

Neural crest cell migration and pigment pattern formation in urodele amphibians

HANS-HENNING EPPERLEIN^{1*}, JAN LÖFBERG² and LENNART OLSSON^{2,3}

¹Anatomisches Institut, Technische Universität Dresden, Dresden, Germany, ²Department of Environmental and Developmental Biology, Uppsala University, Uppsala, Sweden and ³Environmental, Population and Organismic Biology, University of Colorado, Boulder, USA

ABSTRACT This review deals with research on the development and differentiation of the neural crest (NC) in amphibians carried out during the past twenty years. First, earlier studies on the migration and differentiation of NC cells *in vitro* are summarized. These studies include the modes of NC cell migration and their differentiation into chondroblasts, perichondral cells, neurons, Schwann cells and pigment cells (melanophores and xanthophores). Then a summary is given on the development of cranial sensory ganglia and enteric ganglia in *Xenopus laevis*. In the subsequent sections, mechanisms of NC cell migration are investigated in *Ambystoma mexicanum*, the Mexican axolotl (wild-type and white mutant) using ultrastructural, immunohistochemical and biochemical methods. In wild-type or dark axolotl embryos, pigment cells leave the NC and migrate out under the epidermis, whereas in the white mutant, pigment cells remain closely confined to the original position of the NC. This system provides an excellent model for analyzing NC cell migration in vertebrate embryos. Further sections deal with the development of larval pigment patterns in *Triturus alpestris*, (horizontal melanophore stripes) and *Ambystoma mexicanum* (vertical melanophore bars). Comparing the formation of these patterns shows that two different principles exist in the distribution of pigment derivatives of the NC: patterns following environmental cues (*Triturus*) and those ignoring these cues, relying solely on cell-cell interactions (*Ambystoma*). Other studies relate to evolutionary perspectives in pigment pattern formation. They are based on phylogenetic analyses of North American ambystomatids, combined with data on pigment patterns and their formation where such data are available. These studies have shown that vertical bars which develop from aggregates in the NC string are an evolutionary innovation, compared to the more primitive horizontal stripes lacking aggregates in the NC. Thus, in this review we show that the NC of amphibians (*T. alpestris*, *Xenopus laevis*, dark and white axolotls and other ambystomatids) may be used for various analyses concerning the migration and differentiation of its derivatives, as well as for studies on the formation and evolution of pigment patterns.

KEY WORDS: *amphibians, neural crest, migration, differentiation, pigment pattern*

Introduction

The scientific interests of the first author have been directed towards the development of the neural crest (NC) in vertebrates, research being concentrated on investigations into the migration, differentiation and localization of NC cells in amphibian embryos (*Triturus alpestris*, *Ambystoma mexicanum* and *Xenopus laevis*). Work on this has been carried out since 1972; initially in Germany and later in collaboration with colleagues and friends abroad. For this reason, it is not really possible to confine a report on this work solely to scientific activities in Germany. Of the contacts with foreign groups, the closest has been that with J. Löfberg and coworkers in Uppsala (Sweden), which began in 1981 and is still continuing. Projects of common interest included studies on NC cell migration in wild-type (dark) and white

mutant embryos of *Ambystoma mexicanum* (the axolotl) as well as pigment pattern development in *Triturus alpestris* and dark axolotls. Since pigment cells are the most conspicuous derivatives of the NC and externally visible, the divergent patterns in *Triturus* and the dark axolotls could function as models for studying the various ways in which NC cells are distributed during amphibian morphogenesis.

The typical feature of the neural crest is its production of a wide diversity of phenotypes including pigment cells, neurons and glia of the peripheral nervous system, endocrine cells and mesenchymal derivatives of the skull. From their origin on the neural tube, NC cells migrate along various defined routes

Abbreviations used in this paper: NC, neural crest; ENS, enteric nervous system; BM, basement membrane; ECM, extracellular matrix.

*Address for reprints: Anatomisches Institut, Technische Universität Dresden, Fetscherstr. 74, D-01307 Dresden, Germany. FAX: 351.4417429. e-mail: h.h.epp@vcs.urz.tu-dresden.de

