AMER. ZOOL., 37:160–171 (1997)

Mechanistic Basis of Life-History Evolution in Anuran Amphibians: Direct Development[1]

JAMES HANKEN, DAVID H. JENNINGS[2] AND LENNART OLSSON[3]

Department of Environmental, Population, and Organismic Biology, University of Colorado, Boulder, Colorado 80309-0334

SYNOPSIS. **The primitive, or ancestral reproductive mode for Recent amphibians involves a complex, biphasic life history. Yet evolutionarily derived, alternate modes are seen in all three living orders and predominate in some clades. Analysis of the consequences and mechanistic bases of one such mode—direct development—can provide insights into the evolutionary opportunities and constraints conferred by the ancestral metamorphic ontogeny. Direct development in the anuran genus *Eleutherodactylus* involves fundamental alterations to many features of embryonic and posthatching development. At hatching, young emerge as fully formed, albeit tiny versions of the adult; most larval features are absent. Pervasive changes in ontogenetic timing, in particular the precocious (embryonic) formation of many adult structures, appear to be correlated with early development of the thyroid axis, although responsiveness to exogenous thyroid hormone is diminished or even lacking in at least some peripheral tissues. Changes in cranial patterning are likely mediated by the embryonic neural crest, although many gross features of crest biology are highly conserved. Laboratory-based analyses of direct development and other derived reproductive modes in amphibians, using contemporary methods developed for more standard, "model" organisms, may contribute important insights into life-history evolution that complement those derived from analyses of morphology, ecology and phylogeny.**

INTRODUCTION

Animal metamorphosis comprises a concentrated period of postembryonic development (Alberch, 1989; Rose and Reiss, 1993). In many amphibians, metamorphosis effects anatomical and functional transformation between two discrete, free-living life history stages—aquatic larva and terrestrial adult. The ecological and evolutionary significance of metamorphosis and the complex, biphasic life history of which it is a part has long been appreciated. A primitively complex life history, for example, confers on amphibians a tremendous potential for adaptive diversification, which is realized in the spectacular array of alternative reproductive modes, morphologies, and ecological relationships, which are seen in both extinct and extant taxa (Duellman and Trueb, 1986). Consequently, analysis of both metamorphosis and alternate reproductive modes may contribute a great deal to our understanding of several important topics, such as the origin of morphological and functional novelty and the evolution of complex features and morphological integration (Hanken, 1992; Wake and Hanken, 1996).

Among the most extreme evolutionary modifications of the ancestral, complex life history seen in Recent amphibians is direct development. Typically, eggs are laid on land and the young emerge as fully formed, albeit tiny versions of the adult; there is no free-living, aquatic larva (Fig. 1A). Direct development is the characteristic reproductive mode of many hundreds of extant spe-

[1] From the Symposium *Amphibian Metamorphosis: An Integrative Approach* presented at the Annual Meeting of the American Society of Zoologists, 27–30 December 1995, at Washington, D.C.

[2] Present address of David Jennings is Department of Zoology, Arizona State University, P.O. Box 871501, Tempe, AZ, 85287-1501

[3] Present address of Lennart Olsson is Department of Environmental and Developmental Biology, Uppsala University, Norbyvägen 18A, 752 36 Uppsala, Sweden.

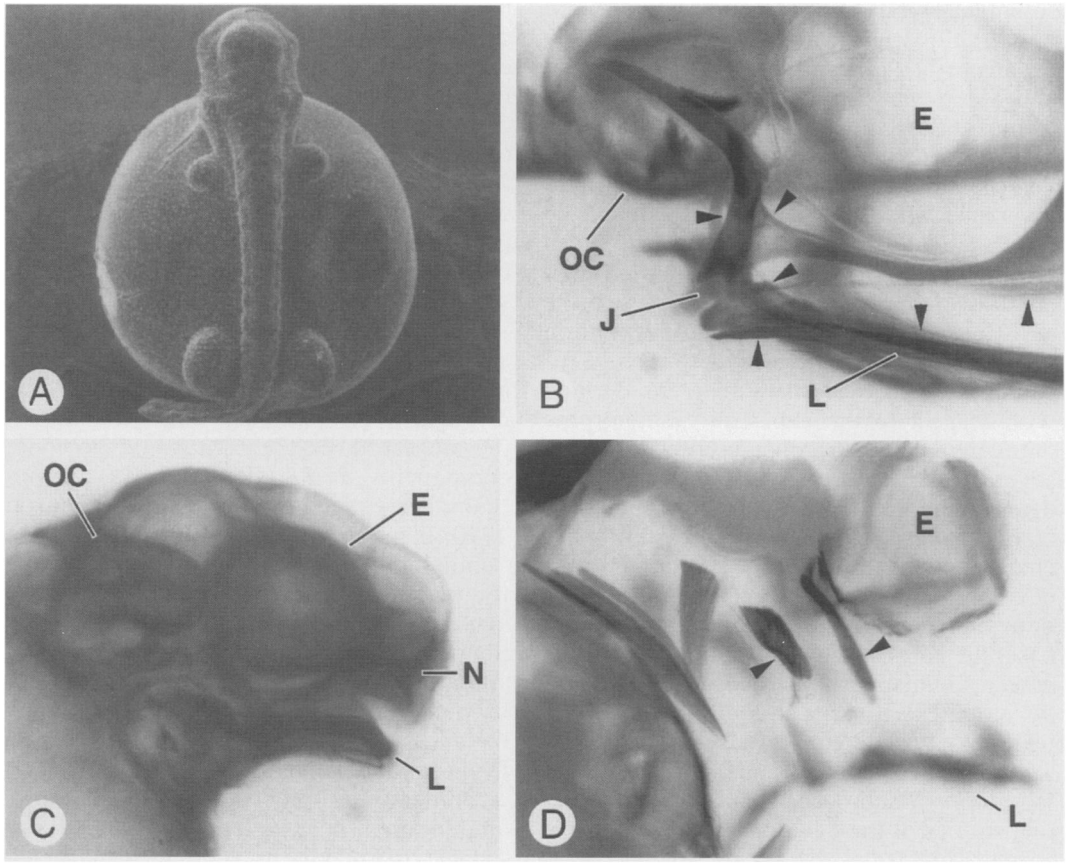

FIG. 1. (A) Direct development in *Eleutherodactylus coqui* comprises 15 embryonic stages from fertilization to hatching (Townsend and Stewart, 1985). This embryo, photographed with scanning electron microscopy, is at stage 5. Fore- and hindlimb buds are prominent swellings on either side of the body axis. Dorsal view; anterior at top. (B) Cleared-and-stained jaw region of a hatchling froglet (stage 15), showing cranial bones (arrowheads) and cartilages. Lateral view, anterior at right. E, eye; J, jaw joint; L, lower jaw; OC, otic capsule. (C) Cartilaginous skull (stage 11), prepared using whole-mount immunohistochemistry with an antibody to type-II collagen (Hanken *et al.*, 1992). The cranial form is adult-like; most larval-specific features are absent. Nasal cartilages (N), for example, are a postmetamorphic feature. Same orientation as B. (D) Cranial musculature (stage 12), prepared as in C but with an antibody to fast-muscle myosin (Klymkowsky and Hanken, 1991). Evolutionary changes in the development of many muscles, especially those responsible for opening and closing the jaw (arrowheads), closely parallel those of adjacent skeletal elements. Same orientation as B.

