994) and reorganization of brainstem motor nuclei (Wake *et al.,* 1988; Nishikawa *et al.,* 1991). These are all changes in which heterochronic shifts are associated with new directions for developmental pathways. Thus, in bolitoglossines early stages of the ancestral pattern of retinotectal pathway formation are retained but then amplified; these salamanders thereby gain extensive ipsilateral projections comparable to those in mammals that relate to improved visual system performance associated with feeding (Rettig and Roth, 1986; Roth, 1987; Wiggers and Roth, 1991; Roth *et al.,* 1993). At the same time, brainstem motor nuclei fail to undergo differentiation events typical of other amphibians, e.g., frogs. Possibly as a result, ancestrally connected movements involved in feeding and lunging are decoupled in these salamanders, resulting in improved feeding performance and the ability to feed in exposed (e.g., arboreal) sites without attracting the attention of predators or losing physical position in the microhabitat (Roth and Wake, 1985).

Ontogenetic repatterning is an evolutionary concept that derives from comparative study of developmental trajectories; it hypothesizes individual changes in developmental processes in particular lineages. It is best understood when specific developmental modifications are interpreted within an explicit phylogenetic and functional context. It postulates that recombination and dissociation of developmental events within ontogenetic trajectories place embryonic rudiments/anlagen in different spatial and temporal relations in comparison to those found in related taxa. These new relationships may produce novel systems of developmental interaction and morphological and functional integration (Wake and Roth, 1989; Roth and Schmidt, 1993).

Altered pattern formation in organogenesis

Direct-developing plethodontids provide several additional instances of fundamental alterations in early embryonic patterning that apparently have accompanied the evolution of this derived reproductive mode and consequent loss of the free-living larval stage. One example involves limb-skeletal patterning in bolitoglossines, which also provides a fascinating case of convergent evolution with amniotes. Traditionally, salamanders have been thought to differ from frogs and amniotes in two major aspects of limb development (Shubin and Alberch, 1986). In urodeles, the basale commune, a skeletal element that lies at the base of digits

one and two, forms early and at a considerable distance distal to the main (proximodistal) limb axis, and there is a subsequent preaxial-to-postaxial gradient of development in the manus/pes ("preaxial dominance"). In other tetrapods, the main limb axis extends to digit four (including the basale commune), and there is a subsequent postaxial-to-preaxial gradient of development in the manus/pes ("postaxial dominance"). Limb development in direct-developing salamanders does not follow the above "urodele" pattern, which proves to be characteristic only of metamorphosing species. Instead, in direct-developers both early, distal development of the basale commune and preaxial dominance are less pronounced (Shubin and Wake, 1991; Shubin, 1995; S. Marks, N. Shubin and D. Wake, personal communication). Pre-chondrogenic patterns of segmentation and connectivity also are more similar to those in amniotes than in other salamanders (Fig. 6).

Differences in pattern formation are mirrored by differences in the relative timing of limb development, which also varies according to life history. In taxa with pond larvae, forelimb buds are small at hatching; hind limb buds are smaller, or even absent. In species with stream larvae, there is less disparity in the time of formation of fore- and hind limb buds, and hind limbs are well developed at hatching (e.g., *Desmognathus;* Collazo and Marks, 1994). Limb development is accelerated even further in species with direct development; hind limb buds develop relatively early and nearly simultaneously with the forelimbs (Piersol, 1908-09; Marks and Collazo, 1988; Marks *et al.,* 1992; Wake and Shubin, 1994; Fig. 7A), as in many direct-developing frogs (Elinson *et al.,* 1990; Hanken *et al.,* 1992; Fig. 7B).

These and other recent observations of direct-developing plethodontids may call for a reevaluation of which features should be considered "typical" of salamander development. Many traits long considered characteristic of all or even most salamanders may prove to be caenogenetic features, i.e., specialized embryonic or larval adaptations to particular functional demands or developmental conditions. For example, the pattern of limb development long regarded as characteristic of all urodeles (see above) may instead be tightly linked with the extracapsular development of limbs and the unique functional demands experienced by species with pond larvae, in which the limbs are used as they form. Accordingly, intracapsular development of limbs in salamanders with direct development, internal development of forelimbs in tadpoles, and embryonic development of limbs in amniotes may all represent variations on an early tetrapod ground plan (Shubin and Wake, 1991; Wake and Shubin, 1994, unpublished data).

