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Early Cranial Neural Crest Migration in the Direct-Developing Frog, *Eleutherodactylus coqui*

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Neural crest
Head
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Abstract
Direct development is a common reproductive mode in living amphibians characterized by absence of the free-living, aquatic larval stage. In *Eleutherodactylus,* a species-rich genus of New World frogs, evolution of direct development from the ancestral biphasic ontogeny is correlated with a comprehensive modification in embryonic cranial patterning, including the loss of many larval-specific components and the precocious formation of many adult (postmetamorphic) structures. We use scanning electron microscopy (SEM) to examine the emergence and early migration of cranial neural crest cells in *Eleutherodactylus coqui* to begin to assess the possible role of the neural crest in mediating these evolutionary changes. As in metamorphosing frogs, cranial crest cells emerge prior to neural fold closure and assemble into three streams: rostral, rostral otic, and caudal otic. These streams contribute to the face and first visceral (mandibular) arch, to the second (hyoid) arch, and to posterior (branchial) arches, respectively. Rostrocaudal position, morphology, and/or migration patterns distinguish subpopulations of cells within the rostral stream and caudal otic stream. With the possible exception of the small size of the rostral otic and caudal otic streams, evolution of direct development in *E. coqui* has not altered basic patterns of neural crest emergence or early migration as assessed by SEM. If observed evolutionary changes in embryonic cranial patterning are mediated by the neural crest, then they likely involve later aspects of crest migration or more subtle features related to pattern formation such as cell behavior and commitment, or gene expression.

Introduction

The neural crest is a paradigm for studying a variety of basic processes in developmental biology. It is also of interest to evolutionary biologists because of its prominent role in the origin and diversification of a number of key vertebrate features, especially in the head. Much of our knowledge of neural crest biology comes from studies using amphibians [many reviewed by Le Douarin, 1982; Hall and Hörstadius, 1988]. Most of these studies utilize species such as *Xenopus laevis* and *Ambystoma mexicanum* in which embryogenesis culminates in the hatching of a free-living larva; few consider species that display any of the alternative reproductive modes that have evolved from the primi-

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tive biphasic life history [Duellman, 1989]. One such alternative mode is direct development: breeding is terrestrial, the free-living larval stage is absent, and embryogenesis culminates in the birth of a fully formed – albeit juvenile – amphibian [Wake, 1989]. Direct development is characteristic of hundreds of living species; in anurans alone it has evolved independently at least 10 times [Duellman and Trueb, 1986].

In many amphibian lineages, the evolutionary shift from indirect to direct development has had numerous effects on embryogenesis, including pervasive changes in cranial patterning [Elinson, 1987a, b, 1990; Elinson et al., 1990; Wake and Hanken, 1996]. In *Eleutherodactylus,* a genus of more than 450 species of direct-developing frogs [Hedges, 1989], many larval-specific cranial cartilages never form, while other cartilages initially assume an abbreviated, mid-metamorphic configuration that is remodeled to the adult form by the time of hatching [Lynn, 1942; Hanken et al., 1992]. Similar modifications affect the jaw and visceral-arch musculature, brain and cranial nerves, gills, and oral integument [Lynn, 1942; Hughes, 1959; Elinson, 1990; Schlosser, 1995]. The central role of the neural crest in both the derivation and embryonic patterning of many cranial tissues [Noden, 1988, 1991a, b; Langille and Hall, 1993] suggests that changes in its development underlie, at least in part, these evolutionary events [Hanken and Thorogood, 1993]. Yet even basic aspects of neural crest biology, which are needed to begin to assess the crest's role in mediating cranial evolution in these vertebrates, remain virtually unknown in any direct-developing amphibian.

Direct-developing species also provide an excellent opportunity to determine the extent of neural crest contribution to many adult cranial features, such as the osteocranium and cranial musculature, whose embryonic origin in amphibians and other anamniotes in general remains poorly documented [Hall, 1980; Hall and Hörstadius, 1988]. Because adult features typically form during embryogenesis in direct-developers, rather than at metamorphosis (i.e. weeks or months after hatching) as in many biphasic species, direct-developers are in this respect more amenable to the use of many labelling methods for evaluating cell lineage and embryonic origin [Krotoski et al., 1988; Collazo et al., 1993]. Here again, however, such experimental studies require information regarding the basic patterns and timing of neural crest emergence and migration, which is largely unavailable for these taxa.

In this study, we use scanning electron microscopy (SEM) to assess the timing and pattern of cranial neural crest cell emergence and early migration in the Puerto Rican frog, *Eleutherodactylus coqui*. We have two specific aims. First, to test the hypothesis that differences in embryonic cranial patterning between direct-developing and metamorphosing amphibians are correlated with differences in basic aspects of cranial neural crest emergence and migration, such as the timing of cell migration and the relative size, number, and configuration of migratory streams. Second, to derive comprehensive baseline data that are prerequisite to planned experimental analyses of neural crest biology in these frogs, including studies of cell lineage and potency, adult derivatives, and pattern formation.

Materials and Methods

Acquisition and Maintenance of Embryos

Embryos of *E. coqui* were obtained from spontaneous matings among wild-caught adults maintained as a laboratory breeding colony at the University of Colorado following standard procedures [Elinson et al., 1990]. Animal collection and care were in accordance with the regulations of the Puerto Rican Department of Natural Resources and the University of Colorado, Boulder, Colo., USA. Fertilization is internal; egg clutches were removed from the brooding male within 24 h of deposition and incubated at 22–24 °C in Petri dishes on toweling moistened with 10% Holtfreter's solution [Jacobson, 1967]. Embryos were staged according to the table of Townsend and Stewart [1985], which defines a total of 15 stages from oviposition to hatching. These stages, especially early ones, are extremely broad in comparison to those described for metamorphosing amphibians [Elinson et al., 1990]. Most of neurulation, for example, occurs within a single stage (stage 3) which lasts approximately 1 day at 23 °C. We therefore used the degree of neural fold closure to define three subdivisions of stage 3: stage 3.1 (first sign of neural folds around the thickened neural plate), stage 3.2 (distinct neural folds rolling towards midline, but open neural plate still visible), and stage 3.3 (neural folds meet at midline, but epithelial fusion has not occurred). These stages correspond to stages 14, 15, and 16 of Gosner's [1960] table for anurans having an aquatic larval stage.