cies and has evolved independently in all three living orders—frogs, salamanders, and caecilians. It is the predominant reproductive mode in some clades, *e.g.*, plethodontid salamanders (Wake and Hanken, 1996), and likely evolved at least 10 times in anurans alone (Duellman and Trueb, 1986). The evolution of direct development can have important ecological and evolutionary consequences for the lineages involved; these range from emancipation from aquatic breeding sites (McDiarmid, 1978), to the removal of larval constraints on adult morphology associated with the metamorphic ontogeny (Alberch, 1987, 1989; Wake and Marks, 1993).

Despite the considerable importance of direct development to the evolutionary biology of amphibians, its *developmental* basis—especially the ways in which the ancestral metamorphic ontogeny is perturbed to achieve it—remains poorly understood (Elinson, 1990). Yet, greater knowledge of the developmental mechanisms underlying this pronounced evolutionary shift in reproductive mode is essential for a critical as-

sessment of the evolutionary constraints and opportunities associated with the complex life history (Wake and Hanken, 1996; Wake and Marks, 1993). It also would contribute to our understanding of the role played by development in mediating evolutionary diversification generally (Hanken, 1992). In this paper, we review several aspects of the developmental biology of the Puerto Rican direct-developing frog, *Eleutherodactylus coqui,* which are being used to address these questions. We begin with a brief description of the embryonic ontogeny in *E. coqui,* focusing on those features that are derived with respect to the ancestral metamorphic ontogeny. We then consider the results of several recent and ongoing studies that address some of the developmental mechanisms that may underlie these evolutionary changes, especially those associated with developmental timing and pattern formation. Information about *E. coqui* provides a baseline for evaluating the consequences and mechanisms of the evolution of direct development in this and other amphibian lineages. In reviewing it, we hope to underscore the feasibility and desirability of laboratory-based descriptive and manipulative studies of the developmental biology of amphibian species displaying this and other alternate reproductive modes.

Ontogeny of Direct Development

The ontogeny of direct development in *E. coqui* provides the necessary morphological context with which to assess underlying developmental mechanisms. Almost by definition, direct development in amphibians involves the precocious, embryonic formation of most adult features, which typically form at metamorphosis in the ancestral, complex life history (M. Wake, 1989). Nevertheless, the ontogenies of direct-developing species vary considerably, especially regarding particular modifications to early development and the extent to which the ancestral larval developmental program is retained, either before or after hatching. *Eleutherodactylus* has long been regarded as extreme among anurans with respect to the degree to which its development deviates from the ancestral ontogeny (*e.g.,* Lynn, 1961); evolution of direct development in this lineage has had pronounced and obvious consequences for embryogenesis (Elinson, 1990; Lynn, 1942; Fig. 1). Novel features of embryonic development in *Eleutherodactylus* in comparison to metamorphosing frogs include tissue type (bone), organs (paired limbs, keratinized egg tooth), function (use of the tail as the principal respiratory organ), and organ and tissue patterning (which is adult; most larval features are absent).

The derived pattern of embryonic development in *Eleutherodactylus* has been documented most thoroughly in the head, which displays a comprehensive modification of the ancestral cranial ontogeny involving components of the skeleton, musculature, brain, nerves, and oral integument (Elinson, 1990; Fang and Elinson, 1996; Lynn, 1942; Schlosser, 1995). In metamorphosing frogs, for example, bone formation occurs posthatching; the larval skull is exclusively cartilaginous. In *Eleutherodactylus,* cranial ossification has been advanced into the embryonic period. Indeed, 13 of the 17 different adult skull bones are present at hatching in *E. coqui* (Hanken *et al.,* 1992; Fig. 1B). Accompanying precocious ossification is altered embryonic patterning of cranial cartilages; many larval-specific cartilages do not form, whereas others initially assume a mid-metamorphic configuration which is subsequently remodeled to the adult configuration before hatching (Fig. 1C). Modifications in cranial ontogeny show a high degree of concordance and integration among tissue types. Derived features of embryonic development of the jaw-opening musculature, for example, closely parallel those of adjacent skeletal elements (Fig. 1D). Overall, these modifications comprise two broad classes of change—developmental timing and embryonic patterning.

Endocrine Control

In the ancestral, complex life history, which is retained by many Recent amphibians, the definitive, adult body form is attained during metamorphosis. The appearance of adult features follows the loss and remodeling of many larval components and de novo formation of other components specific to the adult (Yoshizato, 1989). All

these events are mediated by a complex and highly integrated system of neuroendocrine control. An especially prominent role is played by the suite of secretory organs, chemical messengers, and responding tissues that comprise the thyroid axis, which itself is first activated after hatching and becomes fully functional only at the end of the larval period, during metamorphic climax (Kikuyama *et al.*, 1993).

Because of the predominant role of the thyroid axis in mediating the metamorphic development of adult features in the ancestral life history, changes to the thyroid axis have been implicated in the evolution of many alternative life histories in Recent taxa (Dent, 1968; Elinson, 1990; Lynn, 1936; Matsuda, 1987; Rose, 1996; Shaffer and Voss, 1996). Most comparative studies, however, have focused on understanding the hormonal basis of one or more instances of metamorphic failure, or "neoteny" (see references in Rose, 1996; Shaffer and Voss, 1996; Wake and Hanken, 1996), which involve absence of a discrete metamorphosis and most if not all features unique to the adult stage. Few studies have attempted to assess the changes to the ancestral system of endocrine control that underlie the evolution of other reproductive modes, such as direct development (*e.g.*, Lynn and Peadon, 1955; Rose, 1995). In particular, there have been very few empirical attempts to distinguish explicitly among the several possible evolutionary changes to the ontogeny and function of the thyroid axis by directly assessing the role of various axis components in mediating the embryonic development of adult features in direct-developing amphibians (Fig. 2). Moreover, most of these studies were completed before the advent of a wide variety of molecular tools and assays that now permit resolution of thyroid-axis ontogeny and function to a much finer scale than previously available (Shi, 1994; Tata, 1993).