Evolutionary and phylogenetic consequences and correlates

Loss of larval constraints

In metamorphosing amphibians, free-living larvae must function as moving, feeding organisms. This ecological requirement at least potentially constrains adult morphology. Indeed, in most frogs, highly specialized tadpoles are transformed into adults by an abrupt and comprehensive metamorphosis, a dramatic transition that may have evolved as a means of minimizing larval constraints on the adult while retaining a complex life history. A far less pronounced metamorphosis in salamanders means that many larval features are retained in the adult. One example is the adult retention of larval features of the hyobranchial apparatus that play critical roles in both aquatic and terrestrial feeding (Lombard and
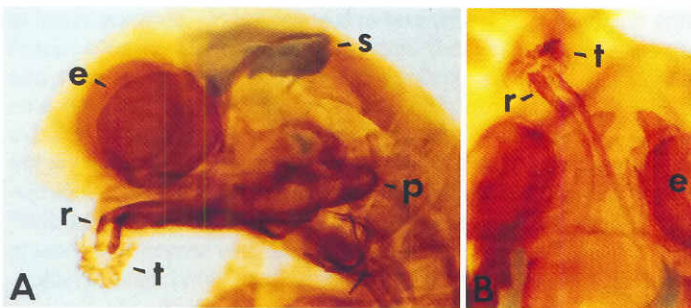
Fig. 5. Whole-mount embryo of *Bolitoglossa subpalmata* preserved several weeks before hatching and stained with an antibody to slow-twitch fiber striated muscle myosin. (A) *Lateral view showing the advanced stage of development of the entire head, but especially the tongue, which is protruding from the mouth. Retractor muscles (r) already assume an essentially adult configuration and insert into the tongue pad. Tongue pad muscles (t) also are well developed and radiate into the pad from their origin on the basibranchial cartilage (not visible). The large, bulb-like protractor muscle (p) is wrapped around the elongate epibranchial cartilage. Both the very large eyes (e) and calcified endolymphatic sacs (s) are typical of direct-developing embryos.* (B) *Dorsal view showing tongue retractor and pad muscles. Note the forward rotation of the eyes, which does not occur until metamorphosis in species with larval development.*

Wake, 1977; Wake, 1982). The evolution of direct development, through its elimination of the larval stage, represents a potential means of escaping such constraints. Moreover, it is one that apparently has been achieved in some, but not all, plethodontid lineages. The supergenera *Hydromantes* and *Bolitoglossa*, for example, have achieved an extreme degree of adult hyobranchial specialization (e.g., the skeleton folds in a novel way during tongue protraction) that would appear to be highly unlikely, if not impossible, in the absence of a much more comprehensive metamorphosis than exists in any urodele. In contrast, direct-developing species of Plethodontini retain the ancestral adult mechanical configuration. Direct development thus represents a necessary but not sufficient condition for the evolution of biomechanical specialization (Roth and Wake, 1985).

Conservation of ontogenetic trajectories

An ontogenetic trajectory is a conserved sequence of developmental events that is associated with extensive self-organization and self-stabilization (Alberch *et al.,* 1979; Wake and Roth, 1989). It can be addressed at many taxonomic levels. At the level of sister species the degree of evolutionary conservation may be virtually complete, while there is a general but nonlinear decay correlated with increasing taxonomic distance. Ontogenetic trajectories are conserved even when caenogenetic features are added. For example, while the presence of a larval stage is a plesiomorphic (i.e., ancestral) state for Recent amphibians, there has been accentuation of larval features in living taxa, especially in frogs (Wassersug and Hoff, 1982), but to a lesser degree also in salamanders. The ancestral state in urodeles may have comprised a relatively long-lived larva that proceeded gradually along an ontogenetic trajectory and underwent only a modest metamorphosis (as in Paleozoic relatives of the Lissamphibia; Schoch, 1992). The rapid and more pervasive metamorphosis observed in many living amphibians thus would represent a caenogenetic feature (Elinson, 1990). Direct development in effect restores a significant

component of the conserved, ancestral ontogenetic trajectory, almost by default.