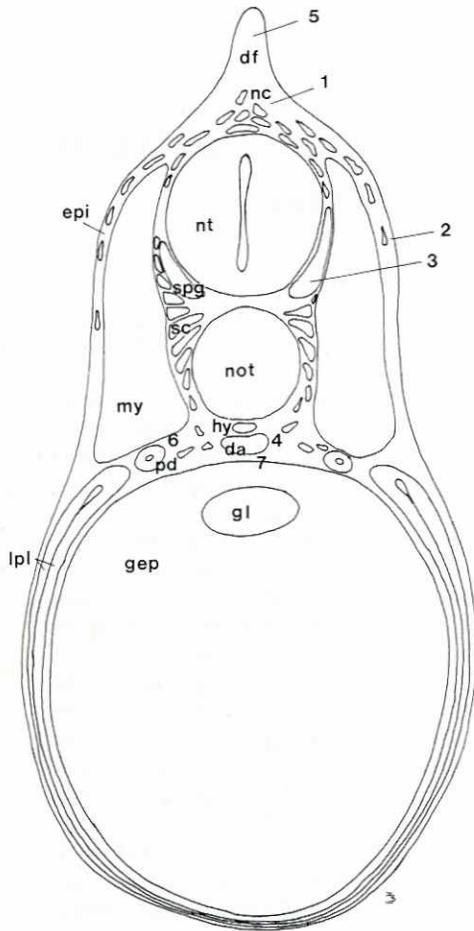


Fig. 1. Distribution of NC derivatives in a transverse section through the midtrunk of an axolotl larva (about stage 40). Data are based on previous findings (Vogel and Model, 1977; Epperlein and Löfberg, 1990, 1993) and labeling with the fluorescent probe PKH 26 (see Figs. 2-7). Numbers 1-7 indicate the first appearance of NC cells at a particular site. Temporal correlations are made to these sites by setting (1) the premigratory NC, 92.5 h at 20°C, stage 28, to 0. Then the particular sites have the following times: (2) dermal pigment cells in the subepidermal position of the dorsolateral flank, stages 33-35; 20.5-29.5 h, (3) site of dorsal root ganglia, stage 34, 22.5 h, and (4) site of sympathetic ganglia, stage 35, 29.5 h, on the ventromedial route; (5) mesenchyme forming the dorsal fin, stages 33-35, 20.5-29.5 h, (6) pigment cells and enteric neurons/glia at the peritoneal lining, stage not determined, about stage 40, 9.3 mm, 140 h. (7) Pigment cells and enteric neurons/glia at the mesenteries, stage not determined, about stage 40, 9.3 mm, 140 h. NC, neural crest; df, dorsal fin; nt, neural tube; not, notochord; hy, hypochord; da, dorsal aorta; gl, gut lumen; gep, gut epithelium; sc, sclerotome; my, myotomes; pd, pronephric duct; lpl, lateral plate; epi, epidermis; mesenteries are not indicated.

through the embryo to give rise to the different cell types before, during or after migration. Basic questions arise concerning the mechanisms of determination, pathway selection and final localization of NC cells. An important aim of NC research would be to identify the molecules in the embryonic environment and in the NC cells themselves which regulate their differentiation and distribution. As the crest is essential for the life of vertebrates including humans, not only their normal, but also their abnormal

distribution is of interest, since this may, for example, have severe consequences for the development of the face, heart or enteric nervous system (for review see Hall and Hörstadius, 1988; Peters-v.d. Sanden, 1994).

The research of the first author consists of four major topics: (I) migration and differentiation of urodele NC cells *in vitro*, (II) development of cranial sensory ganglia and enteric ganglia in *Xenopus*, (III) influence of the extracellular matrix (ECM) on migration and differentiation of NC cells and (IV) development of the larval pigment patterns in the urodeles *Triturus alpestris* and *Ambystoma mexicanum*. While topics III and IV will be given a more detailed treatment under different headings in the sections following the introduction, I and II are briefly mentioned here.

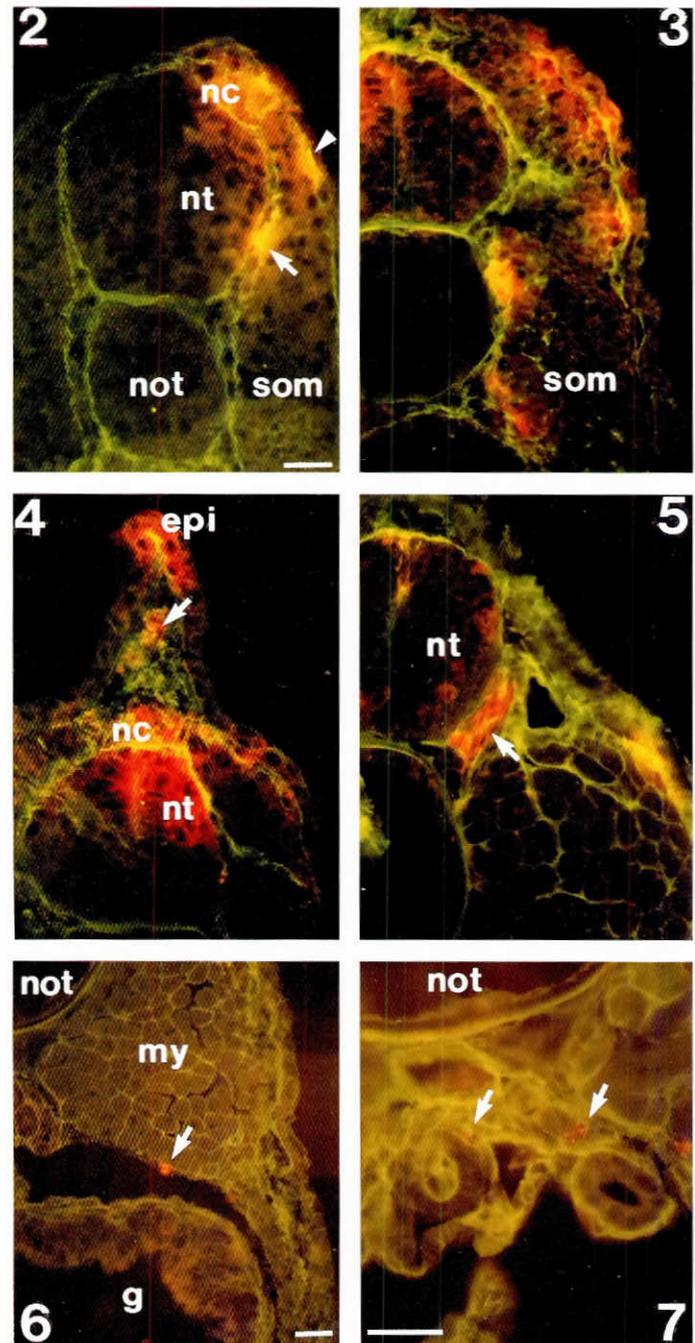
In topic I, the course of migration and differentiation of NC cells has been followed *in vitro* (Epperlein, 1974, 1978; Epperlein and Lehmann, 1975). Head neural fold explants were cultured either alone or at a little distance from a piece of pharyngeal endoderm. The latter is known to stimulate the development of cartilage (Okada, 1955; Holtfreter, 1968; Drews *et al.*, 1972). Principal questions referred to (a) the mode of NC cell migration (random/directional), (b) conditions of cartilage formation from NC cells (contact/diffusion of "inductive" molecules, (c) linkage of DNA content and developmental bias of NC cells and (d) discrimination between NC-derived neuro- and melanoblasts or adrenergic and cholinergic cellular traits. Results showed that migration of NC cells from pieces of neural fold tissue was random whether or not an explant of pharyngeal endoderm was present, thus following contact inhibition of cell movement (Abercrombie, 1961). Among the differentiated cell types observed were chondroblasts, perichondral cells, neurons, Schwann cells and pigment cells (melanophores and xanthophores). Prospective chondroblasts had a 4c DNA content or were in S-phase, which might be due to their marginal (not contact-inhibited) position close to the pharyngeal endoderm. Cartilage only developed if an explant of pharyngeal endoderm was in the culture and if NC cells had contact with it. Thus, long-range diffusion of inducing molecules seemed to be a less likely transmission mechanism for differentiation. The neurons differentiating in the culture system were catecholaminergic, but acetylcholine esterase-negative. They could be distinguished from melanophores by failing to incorporate ³H-dopa. Melanophores developed only randomly within the NC cell outgrowth but were associated more frequently with epidermal cell sheets. Schwann cells became associated with brain derived axons. The molecular mechanisms operating during these interactions of NC cells with other tissues are still unclear.