Precocious onset and activation of the thyroid axis is one potential mechanism for effecting early (embryonic) development of adult features in direct-developing amphibians. Yet, early studies of *Eleutherodactylus* failed to yield an unequivocal assessment of the role of thyroid-axis components in mediating embryonic development in any species; the ancestral system of endocrine control appeared to be retained in the development of some features, but not others (Hughes, 1966, 1968, 1974; Lynn, 1936, 1948). This problem has been reexamined in several recent studies of *E. coqui*. These studies are beginning to provide a more definitive understanding of the role of the thyroid axis, both in embryonic development and in the evolution of direct development.

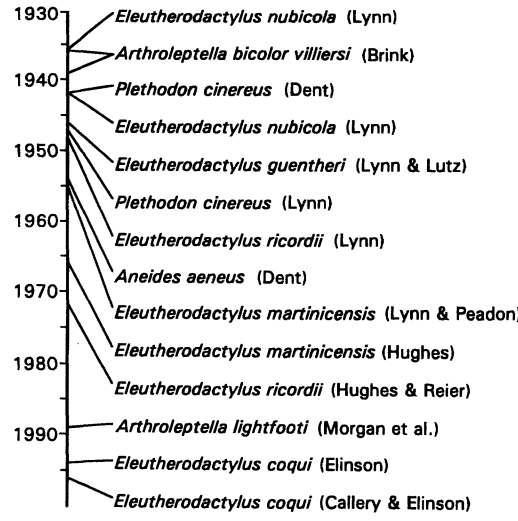

FIG. 2. Only 15 studies, published over the last 60 years, directly assess hormonal control of direct development in Recent amphibians. The studies address a total of four genera (*Plethodon* and *Aneides* are urodeles, *Eleutherodactylus* and *Arthroleptella* are anurans). Two of the citations are for published abstracts (Dent, 1954; Lynn, 1947).

Elinson (1994) examined embryonic limb development in a series of analyses that included exogenous administration of thyroid hormones (TH) triiodothyronine and thyroxine (T_3 and T_4, respectively) to intact embryos, to hindlimb-and-tail explants, and to leopard frog *(Rana pipiens)* embryos bearing *E. coqui* limb transplants. Results yielded no evidence of any role for TH in limb development in *E. coqui,* during either initial stages of limb morphogenesis or later stages of growth and differentiation. Tail regression was enhanced by exogenous TH, but only at dosages two orders of magnitude higher than that required to elicit comparable changes in metamorphosing frogs, such as *Xenopus*.

Callery and Elinson (1996) assessed the

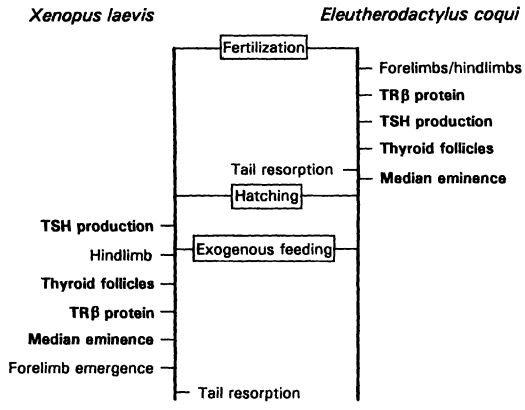

FIG. 3. Heterochrony in thyroid-axis development of frogs with different life histories. Ontogenetic trajectories are standardized to the same time axis relative to fertilization, hatching, and the onset of exogenous feeding; absolute times differ considerably between species. For example, *Xenopus* larvae typically hatch within 50 hr postfertilization (Nieuwkoop and Faber, 1967), whereas *E. coqui* hatch after 17–21 d (Townsend and Stewart, 1986). TRβ, thyroid-hormone receptor beta; TSH, thyroid-stimulating hormone.

possible role of TH in developmental regulation of the urea-cycle enzyme arginase in late-stage embryos. Treatment with exogenous T_3 increased both the amount of arginase protein and enzyme activity, but, as with tail regression (Elinson, 1994; see above), only at a dosage far above those that typically induce metamorphosis in larval anurans.

Jennings (1994, 1997) recently completed a comprehensive examination of the development and activation of several thyroid-axis components in *E. coqui* using conventional histology, immunohistochemistry, and radioimmunoassay. His main findings include histodifferentiation of both the thyroid gland and the median eminence of the hypothalamus, and pituitary production of thyroid-stimulating hormone (TSH), within the last third of embryogenesis (stages 10–15); expression of thyroid-hormone receptors (TRβ) within many tissues (*e.g.*, kidney, notochord, brain, heart, limb bud, gut epithelium) beginning as early as stage 5; and maternal provisioning of oviposited, fertilized eggs with T_3 and T_4. Timing of the above features relative to other prominent developmental events in *E. coqui* is summarized in Figure 3. Comparable data are included for the clawed frog, *Xenopus laevis*, the best studied anuran in this regard (Eliceiri and Brown, 1994; Goos *et al.*, 1968; Moriceau-Hay *et al.*, 1982; Nieuwkoop and Faber, 1967). Basic features of thyroid-axis onset and activation in all species of metamorphosing frogs examined to date are consistent with the pattern found in *Xenopus* (Kikuyama *et al.*, 1993), which we presume to be the ancestral condition for all living anurans. Maternal provisioning of TH in unfertilized eggs has not been assessed in *Xenopus* but has been documented in three other species of metamorphosing frogs (*Bombina orientalis*—Jennings, 1997; *Bufo marinus*—Weber *et al.*, 1994; *Rana catesbeiana*—Fujikara and Suzuki, 1991); it likely represents an additional ancestral feature. The most conspicuous difference between the two chronologies involves the time of thyroid-axis development relative to hatching. In metamorphosing anurans, onset and activation of the thyroid axis occur posthatching. In *Eleutherodactylus*, the thyroid axis forms during embryogenesis.