Embryonic jelly layers were removed prior to fixation by using a chemical solution (0.63 g cysteine HCl, 0.12 g NaCl, 24 ml water, adjusted to pH 7.9–8.1 using 5 *N* NaOH) and watchmaker's forceps. Dejellied embryos that were not used immediately were maintained in 10% Holtfreter's solution at 22–24 °C [Elinson, 1987a].

Early embryos (through stage 4) of *E. coqui* are large, yolky, and easily damaged during removal of the vitelline membrane. These embryos were partially fixed after removing the jelly but before attempting to demembranate them. Fixation solidifies the yolk mass, making it less likely to rupture during manipulations. Vitelline membranes of older embryos were removed prior to fixation.

Scanning Electron Microscopy

Whole mounts, dissections, and fractures of more than 200 embryos were examined; at least 8 embryos were examined from each stage or substage described below. Embryos were fixed in half-strength Karnovsky's fixative [Karnovsky, 1965] in 0.1 *M* cacodylate buffer (pH 7.4) for 1–1.5 h. Following secondary fixation for 1 h in 1% OsO_4 in cacodylate buffer, they were dehydrated rapidly through an ethanol series, critical-point-dried using liquid CO_2 as the exchange

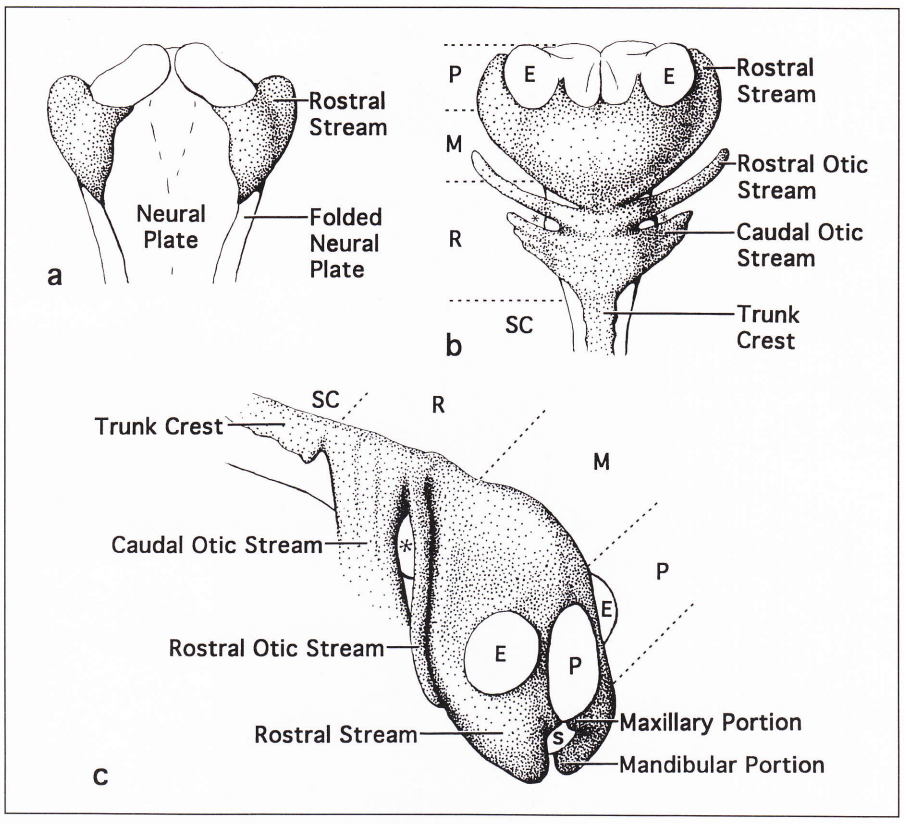


Fig. 1. Basic migratory patterns of cranial neural crest cells (stipple) drawn with the epidermis removed. **a** Stage 3.2. **b** Early stage 4. **c** Late stage 4. Each image is a composite based on several embryos. Dorsal views (**a**, **b**): anterior at top; anterolateral view (**c**): anterior at right. P = Prosencephalon; M = mesencephalon; R = rhombencephalon; SC = spinal cord; E = optic vesicle; S = stomodeal endoderm; * = position of the otic placode/vesicle.

medium, mounted on stubs, sputter-coated with gold, and viewed on an ISI SX-30 scanning electron microscope at 30 kV.

To see individual neural crest cells, in some specimens the overlying epidermis was removed with watchmaker's forceps after primary or secondary fixation. In others, the epidermis was removed with tape or rubber cement after critical-point drying [Tosney, 1978]. Other tissues (e.g. somites, mesenchyme, crest cell masses) were removed in similar ways. Additional specimens were cross-fractured during processing to reveal tissue morphology.

Histological and Anatomical Terminology

When examining neural crest cells, we assumed that individual cells were moving in the same direction as the cell mass (e.g. away from the neural folds/tube). Thus, we consider the lateral edge of a crest cell to be its leading edge and the medial edge to be its trailing edge.

Chan and Tam [1988] report that the 'mesencephalic' crest of the mouse consists of closely packed cells (as opposed to the more loosely organized mesodermal mesenchyme). However, they and some other investigators [Jacobson and Tam, 1982; Meier and Packard, 1984; Meier and Tam, 1982] consider the pre-otic sulcus to be the boundary between the mesencephalon and the rhombencephalon. We follow some other investigators [Tan and Morriss-Kay, 1985, 1986; Nichols, 1987] who consider the pre-otic sulcus to lie completely within the rhombencephalon. Therefore, Chan and Tam's 'mesencephalic' crest includes our 'hindbrain crest rostral to the pre-otic sulcus'.