In topic II, the development and localization of cranial sensory ganglia and enteric ganglia in *Xenopus* were studied using the *Xenopus laevis/borealis* marking system (Thiébaud, 1983). The interesting aspect of developing sensory ganglia is their questionable origin from the crest (Corning, 1899), from the inner, sensory layer (placodes) of the epidermis (Sobotta, 1935; Chibon, 1967) or from both (v. Kupfer, 1894; Stone, 1922; Knouff, 1927; Yntema, 1937, 1943; Starck, 1963; Sadaghiani and Thiébaud, 1987). Since NC and placodal material is, to a great extent, continuous in *Xenopus*, discrimination and choice of the right tissue for exchange experiments is difficult (Hausen and Riebesell, 1991; Epperlein and Löfberg, 1993). Therefore, the true composition of cranial sensory ganglia in *Xenopus* can-

not be elucidated unless an unambiguous marker for NC or epidermis exists. Using, however, the interspecific grafting technique (Sadaghiani and Thiébaud, 1981; Griebhaber and Epperlein, unpubl.) or a polyclonal antibody against an epidermal protein (Cornish *et al.*, 1992), preliminary results were obtained. These imply that the ganglia of cranial nerves V, VII, IX and X are of NC/placodal origin, whereas those of I and VIII are derived from placodes only. Open questions arise concerning the regulation of the final size, proportion of the different cellular populations and definite localization of these ganglia. Research into the role of the embryonic environment acting via cytokines and growth factors and into the role of the migrating cells themselves is necessary. The same applies to studies on the enteric nervous system (ENS) in *Xenopus*. Although NC cells migrate into the gut of *Xenopus* embryos and occupy positions homologous to the submucosal and myenteric plexuses of higher vertebrates (Epperlein *et al.*, 1990), the mechanisms regulating migration, pathway selection and final localization/aggregation of enteric neurons remain largely obscure. Possibly, as in the case of sensory and sympathetic ganglia, cell-cell and cell-matrix/growth factor interactions are involved during development of the ENS, the molecular nature of which, however, has not yet been determined.

Neural crest cell migration and mechanisms of migration

The NC develops from the apical portions of the neural folds which fuse into the neural tube. In the trunk of urodele embryos, the NC is arranged on the dorsal surface of the neural tube as a flat epithelium (*Triturus*, Fig. 8a) or as a multilayered cellular string (*Ambystoma mexicanum*, Fig. 9a). In the head region of both species, NC cells form a flat epithelium. The cellular structure and extension of the premigratory NC can best be observed in SEM specimens from which the dorsal epidermis has been removed. Soon after forming a continuous structure, NC cells begin to spread laterally in an antero-posterior wave on the surface of the neural tube. In the head, the NC layer becomes divided into dorsolateral tongues of mandibular, hyoid and branchial NC which give rise to connective and supportive tissue of the skull (Hörstadius and Sellman, 1946; Löfberg *et al.*, 1989a). In the trunk, NC cells reaching the apex of the somites begin to disperse into two directions: between the somites and the epider-