Jennings's results are consistent with the hypothesis that the ancestral system of neuroendocrine regulation of metamorphosis involving the thyroid axis is conserved (at least in several key aspects) in *Eleutherodactylus*, at the same time that its formation has been advanced into the embryonic period. Presence of maternally derived TH and early expression of TH receptors may indicate that TH mediation of development begins very early in embryogenesis. This, in turn, would implicate a prominent role for the thyroid axis in the precocious formation of adult features, and suggest that temporal shifts in the development and activation of the thyroid axis are an important mechanism for the evolution of direct development in anuran amphibians. These latter conclusions must be regarded as preliminary, however, pending additional experimental studies that directly evaluate thyroid-axis integration in embryonic *Eleutherodactylus*. They also must be reconciled with the results of Elinson (1994) and Callery and Elinson (1996), which demonstrate a diminished, or lack of, responsiveness to TH by various peripheral tissues. The likely, complementary role of additional endocrine factors in mediating development, such as

prolactin and adrenal steroids (Bern, 1983; Hughes and Reier, 1972; Kaltenbach, 1996; Kikuyama *et al.,* 1993) also remains to be assessed.

NEURAL CREST BIOLOGY

Cranial ontogeny in direct-developing *Eleutherodactylus* differs significantly from that seen in metamorphosing anurans (see above). The evolutionary transformation represented by these differences is of general interest in at least two respects. First, the many individual differences together comprise a comprehensive modification of embryonic cranial differentiation and patterning. Secondly, the transformation is characterized by a high degree of morphological and functional integration among a wide range of otherwise disparate tissues. Analysis of the role of underlying developmental mechanisms in these evolutionary events ultimately must be able to account for both features. Over the last 15 years, the embryonic neural crest has emerged as a principal player in the development and organization of the vertebrate head, where it assumes at least two distinct roles. First, the neural crest is a prominent source of progenitor cells of many cranial components, *e.g.,* the skull and connective-tissue elements of cranial muscles (Couly *et al.,* 1993; Noden, 1983*a;* Olsson and Hanken, 1996). Secondly, the neural crest specifies (at least to a considerable extent) the three-dimensional patterning of these and other cranial components, thereby helping to coordinate their development and form (Noden, 1983*b*).

The central role of the neural crest in both the derivation and embryonic patterning of the vertebrate head suggests that changes in its development underlie, at least in part, evolutionary changes in these features, including those seen in direct-developing *Eleutherodactylus*. One might expect, for example, that precocious (embryonic) formation of adult cranial morphology is associated with changes in the timing of neural-crest migration, or that losses of many larval-specific, neural-crest-derived components are correlated with change in the relative sizes or basic migratory pathways of cranial crest streams. Yet, until very recently studies of amphibian neural crest almost completely ignored direct-developing species; the accumulated comparative data were insufficient to adequately address these questions. Several recent studies have begun to define basic aspects of neural-crest biology in *E. coqui* as a means of explaining its derived pattern of cranial ontogeny. These studies focus on two features that likely are involved in pattern formation and morphogenesis: gross patterns and timing of neural-crest emergence and migration, and gene expression.

Moury and Hanken (1995) described cranial neural-crest-cell emergence and early migration using scanning electron microscopy (SEM; Fig. 4). Unexpectedly, *E. coqui* was found to generally resemble metamorphosing anurans with respect to several basic features, such as the number and configuration of migratory streams and the timing of crest-cell emergence relative to neural tube closure. The only obvious difference between *E. coqui* and a metamorphosing species *(Xenopus laevis),* which involves the relative sizes of the three principal migratory streams, apparently is independent of reproductive mode. In *E. coqui* both the rostral-otic (hyoid) and caudal-otic (branchial) streams are much narrower than the rostral (mandibular) stream, whereas in *Xenopus* all these streams are approximately the same width. Yet, the pattern in *Xenopus* has not been observed in other metamorphosing frogs, which instead resemble *Eleutherodactylus* (Olsson and Hanken, 1996). Thus, at least in *Eleutherodactylus,* evolution of direct development has not altered basic patterns of neural-crest emergence or early migration as seen with SEM.

Molecular data are being used to extend these morphological results. In addition to validating the above features of cranial neural-crest-cell emergence and early migration, they are being used to assess heterogeneity among and within crest-cell populations, which might correlate with altered patterns of cell lineage or fate; and to screen for interspecific differences that correlate with reproductive mode and which might underlie associated differences in cranial patterning. Molecular markers in-

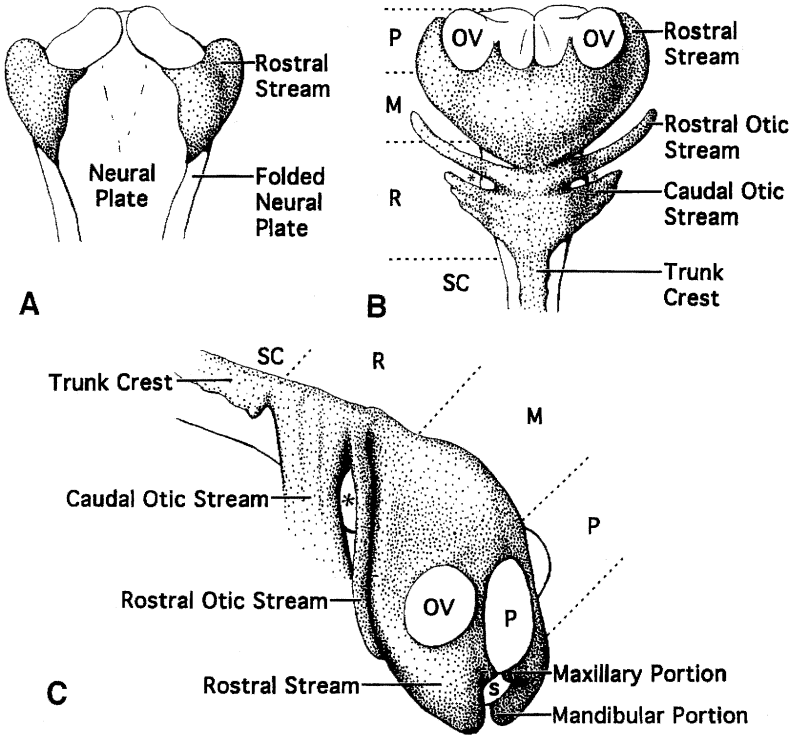

FIG. 4. Early cranial-neural-crest migration in *Eleutherodactylus coqui*. (A) Mid-stage 3. Dorsal view; anterior at top. (B) Early stage 4, orientation as in A. (C) Late stage 4. Anterolateral view; anterior at right. P, prosencephalon; M, mesencephalon; R, rhombencephalon; SC, spinal cord; OV, optic vesicle; S, stomodeal endoderm; *, position of otic placode/vesicle. Modified from Moury and Hanken (1995).