Results

In *E. coqui,* emerging neural crest cells are first seen alongside the mesencephalon (stage 3.2), and later alongside the hindbrain and spinal cord (stage 3.3). Migration begins as soon as the cells emerge and continues through stages 4 and 5. During migration, cranial neural crest cells group into three major streams: rostral, rostral otic, and caudal otic (fig. 1a–c).

Stage 3.1

Cranial neural crest cells have not yet emerged. Epithelium at the boundary between the thick neural plate and thin epidermis begins to elevate as paired neural folds (fig. 2a); it is stratified into superficial and deep cell layers. The epidermis (at least near the neural folds) consists of a thin, superficial layer of squamous-to-low-cuboidal cells, and a slightly thicker, deep layer of cuboidal cells. In the neural plate, the superficial layer is also composed of squamous-to-low-cuboidal cells, but the deep layer is composed of high columnar cells (fig. 2b). Cell processes on the basal surfaces of the deep cells resemble filopodia, and there are numerous spherical, membrane-covered protrusions (fig. 2c).

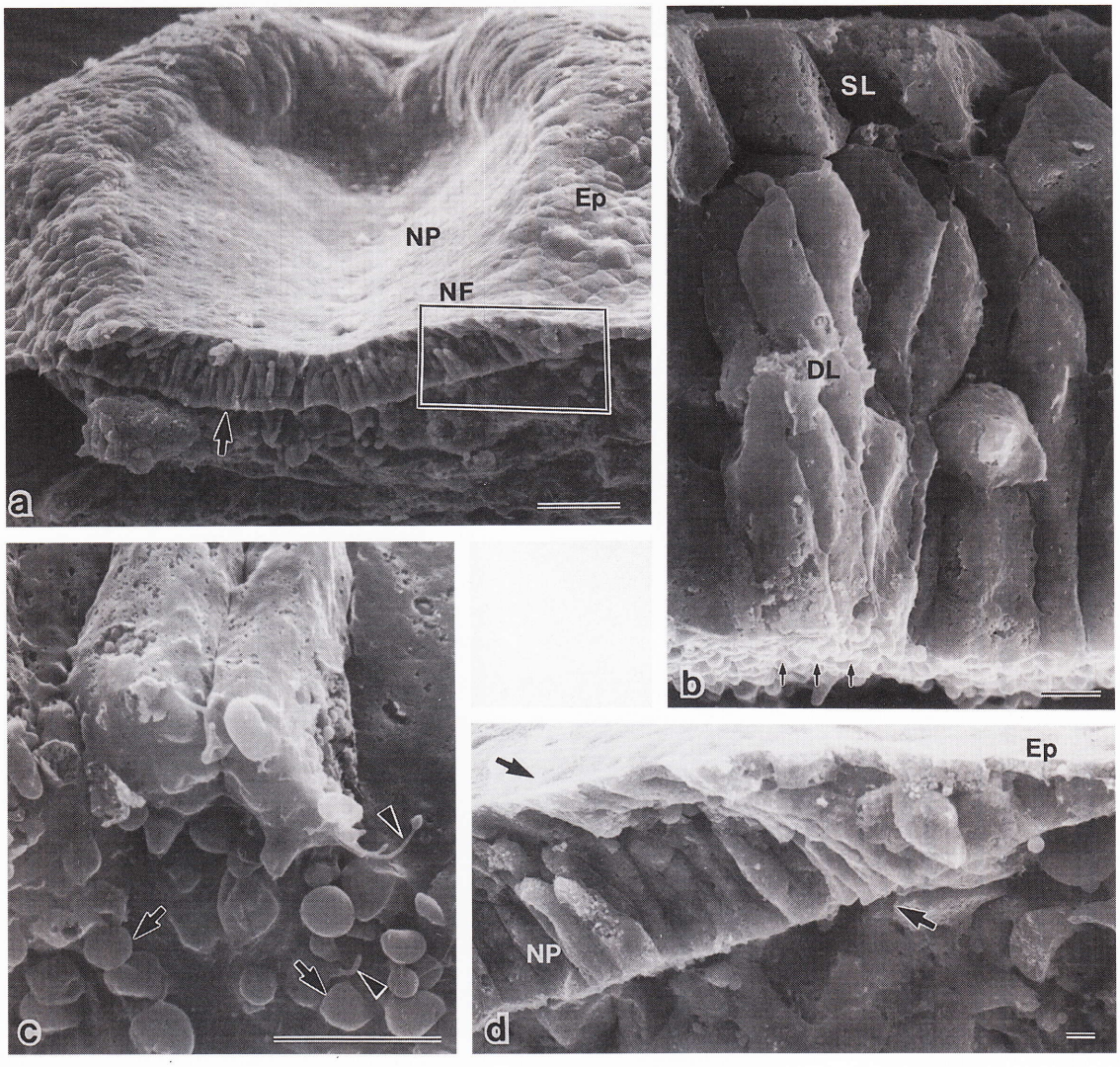


Fig. 2. Cross-fractures through the neural plate (NP), epidermis (Ep), and neural folds (NF) of early neurulae (stage 3.1), before neural crest migration. **a** Dorsal surface of the anterior neural plate and surrounding tissues. The fractured edge of a portion of the neural plate (arrow) from another specimen and the neural fold (box) are enlarged in **b** and **d**, respectively. **b** The neural plate consists of superficial (SL) and deep (DL) layers. Basal surfaces of deep cells (arrows) in another specimen are enlarged in **c**. **c** Basal cell surfaces in the deep layer show elongate processes (arrowheads) and round protrusions (arrows). **d** Epithelial cells are elongated on both sides of the presumptive neural plate-epidermis boundary (arrows). Bars: **a** = 100 µm; **b**–**d** = 10 µm. Dorsal at top.

Spherical protrusions also occur on cells of other tissues (including neural crest) but are more numerous on the basal surface of the neural plate; they remain visible throughout neurulation. In cross-fractures, the boundary between epidermis and neural plate appears as a region of long, wedge-shaped cells (fig. 2d).