Figs. 2-7. First evidence of NC cell migration and distribution in the trunk of axolotl embryos employing a fluorescent probe. Transverse cryosection, labeling of NC cells and NC derivatives with the fluorescent probe PKH 26 (Sigma, München; Horan and Slezak, 1989). At the neurula stage, portions of the left trunk neural fold (prospective midtrunk position) consisting of prospective epidermis, neural fold and neural crest tissue were excised and stained for 25-30 min in the fluorescent dye PKH 26 (final concentration 1×10^{-5} M in Steinberg solution; Steinberg, 1957). After washing in Steinberg solution, the explants were implanted isotopically into an unlabeled host of the same age. Embryos were allowed to develop and sectioned a few days up to several weeks after the operation. The fluorescent probe is insoluble in an aqueous environment and stable following incorporation into the plasma membrane. Its advantage compared to Dil (if applied from outside) is the specific staining. Cryosections through the regions containing the label were counterstained with anti-fibronectin (rabbit anti-human plasma fibronectin, BRL, Basel, Switzerland). **(2)** Initial migration of NC cells, stage 35. Labeled are the premigratory NC (shifted to the side) and NC

cells after dispersion into the dorsolateral (arrowhead) and ventromedial pathway (arrow). **(3)** Labeled NC cells in several places on the dorsolateral and ventromedial route of migration; stage 38. **(4)** Fluorescent cells in the neural tube, NC and epidermis; these tissues derive from the labeled neural fold explant. In addition, NC derived cells in the dorsal fin mesenchyme are also labeled (arrow); stage 38. **(5)** Labeled NC cells in the position of a spinal ganglion (arrow); stage 39/40. **(6 and 7)** At a later stage (15 mm larva, about three weeks), fluorescence is very weak, probably due to many cell divisions and fading of the dye. Labeled cells are found ventrolaterally, at the peritoneum (Fig. 6, arrow) and in the sympathetic region (Fig. 7, arrows). Nt, neural tube; not, notochord; nc, neural crest; epi, epidermis; som, somites; my, myotomes; g, gut. Bars: (2-7) 50 μ m; (2-5, bars of same length).

mis (dorsolateral route) or between somites and neural tube/notochord (ventromedial route). In amphibians, but not in higher vertebrates, a third migratory route of NC cells exists dorsally, into the growing dorsal fin.

In the trunk, the differentiative potential of NC cells varies with respect to the route of migration. Dorsolaterally, pigment cells (melanophores, xanthophores) occur, whereas ventromedially neuronal and glial precursor cells of the peripheral nervous system are found. Dorsally, the trunk NC gives rise to the mesenchyme of the fin, the derivative of mesenchyme from the NC being otherwise typical for the head region. It has been a challenge for more than 60 years, to find evidence for the migration and localization of NC derivatives in the embryo. In the past, nuclear size (Raven, 1931), vital dyes (Detwiler, 1937), radioactive thymidine (Chibon, 1967; Vogel and Model, 1977) or chromatin differences (Thiébaud, 1983) have been used as markers in grafting experiments. Today, retroviral infection (Frank and Sanes, 1991) or fluorescent tracer injection (Bronner-Fraser and Fraser, 1988) are applied in addition to immunohistochemical techniques (HNK-1, Vincent *et al.*, 1983; MEBL-1, Kitamura *et al.*, 1992). In this review, Figure 1 summarizes the migratory routes and differentiative products of the NC. About seven different arbitrary stations of NC cell distribution may be observed (Fig. 1): (1) premigratory NC (stage 28), (2) dermal pigment cells in the subepidermal position of the dorsolateral flank (stages 33-35), (3) site of dorsal root ganglia (stage 34) and (4) site of sympathetic ganglia (stage 35) on the ventromedial route of NC cell migration, (5) mesenchymal cells forming the dorsal fin (stages 34-35), (6) pigment cells and possibly enteric neurons/glia in the peritoneal lining (stage not determined) and (7) pigment cells and possibly enteric neurons/glia in the mesentery (stage not determined). Staging (according to Bordzilovskaya and Dettlaff, 1989) indicates the first appearance of NC cells at a particular site in a position on a midtrunk cross section. Based on labeling with PKH-26, a route of NC cells through the somites could not be discovered.

The mechanisms of NC cell migration in vertebrates may conveniently be investigated in the system of dark (wild-type) and white mutant axolotl embryos. In the dark embryo, pigment cells become distributed in the entire flank, whereas in the white, they remain restricted close to the original position of the NC. The reason for the absence of pigmentation in the flank of the white embryo is an inhibition of the subepidermal migration of pigment cells, not an inhibition of the pigmentation of migrating cells. This, at least, refers to embryonic and larval stages. Two lines of evidence support the conclusion that migration of NC cells is inhibited in the white embryo. (1) In culture, NC cells from neural fold explants of dark or white embryos differentiate in the same way into melanophores and xanthophores (Dalton, 1950b; Epperlein, unpublished). (2) In white embryos, isotopic grafts of dorsolateral fragments of epidermis from the trunk of dark embryos can elicit local migration of pigment cells (DuShane, 1935; Löfberg *et al.*, 1985; Epperlein, 1988). The same effect could be achieved solely with microcarriers (consisting of Nuclepore filter material) containing ECM from the dorsolateral trunk of dark embryos (Löfberg *et al.*, 1989a). From these results it was concluded that the subepidermal ECM of the white embryo has a defect with respect to stimulation of NC cell migration. Searching for structural and biochemical differences responsible for such a defect has so far been only partially suc-