There is, however, persistence of certain larval traits and even metamorphic events in many direct-developing ontogenies. Early observations of the heterogeneous developmental patterns and adult morphologies of many direct-developing plethodontids led to the concept of differential metamorphosis, whereby taxa retain specific, albeit no longer synchronized, features of the ancestral metamorphosis (Wake, 1966). The best understood example is the complicated history of the premaxillary bone (Wake, 1966, 1989, 1991; Wake and Larson, 1987). The ancestral condition is a paired bone at the front of the upper jaw. Early caenogenetic evolution led to the presence in larvae of a single, median premaxillary bone with a specific functional role; in some species, this bone divides at metamorphosis, thereby restoring the ancestral condition in adults. In direct-developing taxa, a single bone still appears in the embryo, but it lacks function; it represents a kind of "phylogenetic memory." This bone remains single throughout life in some taxa; in others it divides near hatching or late in life, following sexual maturation (as in *Batrachoseps wrighti*). Its development is no longer synchronized with other metamorphic events, e.g., remodeling of the hyobranchial system and ossification of septomaxillary and prefrontal bones. Such ontogenetic "shuffling" is rampant within direct-developing plethodontids, especially bolitoglossines, and probably underlies much of the homoplasy that is so prominent in these taxa.

Homoplasy

Homoplasy is evolutionarily derived similarity that results from phenomena other than common ancestry, such as parallelism, convergence or reversal. It is detected as character incongruencies in phylogenetic analysis. Homoplasy is especially common in the direct-developing plethodontids (Wake, 1991), where many similar traits represent the repeated expression of a limited set of phenotypic outcomes of conserved developmental programs. Thus, absence of segmentation and bifurcation of chondrogenic foci within the developing postaxial limb have led to repeated loss of the fifth digit in many species. This trend differs from that in anurans, in which the first toe is repeatedly lost, as a consequence of the contrasting developmental patterns of preaxial versus postaxial dominance in the two groups (Alberch and Gale, 1983, 1985). Other examples include the repeated evolution of similar paedomorphic adult morphologies, characterized by the absence of septomaxillary and prefrontal bones and the retention of cranial fontanelles throughout life, as well as peramorphic adult states such as cranial crests and enlarged jaws and teeth. Homoplasy can also arise from redeployment of components of ancestral ontogenies, such as the functionally significant reorganization of the tarsus in several taxa that is associated with the reappearance of phylogenetically "lost" elements (e.g., Schmalhausen's "m;" Wake, 1991; Shubin *et al.,* 1995).

Research opportunities

The more than 210 species of direct-developing plethodontid salamanders offer excellent research opportunities in contemporary developmental biology. These include phenomena that are unique to direct development, as well as others that are shared with metamorphosing amphibians or vertebrates generally (Elinson et al., 1990). Yet, most studies of direct development in salamanders
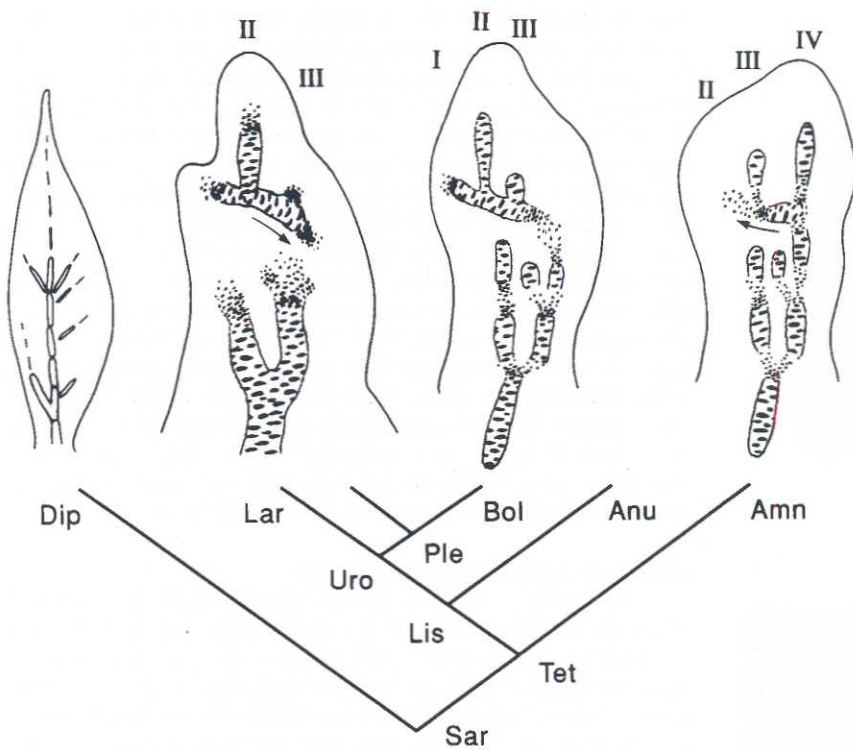
Fig. 6. Forelimb skeletal patterning in sarcopterygian vertebrates (Sar). *Lungfishes (Dip) have a single central axis in the pectoral fin. Among tetrapods (Tet), this axis is retained in amniotes (Amn) and anurans (Anu), where it extends along the postaxial portion of the limb and into digit 4. Evolutionary change occurred in the Lissamphibia (Lis), with urodeles (Uro) diverging from anurans possibly as a result of larval adaptation. Species with larval development (Lar, and the unnamed branch which represents metamorphosing species of Plethodontidae, Ple), have an independent distal element, the basale commune, and the limb axis extend through digit 2. In direct-developing Bolitoglossini (Bol), limb development combines features of both patterns. Roman numerals denote digits. Arrows indicate the direction of differentiation of the digital arch, except in Bolitoglossini where the digital arch forms more or less simultaneously along its entire length. Modified from Shubin (1995).*