Stage 3.2

The rostral neural crest stream is first apparent at the level of the presumptive mesencephalon as the neural folds become more prominent (fig. 1a). The cells begin to migrate ventrolaterally between the neural plate and the epidermis. Additional crest cells at the level of the caudal prosencephalon appear to join the mesencephalic mass that is migrating alongside the neural fold.

Stage 3.3

The large rostral stream continues to migrate rostroventrally as the neural folds approach the dorsal midline (fig. 3a); it extends along the line where the neural plate,

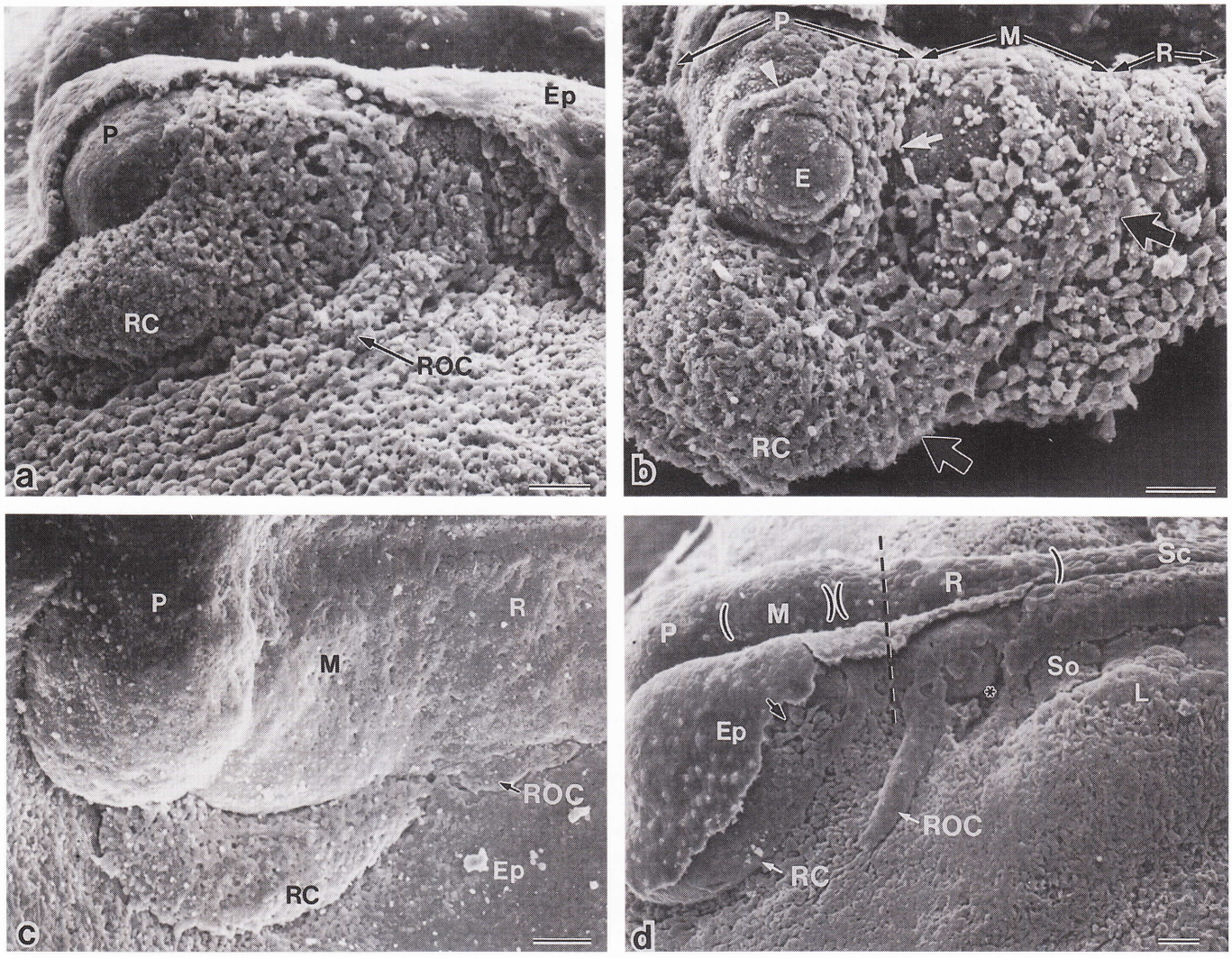


Fig. 3. Rostral (RC) and rostral otic crest streams (ROC) at stage 3.3, seen with the overlying epidermis (Ep) removed. These streams migrate over (or adjacent to) portions of the neural plate that will become the prosencephalon (P), mesencephalon (M), rhombencephalon (R), and spinal cord (SC). **a** Both streams are composed of stellate, loosely organized cells. Lateral view; dorsal at top, anterior at left. **b** Most anterior mesencephalic cells migrate in the cleft between the prosencephalon and mesencephalon (white arrow), but some (arrowhead) migrate medial to the optic vesicle (E). Cells opposite the anterior rhombencephalon form the posterior mass (lateral and caudal edges) of the rostral stream (black arrows). Lateral view, anterior at left. **c** The tip of the rostral otic stream lies caudal to the rostral stream. Ventral view. **d** As the neural folds close, cells in the caudal portion of the rostral stream and in the rostral otic stream become more tightly packed. A few migrating cells remain loosely associated (arrow). Most of the caudal otic stream was removed from this preparation, but its impression (*) remains anterior to the first somite (So) and forelimb bud (L). The dashed line denotes the pre-otic sulcus. Bars = 100 μm.

mesoderm and epidermis meet. As the primary divisions of the brain become distinct, the base of this stream is now seen to lie opposite the mesencephalon and anterior rhombencephalon. It comprises two cell masses with distinct migratory pathways. Cells opposite the mesencephalon form an anterior mass (along the cranial and medial edges of the stream) that primarily migrates ventrally in the cleft between the prosencephalon and the mesencephalon (fig. 3b). The optic vesicle (which now protrudes from the prosencephalon) separates a small, medial group of these cells from the larger, lateral portion. These cells migrate rostrally over the prosencephalon (medial to the optic vesi-

cle) into the facial region. Later (stage 4), cells from the anterior mass will encircle the developing optic stalk. Cells opposite the anterior rhombencephalon form the second, posterior mass of the rostral stream (along its caudal and lateral edges), which migrates primarily between the mesencephalon and rhombencephalon (fig. 3b). A thin layer of cells covers the mesencephalon between the two masses.