clude distalless-gene expression, cholinesterase activity, and HNK-1 immunoreactivity (Fang and Elinson, 1996; Olsson et al., in prep.). Distalless-gene expression is particularly interesting because there is considerable evidence of an important role for homologs of the *Drosophila* distalless gene, *Dll*, in vertebrate head development, including expression of several genes in migrating cranial neural crest in many species (Bulfone *et al.*, 1993; Dirksen *et al.*, 1993; Dolle *et al.*, 1992; Morasso *et al.*, 1995; Robinson and Mahon, 1994; Zhao *et al.*, 1994), and severe head abnormalities in gene-knockout mice produced for *Dlx-2* (Qiu *et al.*, 1995). Moreover, in *Eleutherodactylus*, absence of the cement gland (an oral integumentary structure found in early larvae of many anuran species) is correlated with the loss of anterior distalless-gene expression characteristic of metamorphosing frogs (Fang and Elinson, 1996). Cholinesterase (ChE) activity is a marker for early-migrating neural-crest cells in chicken and mouse (Cochard and Coltey, 1983; Layer and Kaulich, 1991; Martins-Green and Erickson, 1988), but has never before been employed to study neural-crest migration in amphibians. HNK-1 is a monoclonal antibody that recognizes an acidic, sulfated glycosphingolipid (Mailly *et al.*, 1989). Because neural-crest cells of many, diverse vertebrates express the HNK-1 epitope soon after emerging from the neural tube, HNK-1 immunoreactivity has been used to document pathways of crest-cell migration in these species (Bronner-Fraser, 1986; Erickson *et al.*, 1989; Heath *et al.*, 1992; Hou and Takeuchi, 1994; Sadaghiani and Vielkind, 1990). HNK-1 has not been used previously to study neural-crest migration in amphibians because early migrating crest cells are not immunoreactive in many species, including *Xenopus laevis*, the species tested initially (M. Bronner-Fraser, personal communication).

Initial results from the molecular studies underscore the extensive similarity in basic

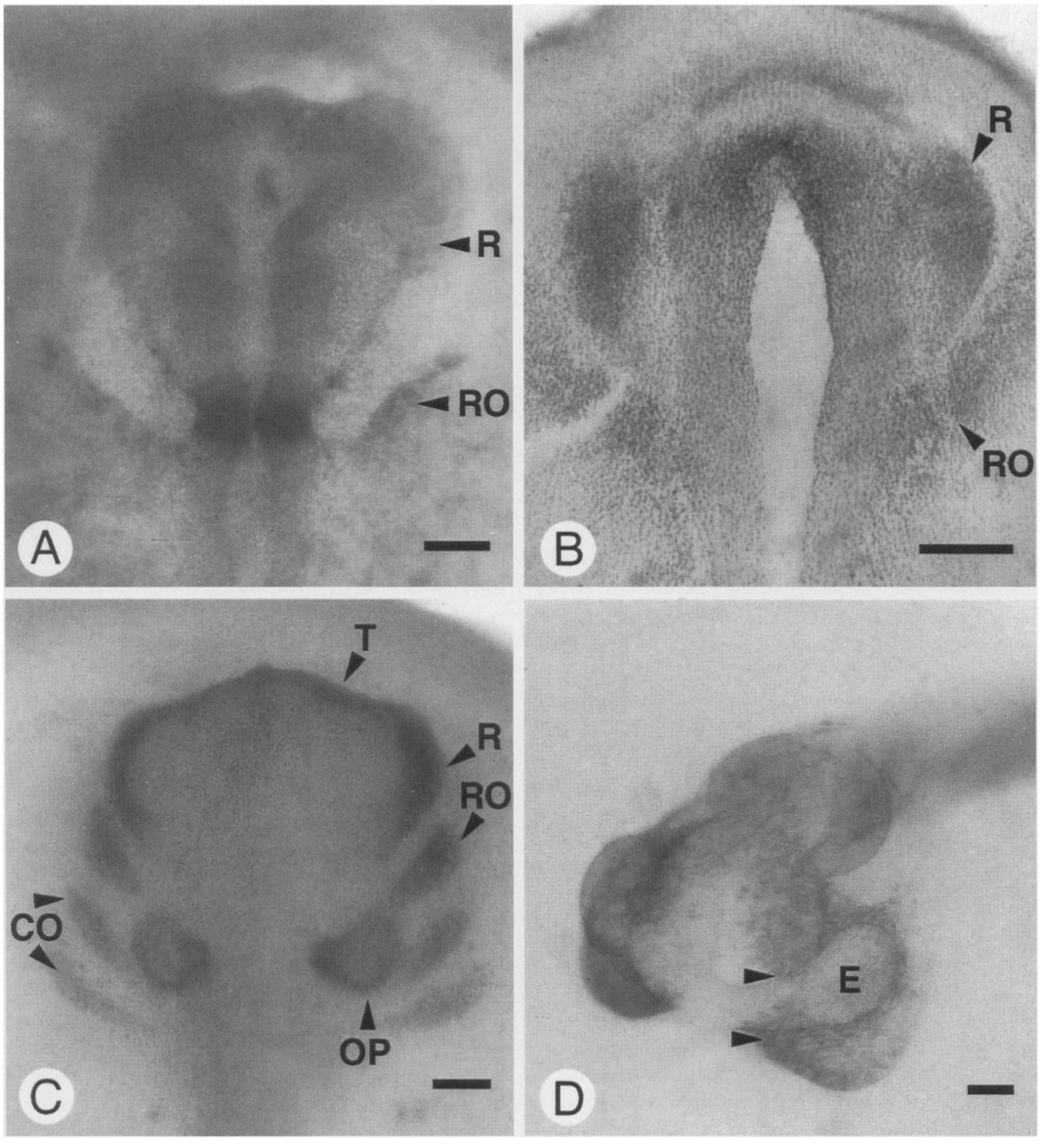

FIG. 5. Expression of three molecular markers in cranial neural crest of *Eleutherodactylus coqui*. (A) Cholinesterase staining (arrowheads) within the lateral portion of the rostral stream (R) and the rostral-otic stream (RO) at stage 3. ChE activity begins in the caudal-otic stream and the transverse neural fold at stage 4, and in the medial portion of the rostral stream at stage 6 (not illustrated). Histochemical methods modified slightly from Karnovsky and Roots (1964); concentrations of all reagents other than fixatives and phosphate buffer were doubled to intensify staining in whole mounts (Klymkowsky and Hanken, 1991). (B) Distalless-protein expression (arrowheads) at stage 3. Crest migration is underway in rostral and rostral-otic streams; the caudal-otic stream has not yet emerged. Immunocytochemical methods (Klymkowsky and Hanken, 1991) used a polyclonal antibody against a conserved region of the *D11* protein in arthropods (Fang and Elinson, 1996; Panganiban *et al.*, 1995). (C) Distalless-protein expression (arrowheads) at stage 4. Distal portions of all three cranial-crest streams express distalless protein, as do the transverse neural fold (T) and the otic placode (OP). The caudal-otic stream (CO) has split into two parallel streams. (D) HNK-1 expression at late stage 4. Immunoreactive cells within the rostral crest stream are migrating around the eye (E) via dorsal and ventral pathways (arrowheads). Methods as in B, except embryos were fixed initially in formalin, followed by postfixation in Dent fixative; antibodies: American Type Culture Collection TIB 200 (Dr. Ruth Nordlander, Ohio State University) and anti-human Leu-7 (CD 57, clone HNK-1; Becton Dickinson, San Jose, CA). A–C, dorsal views; anterior at top. D, anterolateral view; anterior at left. Scale bar: 0.2 mm.