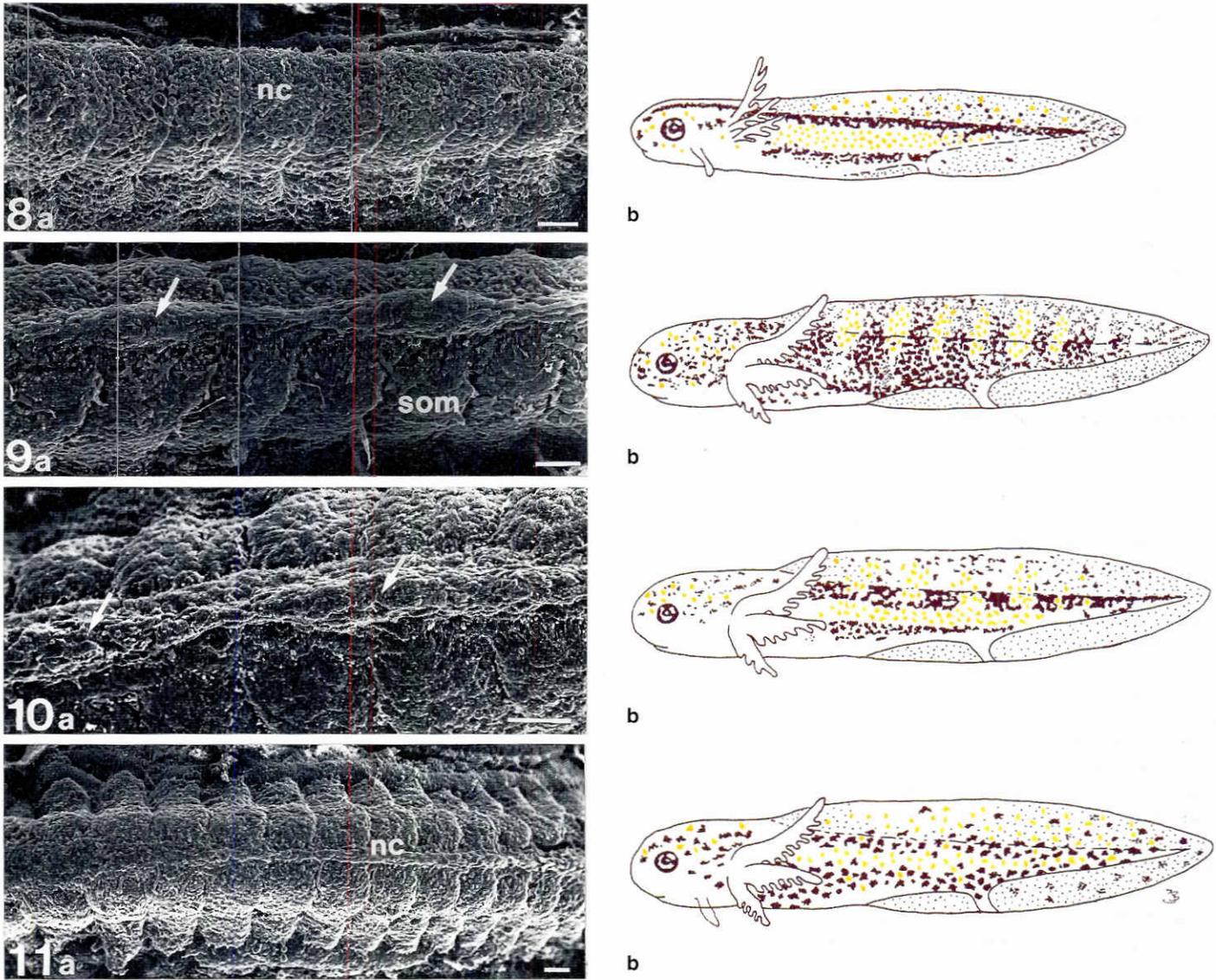
cessful. Possible candidates may be found among the glycosaminoglycans (GAGs), proteoglycans and glycoproteins of the ECM. From SEM findings on the structure of the subepidermal ECM it appeared that in dark embryos there are more electron-dense granules precipitated along collagen fibrils than in white animals (Perris *et al.*, 1990). These granules may represent proteoglycans with GAGs/proteins: Immunostaining for different ECM components (stages 30-35) known to influence morphogenetic processes in other embryonic systems, i.e. collagen*, fibronectin (FN), laminin*, tenascin(TN), chondroitin sulfate proteoglycan (CSPG) and keratan sulfate proteoglycan (*data from Perris *et al.*, 1990; Epperlein and Löfberg, 1993) has revealed a difference mainly for collagen III, which is more dominant in the subepidermal ECM of white embryos. Distribution of FN and TN and their splicing variants was not different in dark and white embryos (Halfter and Epperlein, unpublished). The isolation of pure subepidermal BMs from the dorsolateral trunk epidermis, which has been achieved with Triton and nitrocellulose filters (Epperlein *et al.*, in prep.), allowed the collection of sufficient matrix for biochemical analysis (as compared to matrix previously adsorbed and collected with microcarriers; Perris *et al.*, 1990). In Western blots of one-dimensional gels, BM preparations of dark and white embryos (stages 30, 35, 40) showed various differences in their protein moieties (Epperlein and Löfberg, 1993). The molecular identity of these different proteins, however, has not been analyzed.

In summary, the evaluation of the structural, immunohistochemical and biochemical differences between the ECM of dark and white embryos showed that the matrix of dark embryos may contain additional ECM components which render the subepidermal ECM permissive for NC cell migration. The presence of collagen III, observed immunohistochemically in the white embryo, might exert an inhibiting effect on NC cell migration *in vivo*, since some collagens were not found to support cell migration *in vitro* (Perris and Johansson, 1990). The biochemical data reveal differences in the protein composition of BMs, which, however, have to be further analyzed before a certain role can be ascribed to any particular protein.

Pigment cells and pigment pattern formation in *Triturus alpestris* and *Ambystoma mexicanum* larvae

In amphibian embryos, two other types of pigment cells occur in addition to the black melanophores: yellow xanthophores and silvery iridophores (Bagnara, 1978). The different pigment cells of amphibians may form various pigment patterns during development. In adults, the patterns are more stable, but even then, rapid color changes may occur, which are caused by hormonal or nervous inputs and are brought about by changing the position of pigment organelles within the cells.