The rostral otic stream first appears at this stage, opposite the rhombencephalon and posterior to the rostral stream (fig. 3a). As with the rostral stream, the rostral otic stream initially migrates ventrolaterally between the neural plate and the epidermis, and its tip reaches the basal area of contact between these two tissues (fig. 3c). Unlike the rostral stream, the rostral otic stream does not follow the line where neural plate, mesoderm, and epidermis meet; rather, it migrates laterally and rostrally away from the neural tube between the paraxial mesoderm and epidermis (fig. 3d). The rostral stream and rostral otic stream lie on opposite sides of the pre-otic sulcus, a slight constriction that develops within the neural folds of the presumptive rhombencephalon (fig. 3d).

Late in stage 3.3, just before cranial neural folds fuse, cells in the caudal portion of the rostral stream (posterior mass) and in the rostral otic stream become more tightly packed (fig. 3d). Cells in the rostral otic stream form a thick, compact rod. A thin covering of loose cells (likely crest, but possibly mesoderm) remains near the midline, becoming thinner as development proceeds.

The third and most posterior of the cranial crest streams also becomes visible late in stage 3.3, after the two anterior streams. The caudal otic stream appears opposite the posterior rhombencephalon as a tongue of cells that lies caudal to the rostral otic stream and cranial to the first somite and forelimb bud (fig. 4).

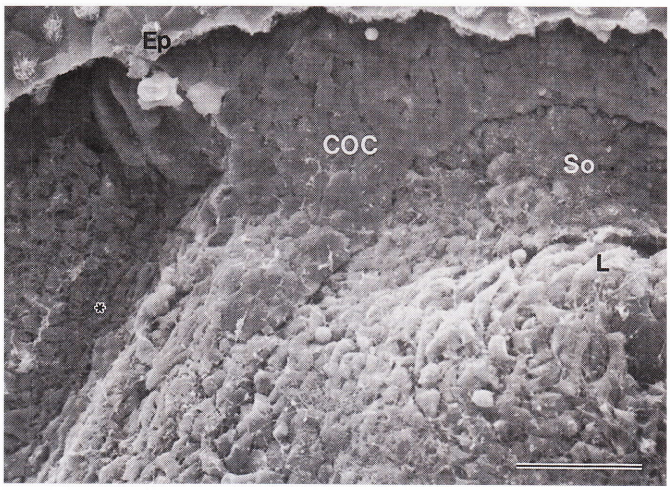


Fig. 4. Caudal otic crest stream (COC) at stage 3.3, seen with the epidermis (Ep) partially removed. Rostral otic crest was also removed from this preparation, leaving a depression (*). Lateral view, anterior at left. So = First somite; L = forelimb bud. Bar = 100 μm.

Stage 4

Midline fusion of the neural folds marks the beginning of stage 4 (early limb and tail buds). A wedge-shaped mass of loose cells – likely neural crest – that lies on the dorsal surface of the neural tube still separates the two sides (fig. 5a). Cranial neural crest streams and the otic placodes are prominent (fig. 1b). All three streams displace the overling epidermis and can be seen superficially as they continue to migrate, more or less rostrolaterally (fig. 5b).

Neural crest is present all along the dorsal midline of the rhombencephalon and is continuous with both mesencephalic crest anteriorly and trunk crest posteriorly. In the otic area, the superficial epidermal layer is wrinkled (fig. 5b), while the deep epidermal layer has thickened to form the otic placode (fig. 5c, d). When the rhombencephalic crest enters this area, it separates into rostral and caudal portions. The rostral otic stream is a single, long, thin mass of cells that migrates cranial to the otic placode; the caudal otic crest is all the cranial crest that lies caudal to the otic placode. The caudal otic stream forms a broad, thin 'sheet' that comprises at least two parallel cell masses (fig. 5d); its migratory path parallels that of the rostral otic stream.

Later in stage 4, the facial and branchial regions of the head begin to take shape. The dorsal body axis rises above surrounding tissues and elongates rostrocaudally, creating the tail bud and causing the head to extend beyond the sphere of yolk and to flex ventrally. This elevation and elongation exposes the ventral portions of the head. Similar movements form the stomodeal plate by bringing the foregut into contact with the epidermis ventral to the prosencephalon. Mesenchyme (probably a combination of neural crest and head mesoderm) now covers much of the neural tube, and visceral clefts begin to form as indentations in the epidermis between adjacent crest streams (fig. 6a, b). Neural crest from the rostral stream completely surrounds the developing eye (fig. 6a).

Near the rostral midline, rostral crest streams from either side begin to encircle the stomodeum. Most of each stream spreads around the lateral and caudal/ventral borders of the stomodeum, while a small mass of cells branches away and begins to move toward the midline between the prosencephalon and the stomodeum (fig. 6b). The main mass and the smaller branch of each rostral stream will become the