patterns of neural-crest emergence and early migration between direct-developing *E. coqui* and metamorphosing anurans (Fang and Elinson, 1996; Moury and Hanken, 1995; Olsson and Hanken, 1996). At the same time, they reveal considerable heterogeneity among and even within crest-cell populations in *E. coqui*. For example, whereas all three cranial migratory streams show ChE activity and express distalless-gene protein (Fig. 5A, C), only the rostral stream shows HNK-1 immunoreactivity (Fig. 5B). Within the rostral stream, lateral and medial portions display an inverse relationship between ChE activity and HNK-1 expression (Fig. 5A; Olsson *et al.*, in preparation). The transverse neural fold, which is not a source of neural-crest cells, expresses ChE and distalless protein (Fig. 5A, C), but not HNK-1 (Fig. 5D). Cranial neural crest will contribute to a wide range of differentiated tissues, including bone, cartilage, muscular connective tissue, and nerves, but the extent to which these early differences in molecular staining properties correlate with eventual differences in cell lineage or fate is unknown.

Preliminary interspecific comparisons of the expression patterns of these three molecular markers within cranial neural crest yield an association with reproductive mode for one but neither of the other two (Olsson *et al.*, in preparation). HNK-1 immunoreactivity is present in direct-developing *E. coqui* but absent in two metamorphosing species, *Xenopus laevis* and *Bombina orientalis*. ChE staining and distalless-protein expression appear to be similar in all three species. Additional data are needed to determine if this association holds for other direct-developing and metamorphosing taxa, and, if it does, to assess whether HNK-1 expression might be causally related to the profound changes in cranial patterning that have accompanied the evolution of direct development in *Eleutherodactylus*.

DISCUSSION

A primary goal of contemporary research on the developmental biology of direct-developing amphibians is to reveal the specific perturbations in developmental mechanism that underlie the evolutionary shift from the ancestral, complex life history (Wake and Hanken, 1996). In *Eleutherodactylus,* this shift is reflected in at least two general classes of ontogenetic change—timing and patterning. While the basis of each class of change may be investigated separately to a considerable extent, both classes of change may prove to share a common developmental basis.

Several of the above studies are in their early stages; some of the conclusions are tentative. Nevertheless, these and other recent studies (*e.g.,* Shaffer and Voss, 1996) demonstrate the feasibility of laboratory-based analyses of the mechanistic basis of life-history evolution in amphibians, including direct development, using contemporary methods developed for more standard, "model" organisms. Important insights derived from such analyses will complement those derived from analyses of morphology, ecology and phylogeny.

ACKNOWLEDGMENTS

Research support was provided by NSF to J. H. (IBN 94–19407) and D. H. J. (IBN 93–21572); L. Olsson received postdoctoral grants from the Wenner-Gren Center Foundations and the Swedish Institute. Adult frogs were collected with the permission of the Puerto Rican Department of Natural Resources, as part of the Long-Term Ecological Research Program in the Luquillo Experimental Forest. We thank Dr. Grace Panganiban, University of Wisconsin-Madison, and Dr. Ruth Nordlander, Ohio State University, for providing antibodies, and three anonymous reviewers for offering excellent criticisms and comments on an earlier version of the manuscript.

REFERENCES

Alberch, P. 1987. Evolution of a developmental process—irreversibility and redundancy in amphibian metamorphosis. *In* R. A. Raff and E. C. Raff (eds.), *Development as an evolutionary process,* pp. 23–46. Alan R. Liss, Inc., New York.

Alberch, P. 1989. Development and the evolution of amphibian metamorphosis. *In* H. Splechtna and H. Hilgers (eds.), *Trends in vertebrate morphology* (Fortschritte der Zoologie, Vol. 35), pp. 163–173. Gustav Fischer Verlag, Stuttgart.

Bern, H. 1983. Functional evolution of prolactin and growth hormone in lower vertebrates. Amer. Zool. 23:663–671.

Brink, H. E. 1936. Die Skildklier en Metamorphose by die Amphibia. Annals Univ. Stellenbosch, ser. A 14:1–111 (in Afrikaans).

Brink, H. E. 1939. A histological and cytological investigation of the thyroids of *Arthroleptella bicolor villiersi* and *Bufo angusticeps* during the normal and experimentally accelerated metamorphosis. Proc. Linn. Soc. Lond. 151:120–125.

Bronner-Fraser, M. 1986. An antibody to a receptor for fibronectin and laminin perturbs cranial neural crest development in vivo. Devel. Biol. 117:528–536.

Bulfone, A., H. J. Kim, L. Puelles, M. H. Porteus, J. F. Grippo, and J. L. Rubenstein. 1993. The mouse *Dlx-2 (Tes-1)* gene is expressed in spatially restricted domains of the forebrain, face and limbs in midgestation mouse embryos. Mech. Devel. 40:129–140.

Callery, E. M. and R. P. Elinson. 1996. Developmental regulation of the urea-cycle enzyme arginase in the direct developing frog *Eleutherodactylus co* → *qui*. J. Exp. Zool. 275:61–66.

Cochard, P. and P. Coltey. 1983. Cholinergic traits in the neural crest: Acetylcholinesterase in crest cells of the chick embryo. Devel. Biol. 98:221–238.

Couly, G. F., P. M. Coltey, and N. M. Le Douarin. 1993. The triple origin of the skull in higher vertebrates: A study in quail-chick chimeras. Development 117:409–429.

Dent, J. N. 1942. The embryonic development of *Plethodon cinereus* as correlated with the differentiation and functioning of the thyroid gland. J. Morphol. 71:577–601.

Dent, J. N. 1954. Observations on iodine metabolism in embryos of the terrestrial salamander *Aneides aeneus*. Anat. Rec. 118:294.