have focused on ecological and evolutionary implications of this derived reproductive mode (e.g., Houck, 1977; Tilley and Bernardo, 1993). Published studies of the developmental biology of direct-developing taxa have largely concentrated on defining basic parameters in one or another species, such as the sequence and timing of discrete developmental events, interspecific variation in egg size and length of the development period, and gross development of specific organ systems (Noble and Marshall, 1929; Bishop, 1941; Wake and Marks, 1993). Few studies have explored basic developmental mechanisms in these vertebrates, which have immediate relevance to both evolutionary and developmental topics. *Here we identify three areas that offer especially promising opportunities for research.*

Cell lineage, compartmentalization, and the embryonic origin of adult structures

Resolution of many fundamental questions regarding the underlying developmental mechanisms and evolutionary consequences of direct development in urodeles will require precise knowledge of the embryonic origin of adult features in both metamorphosing and direct-developing species. Information regarding the extent to which larval and adult structures originate from discrete embryonic cell lineages, for instance, is needed to critically evaluate claims regarding the existence of larval constraints on adult morphology in metamorphosing species (see above). Existing evidence of such developmental constraints in urodeles is largely circumstantial, viz., the extreme diversification of adult morphology in many species that lack larvae. In anurans, many organ systems display a phenomenon similar to the embryonic compartmentalization of adult precursors found in many insects (Fox, 1981). In jaw muscles, for example, all larval myofibers degenerate at metamorphosis and the muscles are repopulated by adult myofibers recruited from quiescent satellite cells residing within the larval muscles (Alley, 1989).

Compartmentalization of larval versus adult cells has been described in the ontogeny of the epibranchial cartilage in the metamorphosing plethodontid *Eurycea bislineata* (Alberch *et al.*, 1985, 1986; Alberch and Gale, 1986; Alberch, 1987, 1989), but the extent to which this developmental pattern is unique to this taxon or more widespread among plethodontids remains to be assessed. Development of reliable methods for tracing the lineage of embryonic stem-cell populations into posthatching ontogeny (e.g., Collazo *et al.*, 1994) has overcome many of the technical difficulties that impeded early efforts to answer these questions (e.g., de Beer, 1947) and made such analyses feasible.