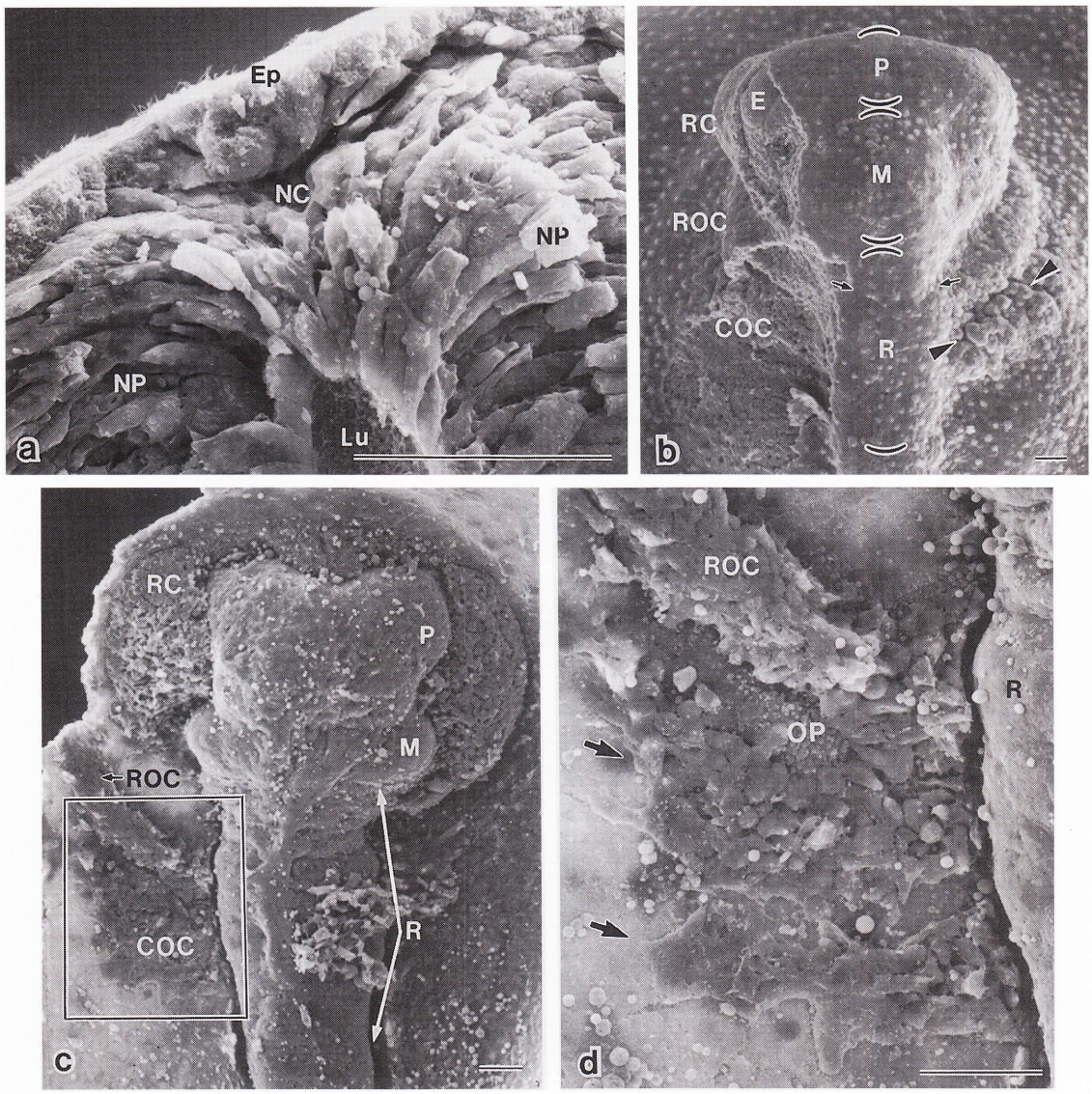
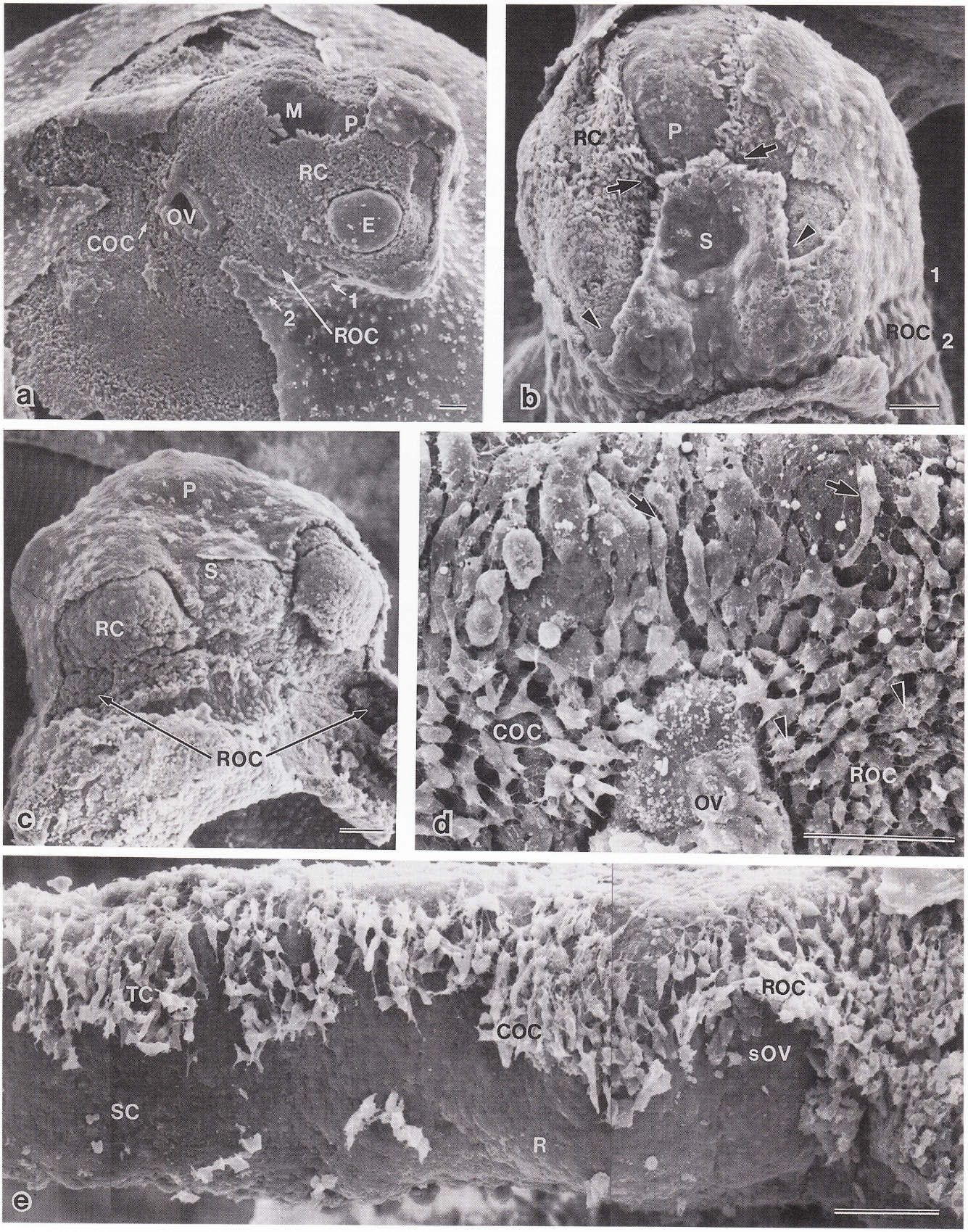