Dent, J. N. 1968. Survey of amphibian metamorphosis. *In* W. Etkin and L. I. Gilbert (eds.), *Metamorphosis, a problem in developmental biology,* pp. 271–311. Appleton-Century-Crofts, New York.

Dirksen, M. L., P. Mathers, and M. Jamrich. 1993. Expression of a *Xenopus* Distal-less homeobox gene involved in forebrain and cranio-facial development. Mech. Devel. 41:121–128.

Dolle, P., M. Price, and D. Duboule. 1992. Expression of the murine *Dlx-1* homeobox gene during facial, ocular and limb development. Differentiation 49:93–99.

Duellman, W. E. and L. Trueb. 1986. *Biology of amphibians.* McGraw-Hill Book Company, New York.

Eliceiri, B. P. and D. D. Brown. 1994. Quantitation of endogenous thyroid hormone receptors α and β during embryogenesis and metamorphosis in *Xenopus laevis*. J. Biol. Chem. 269:24459–24465.

Elinson, R. P. 1990. Direct development in frogs: Wiping the recapitulationist slate clean. Sem. Devel. Biol. 1:263–270.

Elinson, R. P. 1994. Leg development in a frog without a tadpole (*Eleutherodactylus coqui*). J. Exp. Zool. 270:202–210.

Elinson, R. P., E. M. del Pino, D. S. Townsend, F. C. Cuesta, and P. Eichhorn. 1990. A practical guide to the developmental biology of terrestrial-breeding frogs. Biol. Bull. 179:163–177.

Erickson, C. A., J. F. Loring, and S. M. Lester. 1989. Migratory pathways of HNK-1 immunoreactive neural crest cells in the rat embryo. Devel. Biol. 134:112–118.

Fang, H., and R. P. Elinson. 1996. Patterns of distal-less gene expression and inductive interactions in the head of the direct developing frog *Eleutherodactylus coqui*. Devel. Biol. 179:160–172.

Fujikura, K. and S. Suzuki. 1991. Thyroxine and thyroglobulin in eggs and embryos of bullfrog. Zool. Sci. 8:1166.

Goos, H. J. T., J. C. M. Zwanebeek, and P. G. W. J. VanOordt. 1968. Hypothalamic neurosecretion and metamorphosis. II. The effect of thyroxine following treatment with propylthiouracil. Arch. Anat. Embryol. 51:268–274.

Hanken, J. 1992. Life history and morphological evolution. J. Evol. Biol. 5:549–557.

Hanken, J., M. W. Klymkowsky, C. H. Summers, D. W. Seufert, and N. Ingebrigtsen. 1992. Cranial ontogeny in the direct-developing frog, *Eleutherodactylus coqui* (Anura: Leptodactylidae), analyzed using whole-mount immunohistochemistry. J. Morphol. 211:95–118.

Heath, L., A. Wild, and P. Thorogood. 1992. Monoclonal antibodies raised against premigratory neural crest reveal population heterogeneity during crest development. Differentiation 49:151–165.

Hou, L. and T. Takeuchi. 1994. Neural crest development in reptilian embryos, studied with monoclonal antibody, HNK-1. Zool. Sci. 11:423–431.

Hughes, A. 1966. The thyroid and the development of the nervous system in *Eleutherodactylus martinicensis*: An experimental study. J. Embryol. Exp. Morphol. 16:401–430.

Hughes, A. 1968. *Aspects of neural ontogeny.* Logos, London.

Hughes, A. F. 1974. Endocrines, neural development, and behavior. Stud. Devt. Behav. Nerv. Syst. 2:223–243.

Hughes, A. and P. Reier. 1972. A preliminary study on the effects of bovine prolactin on embryos of *Eleutherodactylus ricordii*. Gen. Comp. Endocrinol. 19:304–312.

Jennings, D. H. 1994. Thyroid hormone mediation of embryonic development in a non-metamorphosing frog, *Eleutherodactylus coqui*. J. Morphol. 220: 359.

Jennings, D. H. 1997. Evolution of endocrine control in amphibians with derived life-history strategies. Ph.D. Diss., University of Colorado, Boulder.

Kaltenbach, J. C. 1996. Endocrinology of amphibian metamorphosis. *In* L. I. Gilbert, J. R. Tata, and B. G. Atkinson (eds.), *Metamorphosis: Postembryonic reprogramming of gene expression in amphibian and insect cells,* pp. 403–431. Academic Press, San Diego.

Karnovsky, M. J. and L. Roots. 1964. A "direct coloring" thiocholine method for cholinesterases. J. Histochem. Cytochem. 12:219–221.

Kikuyama, S., K. Kawamura, S. Tanaka, and K. Yamamoto. 1993. Aspects of amphibian meta-

morphosis: Hormonal control. Internat. Rev. Cytol. 145:105–148.

Klymkowsky, M. W. and J. Hanken. 1991. Whole-mount staining of *Xenopus* and other vertebrates. *In* B. K. Kay and H. B. Peng (eds.), *Xenopus laevis*: *Practical uses in cell and molecular biology.* Meth. Cell Biol. 36:419–441. Academic Press, New York.

Layer, P. G. and S. Kaulich. 1991. Cranial nerve growth in birds is preceded by cholinesterase expression during neural crest cell migration and the formation of an HNK-1 scaffold. Cell Tiss. Res. 265:393–407.

Lynn, W. G. 1936. A study of the thyroid in embryos of *Eleutherodactylus nubicola.* Anat. Rec. 64:525–539.

Lynn, W. G. 1942. The embryology of *Eleutherodactylus nubicola,* an anuran which has no tadpole stage. Contrib. Embryol. Carnegie Inst. Washington. Publ. 541:27–62.

Lynn, W. G. 1947. The effects of thiourea and phenylthiourea upon the development of *Plethodon cinereus.* Biol. Bull. 93:199.

Lynn, W. G. 1948. The effects of thiourea and phenylthiourea upon the development of *Eleutherodactylus ricordii.* Biol. Bull. 94:1–15.

Lynn, W. G. 1961. Types of amphibian metamorphosis. Amer. Zool. 1:151–161.

Lynn, W. G. and B. Lutz. 1946. The development of *Eleutherodactylus guentheri* Stdnr. 1864. Bol. Museu. Nacional-Zool. 71:1–46.

Lynn, W. G. and A. M. Peadon. 1955. The role of the thyroid gland in direct development in the anuran, *Eleutherodactylus martinicensis.* Growth 19:263–285.

Mailly, P., A. B. Younes Chennoufi, and S. Bon. 1989. The monoclonal antibodies Elec-39, HNK-1 and NC-1 recognize common structures in the nervous system and muscles of vertebrates. Neurochem. Internat. 15:517–530.