The origin of pigment cells from the NC was established by DuShane (1934). Their exact position within the NC lineage is uncertain. According to a model for higher vertebrates (Weston, 1991), the mesenchymal derivatives are the first to become segregated from the cranial neural folds, then the trunk neural folds give rise to neuron precursors (sensory and autonomic ganglia) and non-neurogenic derivatives (glial and pigment cells). Although the distribution of melanophores seems to be well regulated during early stages of development (fixed number of cells, definite site in the subepidermal position), later on, the



Figs. 8-11. Structure of the neural crest and corresponding larval pigment patterns. *Combination of morphogenetic and evolutionary data. Scanning electron micrographs of the premigratory trunk neural crest in embryos of Triturus alpestris (8a), Ambystoma mexicanum (9a), Ambystoma t. tigrinum (10a) and Ambystoma maculatum (11a), from which the dorsal epidermis has been removed. Anterior is to the left in all figures. (8b, 9b, 10b and 11b) Corresponding larvae with their pigment patterns consisting of black melanophores and yellow xanthophores. Aggregate formation in the trunk NC is an evolutionary innovation that leads to the formation of vertical bars. The bars represent a derived state compared to the more primitive horizontal stripes. NC, neural crest; NC aggregates, arrow; som, somites. Bars, 100 μ m.*

melanophores are also found in various other extracutaneous locations, as, for example, on the ventromedial route of NC cell migration, in spinal ganglia, in the peritoneum or in the gut. Thus the imprecision of pigment cell distribution observed later contradicts the seemingly exact localization of these cells at early stages. The development and distribution of xanthophores and iridophores are similarly unsolved, as are the determination and distribution of melanophores. According to a concept of Bagnara (1987), the different pigment cell types develop from NC stem cells with primordial pigment organelles. However, it is unclear when and to what extent the different types of chromatophores are segregated from the NC lineage.

The development of pigment patterns in urodeles had been studied from an evolutionary point of view long before it was introduced by Twitty (1936) in connection with experimental embryology. At the turn of the century, v. Linden (1900) investigated whether the biogenetic law (Haeckel) is valid also for the development of pigment patterns in animals. Studying pigmentation in different species of *Triturus* (from hatching stages to juveniles), she confirmed the results of Eimer (1881) in *Lacerta* according to which longitudinal patterns represent the lowest onto- and phylogenetic level, followed by rows of spots, transverse stripes and uniformity. v. Linden's studies are mentioned here only for historical reasons, since they have little bearing on

the pigment pattern research initiated by Twitty. Her studies ignore (certainly unintentionally) the early embryonic stages, where pigment cells are first uniformly distributed before they become arranged into stripes.

Studies on pigment pattern development in urodeles based on an embryological background were first carried out by Twitty (1936, 1945, 1966) and Twitty and Bodenstern (1939). The species investigated were mainly *Taricha torosa* (larvae with a prominent dorsal and a weak lateral longitudinal melanophore stripe) and *Taricha rivularis* (larvae with a uniform distribution of melanophores). Of main interest was the limited distribution of melanophores in the embryo, i.e. only in the dorsolateral but not in the ventral regions of the trunk, and their arrangement into defined uniform or banded patterns. Which were the embryonic determinants that control a particular pattern and to what extent is it established by the melanophores themselves? Twitty's exchange experiments of trunk neural fold material between *rivularis* and *torosa* neurulae seemed to furnish clear results: in the host a donor-type pigment pattern always developed. This could only be explained by a cell-intrinsic mechanism of pattern formation. However, as *rivularis* melanophores remain scattered irrespective of the embryonic environment, which, as suggested later (Twitty, 1945), was due to their "inherently lower rate and final level of differentiation", an interaction between melanophores and embryonic tissues cannot be tested in Twitty's system. Later Rosin (1943) used embryos of different urodele species whose melanophores could fully differentiate and reaggregate. After exchanging neural folds, epidermis and musculature between these species, mainly host-type patterns developed. Rosin explained this result as an ability of the melanophores to respond to influences of the embryonic environment. Thus, the explanatory emphasis shifted from intrinsic to extrinsic control of pigment pattern formation. Later, the experiments of Dalton in particular (1950a, 1953) on pigment pattern formation in dark and white axolotl embryos, showed that it is the environment, i.e. the epidermis in his experiments, which stimulates pigment cell migration. Dalton's experiments were refined later and confirmed by other authors (Keller et al., 1982; Löfberg et al., 1985, 1989a,b; Epperlein et al., 1986; Perris and Löfberg, 1986; Epperlein, 1988).

The arrangement of melanophores (and xanthophores) in alternating horizontal stripes, as in *Triturus alpestris* (Fig. 8b), and vertical bars, as in *Ambystoma mexicanum* (Fig. 9b), was studied more recently by Epperlein and Löfberg (1990). The principal problem investigated was how the different patterns develop although the NC seemed to be invariant in both species. Do the differences exist in the NC itself, in the mode of NC cell migration, in the interaction between melanophores and xanthophores or between pigment cells and environmental tissues? Some major results on investigations into the development of the two different pigment patterns are summarized as follows.