Hormonal control

Hormonal control of amphibian metamorphosis has long offered a paradigm for studying the regulation of postembryonic development in vertebrates (Dent, 1968; Gilbert and Frieden, 1981). Recent analyses have begun to reveal the molecular mechanisms that underlie the ordered appearance of the adult phenotype in a cell- and tissue-specific fashion (Tata, 1993; Atkinson, 1994). While it is likely that perturbations to this system of control play a prominent role in mediating the evolutionary changes in developmental rate and timing that characterize amphibian phylogenesis (Hanken and Hall, 1984, 1988; Reilly, 1986, 1994; Emerson, 1987; Rose, 1996), this role, including changes in specific control parameters (e.g., the number of thyroid hormone receptors or their binding properties), remains to be defined in most groups. Most previous comparative and experimental studies of salamanders have focused on instances of gross change in developmental timing involved in the evolution of "neoteny," or larval reproduction (Shaffer, 1993). These include a series of both plethodontids
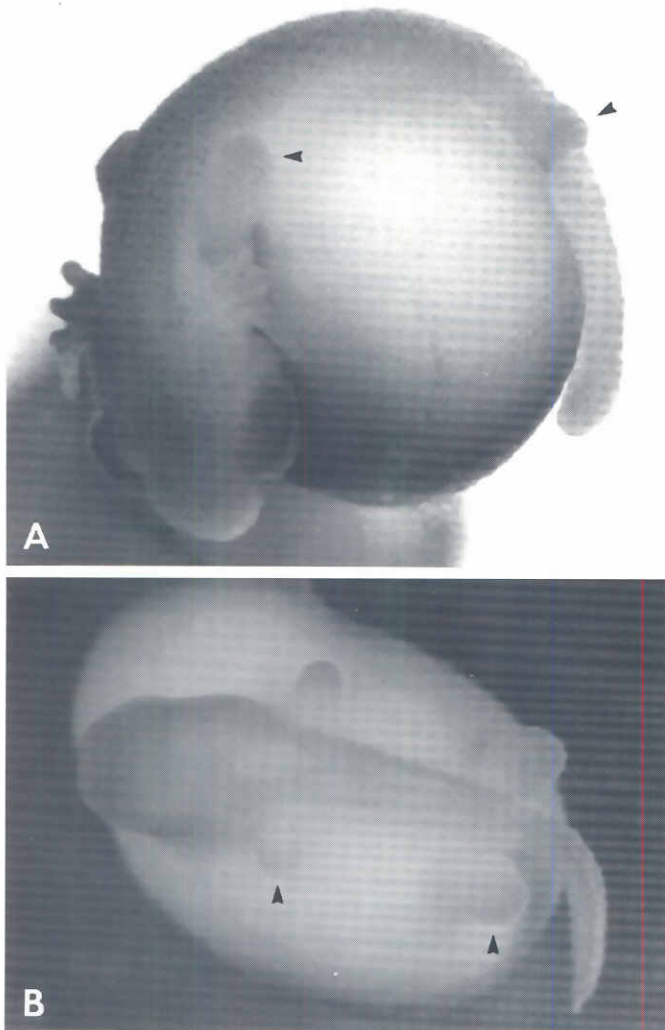
Fig. 7. Direct-developing embryos. (A) *The Mexican plethodontid sala-mander Chiropterotriton magnipes ("early pigmentation" stage; Vial, 1968, Fig. 17).* **(B)** *The Puerto Rican leptodactylid frog Eleutherodactylus coqui (stage 4-5; Townsend and Stewart, 1985). Early, simultaneous develop-ment of fore- and hind limb buds (arrowheads) has evolved independently in direct-developing urodeles and anurans.*

(*Haideotriton* – Dundee, 1961; *Typhlomolge* – Dundee, 1957; Gorbman, 1957; *Gyrinophilus* – Dent *et al.,* 1955; Blair, 1961; Dent and Kirby-Smith, 1963; Yeatman, 1967; Brandon *et al.,* 1986; *Eurycea* – Kezer, 1952; Dundee and Gorbman, 1960) and species in other families (e.g., *Ambystoma* – Darras and Kühn, 1984; Jacobs *et al.,* 1988; Galton, 1992; *Necturus* – Galton, 1985; *Amphiuma* – Kobayashi and Gorbman, 1962; Larsen, 1968). Most evolutionary transitions in the Plethodontidae, however, involve more subtle changes in development than are represented by larval reproduction (Wake, 1966, 1991; Rose, 1995b). While changes in hormonal control may have played as significant a role in mediating these transitions as they have in instances of larval reproduction, until recently they have received almost no attention (Alberch *et al.,* 1985, 1986; Rose, 1995c,d).