Fig. 5. a Cross-fracture through the rhombencephalon, early stage 4. Neural crest (NC) forms a wedge in the dorsal part of the neural tube that extends to the lumen (Lu). Many of these cells resemble adjacent neural plate cells (NP), but some may be derived from the epidermis (Ep). **b** Superficial view. The pre-otic sulcus (arrows) separates the rostral (RC) and rostral otic (ROC) streams, and the convoluted epidermis above the otic placode (arrowheads) separates the rostral otic and caudal otic (COC) streams. Epidermis has been removed on the left side; anterior at top. **c** Epithelium and attached neural crest seen in ventral view. The rostral stream moves beneath the elevating head. Rhombencephalic crest (box) is enlarged in **d**. **d** Neural crest streams adjacent to the rhombencephalon migrate laterally (left). The rod-like rostral otic stream is separated from the caudal otic stream (arrows) by the developing otic placode (OP). E = Optic vesicle; M = mesencephalon; P = prosencephalon; R = rhombencephalon. Bars = 100 µm.

mandibular and maxillary portions, respectively, of the first visceral (mandibular) arch (fig. 1c).

The rostral otic stream approaches the ventral midline of the embryo in the area of the second (hyoid) arch, just caudal to the rostral stream, but remains connected to the rhombencephalon by a thin line of cells (fig. 6c). The large otic vesicle, now partially covered by mesenchymal cells, still separates the rostral otic and caudal otic streams (fig. 6a, d, e).

Neural crest cells that contribute to head mesenchyme resemble mesenchymal cells of mesodermal origin. Even so, the rostral and rostral otic streams remain distinct – es-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pecially near the midline – due to their tightly packed cells and the developing visceral clefts that form between them. The caudal otic stream is less obvious because many of its cells merge with the adjacent mesenchyme.

Stage 5

Limb buds and tail bud, which first became visible during stage 4, elongate during stage 5. A few cranial neural crest cells continue to emerge and migrate after fusion of the neural folds. By this time, the larger crest masses have moved ventrally and mesenchyme covers most of the head. This mesenchyme obscures the underlying neural tube and fills the space between the otic vesicles and the epidermis; only the rim of the optic cup remains exposed. The endodermally derived pharyngeal pouches fuse with the visceral clefts, thereby separating adjacent visceral arches.

Discussion

Embryonic cranial patterning in direct-developing *Eleutherodactylus* is dramatically different from that characteristic of metamorphosing anurans (see 'Introduction'). Are these differences in patterning, as well as in other aspects of early development, correlated with differences in basic aspects of neural crest emergence and early migration? A prominent feature of the evolution of direct development, for example, is change in the relative timing of developmental events, or heterochrony [Elinson, 1990; Hanken, 1992; Wake and Hanken, 1996]. Have these changes, which in *Eleutherodactylus* include a broad range of tissues and organs, affected the timing of neural crest migration? Are losses of larval-specific cranial cartilages accompanied by change in the relative sizes or basic migratory pathways of cranial crest streams? The extremely limited database regarding neural crest biology in most amphibian species precludes detailed interspecific comparisons. Comprehensive descriptions of emergence and migratory pathways of cranial crest using SEM are available for only two metamorphosing species, the African clawed frog, *Xenopus laevis* [Sadaghiani and Thiébaud, 1987] and the California newt, *Taricha torosa* [Jacobson and Meier, 1984], and our account is the only one for a direct-developing amphibian. Here we offer preliminary comparisons, both to metamorphosing amphibians and to other vertebrates.

Emergence of Neural Crest Cells

Neural crest emergence and early migration in metamorphosing anurans are distinctive in at least two respects. First, in contrast to the simple or pseudostratified neuroepithelium seen in many vertebrates [Erickson and Weston, 1983; Hirano and Shirai, 1984; Nichols, 1986, 1987; Chan and Tam, 1988], the neuroepithelium in metamorphosing anurans is stratified into superficial and deep layers. Either layer might contribute cells to the neural crest. In *Xenopus,* the superficial layer gives rise to the ependymal layer of the neural tube; the deep layer contributes to the neural crest and forms most of the remainder of the neural tube [Nieuwkoop and Florschütz, 1950; Keller, 1975, 1976; Sadaghiani and Thiébaud, 1987]. The second distinctive feature concerns the timing of neural crest migration. In metamorphosing anurans, including species as distantly related as *Xenopus* [Sadaghiani and Thiébaud, 1987] and *Rana* [Stone, 1929], crest migration is well underway before neural fold closure. This is much earlier than migration in newts [Jacobson and Meier, 1984] and chickens [Tosney, 1978; Le Douarin, 1982], which begins after fold closure. 'Precocious' crest migration is, however, also typical of some rodents [Tan and Morriss-Kay, 1985, 1986; Nichols, 1986, 1987; Chan and Tam, 1988]. A third characteristic, if not distinguishing, feature of metamorphosing anurans is the formation of 'lateral masses' of presumptive neural crest cells within the cranial neuroepithelium at the onset of crest emergence [Sadaghiani and Thiébaud, 1987].