Martins-Green, M. and C. A. Erickson. 1988. Patterns of cholinesterase staining during neural crest cell morphogenesis in mouse and chick embryos. J. Exp. Zool. 247:62–68.

Matsuda, R. 1987. *Animal evolution in changing environments, with special reference to abnormal metamorphosis.* John Wiley & Sons, New York.

McDiarmid, R. W. 1978. Evolution of parental care in frogs. *In* G. M. Burghardt and M. Bekoff (eds.), *The development of behavior*: *Comparative and evolutionary aspects* pp. 127–147. Garland STPM Press, New York.

Morasso, M. I., K. A. Mahon, and T. D. Sargent. 1995. A *Xenopus* distal-less gene in transgenic mice: Conserved regulation in distal limb epidermis and other sites of epithelial-mesenchymal interaction. Proc. Natl. Acad. Sci. U.S.A. 92:3968–3972.

Morgan, B. E., N. I. Passmore, and B. C. Fabian. 1989. Metamorphosis in the frog *Arthroleptella lightfooti* (Anura, Ranidae) with emphasis on neuroendocrine mechanisms. *In* M. N. Bruton (ed.), *Alternative life-history styles of animals,* pp. 347–370. Kluwer Academic Publishers, Dordrecht.

Moriceau-Hay, D., J. Doerr-Schott, and M. P. Dubois. 1982. Immunohistochemical demonstration of TSH-, LH-, and ACTH-cells in the hypophysis of tadpoles of *Xenopus laevis* D. Cell Tissue Res. 225:57–64.

Moury, J. D. and J. Hanken. 1995. Early cranial neural crest migration in the direct-developing frog, *Eleutherodactylus coqui.* Acta Anat. 153:243–253.

Nieuwkoop, P. D. and J. Faber. (eds.) 1967. Normal table of *Xenopus laevis* (Daudin): a systematical and chronological survey of the development from the fertilized egg till the end of metamorphosis, 2nd ed. North-Holland Publ. Co., Amsterdam. Paperback reprint. Garland Publishing, Inc., New York, 1994.

Noden, D. M. 1983*a*. The embryonic origins of avian cephalic and cervical muscles and associated connective tissues. Amer. J. Anat. 168:257–276.

Noden, D. M. 1983*b*. The role of the neural crest in patterning of avian cranial skeletal, connective, and muscle tissues. Devel. Biol. 96:144–165.

Olsson, L. and J. Hanken. 1996. Cranial neural-crest migration and chondrogenic fate in the Oriental fire-bellied toad, *Bombina orientalis*: Defining the ancestral pattern of head development in anuran amphibians. J. Morphol. 229:105–120.

Panganiban, G., A. Sebring, L. Nagy, and S. Carroll. 1995. The development of crustacean limbs and the evolution of arthropods. Science 270:1363–1366.

Qiu, M., A. Bulfone, S. Martinez, J. J. Meneses, K. Shimamura, R. A. Pedersen, and J. L. R. Rubenstein. 1995. Null mutation of *Dlx-2* results in abnormal morphogenesis of proximal first and second branchial arch derivatives and abnormal differentiation in the forebrain. Genes & Devel. 9:2523–2538.

Robinson, G. W. and K. A. Mahon. 1994. Differential and overlapping expression domains of *Dlx-2* and *Dlx-3* suggests distinct roles for Distal-less homeobox genes in craniofacial development. Mech. Devel. 48:199–215.

Rose, C. S. 1995. Skeletal morphogenesis in the urodele skull: II. Effect of developmental stage in thyroid hormone-induced remodeling. J. Morphol. 223:149–166.

Rose, C. S. 1996. An endocrine-based model for developmental and morphogenetic diversification in metamorphic and paedomorphic urodeles. J. Zool., Lond. 239:253–284.

Rose, C. S. and J. O. Reiss. 1993. Metamorphosis and the vertebrate skull: Ontogenetic patterns and developmental mechanisms. *In* J. Hanken and B. K. Hall (eds.), *The Skull*: *Vol. 1—Development,* pp. 289–346. University of Chicago Press, Chicago.

Sadaghiani, B. and J. R. Vielkind. 1990. Distribution and migration pathways of HNK-1– immunoreactive neural crest cells in teleost fish embryos. Development 110:197–209.

Schlosser, G. 1995. Comparative studies on the development of the peripheral nervous system in frogs. Ph.D. thesis, University of Bremen.

Shaffer, H. B. and S. R. Voss. 1996. Phylogenetic and mechanistic analysis of a developmentally integrated character complex: Alternative life history

modes in ambystomatid salamanders. Amer. Zool. 36:24–35.

Shi, Y.-B. 1994. Molecular biology of amphibian metamorphosis. Trends Endocrinol. Metab. 5:14–20.

Tata, J. R. 1993. Gene expression during metamorphosis: An ideal model for post-embryonic development. BioEssays 15:239–248.

Townsend, D. S. and M. M. Stewart. 1985. Direct development in *Eleutherodactylus coqui* (Anura: Leptodactylidae): A staging table. Copeia 1985: 423–436.

Townsend, D. S. and M. M. Stewart. 1986. The effect of temperature on direct development in a terrestrial-breeding, neotropical frog. Copeia 1986:520–523.

Wake, D. B. and J. Hanken. 1996. Direct development in the lungless salamanders: What are the consequences for developmental biology, evolution and phylogenesis? Internat. J. Devel. Biol. 40:859–869.

Wake, D. B. and S. B. Marks. 1993. Development and evolution of plethodontid salamanders: A review of prior studies and a prospectus for future research. Herpetologica 49:194–203.

Wake, M. H. 1989. Phylogenesis of direct development and viviparity in vertebrates. *In* D. B. Wake and G. Roth (eds.), *Complex organismal functions: Integration and evolution in vertebrates,* pp. 235–250. John Wiley & Sons, Ltd., Chichester.

Weber, G. M., E. S. Farrar, C. K. Tom, and E. G. Grau. 1994. Changes in whole-body thyroxine and triiodothyronine concentrations and total content during early development and metamorphosis of the toad *Bufo marinus*. Gen. Comp. Endocrinol. 94:62–71.

Yoshizato, K. 1989. Biochemistry and cell biology of amphibian metamorphosis with a special emphasis on the mechanism of removal of larval organs. Internat. Rev. Cytol. 119:97–149.

Zhao, G. Q., S. Zhao, X. Zhou, H. Eberspaecher, M. Solursh, and B. DeCrombrugghe. 1994. *RD1x,* a novel distal-less-like homeoprotein is expressed in developing cartilages and discrete neuronal tissues. Devel. Biol. 164:37–51.