Opportunities for probing the role of changes in hormonal control in phyletic diversification are particularly promising in the case of direct development. Is the development of adult features, which now form in the embryo, still critically dependent on thyroid hormone (TH) as it is in metamorphosing taxa? Or, instead, is adult ontogeny "emancipated" from TH control (Lynn and Peadon, 1955)? If the system of hormonal control has been retained, what is the source of TH and other hormones – the embryo, or maternally derived yolk? Have all adult features been affected equally, or have some retained hormonal dependence while others lost it? To what extent does the gross change in hormonal control constrain or facilitate opportunities for morphological evolution? To date, these questions have been pursued most extensively in anurans (e.g., *Eleutherodactylus* – Lynn, 1936, 1948; Lynn and Peadon, 1955; Hughes, 1966; Hughes and Reier, 1972; Elinson, 1994; Jennings, 1994; *Arthroleptella* – Brink, 1939; Morgan *et al.,* 1989). Compara-ble analyses of direct-developing plethodontids were initiated more than 50 years ago (*Plethodon* – Dent, 1942; Lynn, 1947; *Aneides* – Dent, 1954), but these studies were never completed and deserve to be followed up (e.g. Rose, 1995c,d).

Genes, development and evolution

There is intense current interest in the molecular-genetic basis of pattern formation. Increasingly, such studies are being applied to problems in evolution, such as the role of various kinds of developmental control genes in morphological diversification (Gaunt, 1994). To date, most studies follow an essentially typologi-cal approach; evolutionary inferences are made from broad com-parisons among distantly related taxa, often with little considera-tion paid to known ancestor-descendant relationships or character state transitions (Gendron-Maguire *et al.,* 1993; Rijli *et al.,* 1993). Direct-developing plethodontid salamanders offer an excellent system with which to assess the molecular-genetic basis of mor-phological diversification within an explicitly defined phylogenetic, functional, and ecological context, a context that typically is not afforded by the typological approach. A practical consideration that precluded such studies in the past is the high cost and effort involved in preparing gene-specific probes and other molecular tools that are used in analyses of this kind, which limited them to a few "model" taxa. Recent technical innovations, however, such as effective methods for cross-species *in situ* hybridization (A. Nieto and D. Wilkinson, personal communication), have brought more fine-scaled comparative studies within reach.

Numerous opportunities exist. Several studies, for example, have documented a prominent role of various homeobox and other segment-identity genes in specifying regional identity within the hindbrain and associated visceral arch derivatives. In different lineages of plethodontid salamanders, the evolution of direct development has affected the initial embryonic patterning of these same components to different extents – in Desmognathinae and Plethodontini, initial patterning has changed relatively little; in Bolitoglossini, changes in patterning are much more profound. If homeobox and other segment-identity genes have played an important role in mediating these evolutionary changes, then we might expect to see differences in patterns of gene expression between direct-developing desmognathines and bolitoglossines. Similarly, gene expres-sion patterns should be more similar between direct-developing desmognathines and metamorphosing outgroups than between bolitoglossines and metamorphosing taxa. Similar opportuni-ties exist to explore the genetic basis of morphological diversi-fication of the hyobranchial skeleton (Lombard and Wake,

1986) and the hindbrain and cranial nerves (Wake, 1993b) within major lineages of direct-developers.

Conclusions

Throughout much of the 20th century, developmental biologists have shown an increasing preoccupation with a small number of "model" organisms (Bolker, 1995); for those working with urodeles, the primary model is, of course, the axolotl (Armstrong and Malacinski, 1989; Shaffer, 1993). Non-model organisms offer unique opportunities to test the validity and generality of many observations derived from the study of model taxa, and to investigate unique and important developmental problems that are not represented by them (Hanken, 1993). Plethodontid salamanders provide the greatest number and range of such opportunities among urodeles, especially in the context of analyses of the complex interplay between development and phylogeny during evolutionary diversification.

Earlier studies of amphibian developmental biology included a much broader range of taxa than are now commonly used, including many plethodontids (e.g., Goodale, 1911; Emmel, 1924; Noble and Richards, 1932). They also devoted much greater attention to the role of developmental biology in organismal adaptation and phylogenesis than prevails today (Moran, 1994). In this sense, we call for a return to the early exploratory, integrative spirit that once typified the field. Combined with the dramatic discoveries and technological innovations that are so much a part of contemporary research, such an approach is likely to provide an abundance of fundamental insights into both developmental and evolutionary biology.

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