In *Triturus*, melanophores and xanthophores are mixed in the premigratory crest, which shows a homogeneous distribution (Fig. 8a). From stage 28 onwards (early tailbud; staging according to Epperlein and Junginger, 1982), they start to migrate laterally and become distributed over the flank. Then melanophores start to accumulate at the dorsal and lateral

somite margins, where they aggregate into compact bands or stripes in hatching larvae (stage 34). Xanthophores withdraw from the sites of melanophore stripes and recede into the interband area or the dorsal fin. Stripe formation of melanophores seems to be induced by specific cues arising in the subepidermal ECM along the trunk by stages 31/32 (Epperlein and Löfberg, 1990). Following the results of embryonic transplantations, SEM and immunohistochemical findings, pigment pattern formation in *Triturus* can be explained as follows. Melanophore distribution changes from uniform to banded, because the appearance of TN and CSPG in the interband region (probably of mesodermal origin) prevents their adhesion to a substrate still rich in FN. Melanophores thus recede to the more adhesive dorsal and lateral areas with unchanged distribution of FN (these are the cues for stripe formation). In the dorsal fin, TN and CSPG prevent the invasion of melanophores. Ventral to the lateral stripe, melanophores are absent because the substrate is unsuitable for migration. Conversely, where melanophores occur, xanthophores are excluded. Xanthophores, however, may persist in the interband area and dorsal fin, i.e. in regions containing TN and CSPG. A main implication for this "exclusion hypothesis" is that melanophores and xanthophores possess different cell surface receptors for different components of the ECM.

In dark (wild-type) embryos of *A. mexicanum*, the melanophores migrate out first and become scattered over the entire dorsolateral trunk (stage 35; staging according to Bordzilovskaya and Dettlaff, 1989). At that time, several mixed chromatophore groups are recognizable along the premigratory NC (see Fig. 9a). They constitute a prepattern of the barred or transverse band pigment pattern of the axolotl larva (Fig. 9b). The formation of the mixed groups seems to be caused by xanthophores adhering more tightly to each other and to the neural tube than do melanophores. During further development, xanthophores spread more and more radially, while melanophores recede towards adjacent regions populated by those melanophores which dispersed earlier from the NC. As a consequence of this separation or sorting out of melanophores and xanthophores, alternating transverse bands of melanophores and xanthophores are formed.

If both patterns are compared, the decisive difference is that, in *T. alpestris*, randomly scattered chromatophores follow different environmental cues when sorting out into the longitudinal zones, whereas in the axolotl alternating transverse melanophore and xanthophore bands arise without environmental influences but through cell-cell interactions. As transplantation of NC material between *Triturus* and *Ambystoma* has shown, *Triturus* melanophores can form broad longitudinal stripes in the axolotl. So the stripe forming cues seem not to be lost in the axolotl, but axolotl melanophores are unable to respond to these cues in their own embryo.

In conclusion, morphogenetic analysis of pigment pattern development in *Triturus* and *Ambystoma* demonstrates divergent ways of interaction between NC derivatives and the embryonic environment. If more about the different types of pigment cell and their surface receptors and more about the external influences (ECM, growth factors) were known, perhaps a molecular explanation of these cell-cell and cell-environmental interactions could finally be given.