Both distinctive features of neural crest emergence and early migration in metamorphosing anurans are also characteristic of *Eleutherodactylus:* the neuroepithelium is stratified into superficial and deep layers (fig. 2a, b, d), and neural crest emergence and migration begin well before neural fold closure (fig. 1a, 3). Lateral masses of neural crest sim-

Fig. 6. Cranial neural crest migration at late stage 4, seen with the epidermis partially or totally removed. **a** Mesenchyme covers much of the head, but cells are sparse in the dorsal part of the neural tube. Absence of cells atop the caudal prosencephalon (P) and mesencephalon (M) is a preparation artifact. Lateral view; anterior to right. **b** Some cells of the rostral stream (RC) move into the maxillary region (arrows) between the prosencephalon and the stomodeum (S). Other cells migrate into the mandibular region (arrowheads) lateral and ventral to the stomodeum. Frontal view; dorsal at top. **c** The rodlike rostral otic stream (ROC) migrates toward the ventral midline. Its cells contribute to the second visceral (hyoid) arch. Ventral-frontal view; anterior at top. **d** The otic vesicle (OV) separates rostral otic and caudal otic (COC) streams. Spindle-shaped, migrating neural crest cells (arrows) become stellate as they join the dense head mesenchyme (arrowheads). Lateral view; anterior to right. **e** Removal of overlying mesenchyme and somites from the rhombencephalon and anterior trunk reveals neural crest cells migrating on the neural tube. Site of the otic vesicle (removed) is visible as a spherical depression (sOV) separating migrating crest streams. Lateral view; anterior to right. E = Optic vesicle; 1, 2 = visceral clefts 1 and 2. Bars = 100 μm.

ilar to those seen in *Xenopus* are also visible on either side of the cranial neural folds (fig. 3a–d, 5b–d), although in *E. coqui* these masses appear to be composed of migrating rather than presumptive neural crest cells that have not yet emerged from the neuroepithelium. Because morphology alone is insufficient to determine where a particular cell originated, detailed labelling studies are needed in *Eleutherodactylus* to determine if the fates of the various epithelial cells are the same as in *Xenopus* and other metamorphosing species. Similar labelling studies will be required to determine which mid- and hindbrain regions contribute to the three cranial neural crest migratory streams in *E. coqui* as well as other amphibians [cf. Bradley et al., 1993], and if this feature has been affected by the evolution of direct development.

Pattern of Cranial Neural Crest Migration

Cranial neural crest migration in *E. coqui* begins shortly after the crest cells begin to emerge, when they coalesce into three major streams (fig. 1a–c). The rostral stream develops first and is the largest of the cranial streams; the rostral otic stream develops next and is the smallest; the caudal otic stream develops last and is of intermediate size. In intact embryos, these streams appear as a series of paired bulges in the dorsal surface of the head; in dissected embryos, they appear as cell streams separated by obvious crest-free zones (fig. 5b). Because the surface morphology of neural crest cells from all three streams is so similar (as seen with SEM), we cannot determine unequivocally the contribution of each stream to different regions of the head. Nevertheless, our results are consistent with the rostral stream contributing cells to the facial region and to the first visceral (mandibular) arch; the rostral otic stream contributing cells to the second (hyoid) arch; and the caudal otic stream contributing cells to the remaining (branchial) arches. In all these basic features, neural crest migration in *Eleutherodactylus* closely resembles that reported for a wide variety of other vertebrates, including metamorphosing frogs and salamanders, turtles, chicks, rats and mice [Anderson and Meier, 1981; Tosney, 1982; Jacobson and Meier, 1984; Meier and Packard, 1984; Tan and Morriss-Kay, 1985, 1986; Sadaghiani and Thiébaud, 1987; Serbedzija et al., 1992; Langille and Hall, 1993; Sechrist et al., 1993].

The only conspicuous feature of early cranial neural crest migration that differs between *Eleutherodactylus* and a metamorphosing anuran, and thus appears to correlate with reproductive mode, is the relative sizes of the three migratory streams. In *E. coqui,* the rostral stream is substantially broader (along the rostrocaudal axis) than either the rostral otic or caudal otic streams; the rostral otic stream is especially narrow (fig. 1b, 3a, c, 5b, d). In *Xenopus,* the only other anuran for which there are comparable (SEM) representations of the cranial migratory streams, the three streams are approximately the same width [Sadaghiani and Thiébaud, 1987] (fig. 3c). Given that rostral otic and caudal otic crest is the source of the skeletogenic cells that form the prominent, posterior hyobranchial skeleton in larval anurans [Hall and Hörstadius, 1988], and that this skeleton is a highly reduced and transient embryonic structure in *Eleutherodactylus* [Lynn, 1942; Hanken et al., 1992], the apparent reduction in the relative size of these two streams might represent a consequence of the evolution of direct development. This interpretation, however, must be regarded as tentative at best pending the examination of other metamorphosing and direct-developing taxa to assess the extent to which (1) patterns of cranial neural crest migration in *Xenopus* are typical of metamorphosing frogs, and (2) differences in the relative size of the three migratory streams correlate with reproductive mode. In fact, recent studies of cranial neural crest migration in a phylogenetically diverse array of other metamorphosing anurans indicate that the pattern in *Eleutherodactylus* is typical of most frogs, regardless of life history, whereas that in *Xenopus* is exceptional [Olsson and Hanken, in prep.].

Neural Crest and Direct Development

Evolution of direct development in *Eleutherodactylus* has not affected the basic features of cranial neural crest emergence and early migration, which instead are highly conserved. This conservatism of early development likely reflects the prominent role of the neural crest in the formation of initial features of cranial organization and anatomy that are characteristic of all vertebrates, regardless of reproductive mode, and the fact that interspecific differences in adult anatomy generally are not manifest until later stages. If modifications to the neural crest do underlie the prominent changes in embryonic cranial patterning that are observed in *Eleutherodactylus,* then these modifications likely involve either later aspects of crest migration or more subtle features related to pattern formation such as cell behavior and commitment, or gene expression. Experimental studies designed to test this hypothesis are currently underway.

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