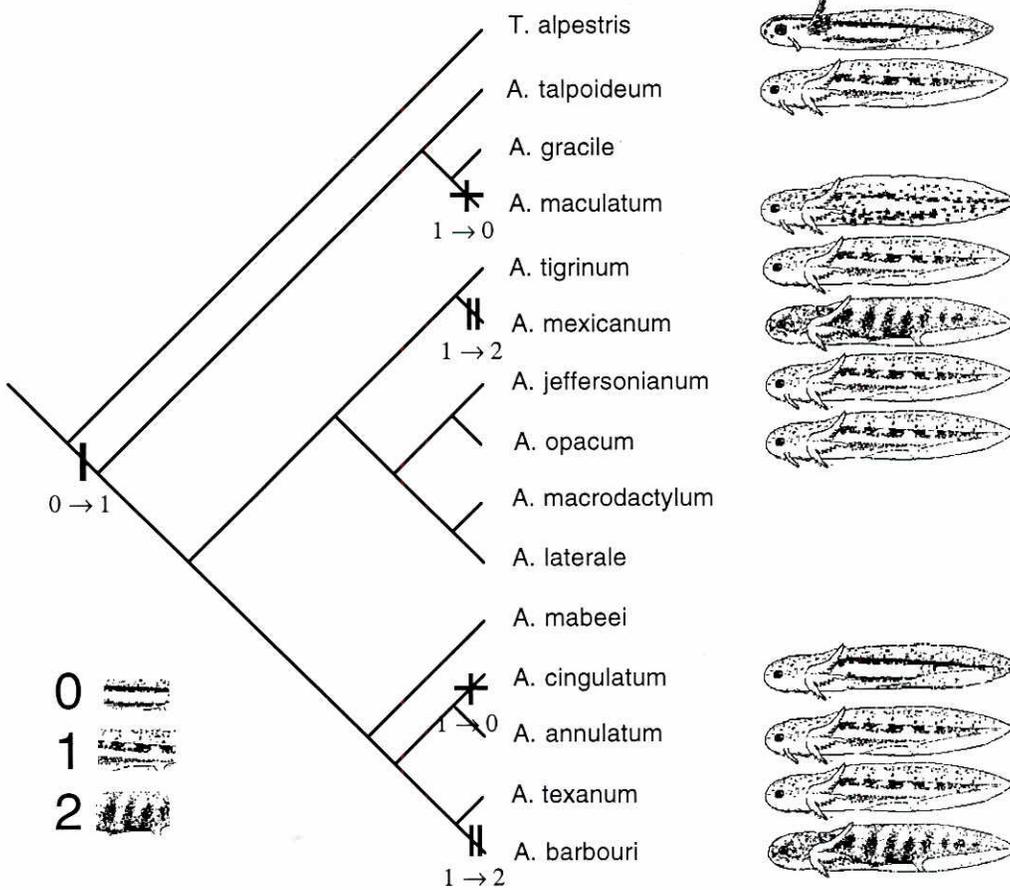


Fig. 12. Salamander phylogeny with the pigment pattern of those larvae in which it is known. Character states are: 0= horizontal stripes; 1= vertical bars and horizontal stripes; 2= vertical bars without horizontal stripes. If the evolution of pigment patterns and their mechanism of formation is traced, the result is that the aggregate formation has evolved only once (0→1) and been lost twice (1→0), in *A. maculatum* and *A. cingulatum*. Two transitions to the axolotl type of pattern (vertical bars without horizontal stripes) are hypothesized to have taken place (1→2), in *A. mexicanum* and *A. barbouri* respectively. An equally parsimonious alternative exists, the only differences being that the primitive state (0) persists in *A. maculatum* and that the transition to aggregate formation, which gives rise to vertical bars (0→1), takes place twice.

Pigment pattern formation – an evolutionary perspective

It has been shown that different morphogenetic mechanisms are responsible for pigment pattern formation in embryos of *Triturus alpestris*, a salamandrid, and in *Ambystoma mexicanum* (the axolotl), an ambystomatid (Epperlein, 1982; Epperlein and Claviez, 1982a,b; Epperlein and Löfberg, 1984, 1990, 1993). However, the way in which these patterns are phylogenetically related and whether one pattern is primitive and the other derived, has not been previously considered. To find an answer to these questions, we used the North American ambystomatids since data for pigmentation exist for them but not for the Mexican group. *A. mexicanum*, however, is an exception, being a sister group to *A. tigrinum*, and is therefore included in the phylogeny (Fig. 12). The evolution of patterns – horizontal stripes and vertical bars - and their pattern-forming mechanisms are discussed here within the phylogeny of the group. Phylogeny and cladogram are used interchangeably. A phylogeny is the proper basis for investigating the way in which characters (such as pigment patterns) have evolved and a phylogeny for the species dealt with is shown in Figure 12. Data for this are summarized from previous work (Olsson, 1993, 1994; Olsson and Löfberg, 1992). Uniform patterns preceding the banded pigment patterns of *Triturus alpestris* and *Ambystoma mexicanum* larvae and constituting the larval pattern in *Taricha rivularis* are not included in this study. The reason is that the relations between the genus *Taricha* and other salamandrids remain largely unresolved.

***Ambystoma t. tigrinum* – horizontal stripes and vertical bars, an intermediate pattern**

The early stages of NC cell development in *Ambystoma t. tigrinum* are very similar to those in *A. mexicanum* (see above). As in the axolotl, the trunk NC forms several elevated aggregates, i.e. mixed chromatophore groups which constitute the prepattern of the definite barred pigment pattern (Fig. 10a). Melanophores migrate out from the NC in an antero-posterior wave and become evenly distributed over the flank of the embryo. Later, xanthophores leave the mixed chromatophore aggregates and move ventrolaterally under the epidermis. The melanophores, having migrated out early, start to form a dorsal and a lateral horizontal stripe. At the sites of ventral spreading of xanthophores, the dorsal melanophore stripe is interrupted. Melanophores that were previously in these sites recede to adjacent melanophore regions. Xanthophores occupy the areas between the melanophore stripes but do not interfere with the lateral melanophore stripe. In this way a pattern is formed with an intact lateral melanophore stripe and a dorsal one interrupted by xanthophore bars (Fig. 10b). The pattern may be regarded as intermediate between that in *A. mexicanum* (Fig. 9b) and *T. alpestris* (Fig. 8b). The initiation of pattern formation is identical to that in *A. mexicanum*.

***Ambystoma maculatum* – horizontal stripes in an ambystomatid**

The premigratory crest forms an even, multilayered string along the trunk neural tube (Fig. 11a) and does not display

aggregates as in other ambystomatids. This condition is similar to the structure of the NC found in *T. alpestris* (Fig. 8a), as is the manner in which melanophores and xanthophores leave the crest. Both cell types start to migrate out dorsoventrally and become evenly scattered over the flank of the embryo. In most cases, the distribution of melanophores changes from a random distribution to a pattern with wide dorsal and lateral horizontal bands (Fig. 11b). The general direction of melanophore migration is dorsoventral. The dorsal stripe is formed by melanophores which stop migrating, the lateral stripe by those cells that have migrated further laterally from the NC string. Xanthophores settle in the interstripe area but are not confined to it, becoming scattered elsewhere in the dorsolateral trunk without a distinctive pattern.

***Ambystoma talpoideum*, *A. barbouri* and *A. annulatum* – vertical bars as a basic pattern**

In addition to *A. mexicanum* and *A. tigrinum*, the development of pigment patterns has been studied in *Ambystoma talpoideum*, *A. barbouri* and *A. annulatum* (Olsson, 1994). In these three ambystomatid species, pigment pattern formation is based on the mechanism of aggregate formation as described for *A. mexicanum* and *A. tigrinum*. The definite larval pigment patterns, however, vary, particularly in *A. talpoideum* because of a very low, and in *barbouri*, a very high number of melanophores. The pattern in *A. annulatum* closely resembles that in *A. mexicanum*.

The evolution of pigment patterns and their mechanisms

In addition to our own investigations on pigment pattern formation (see above), descriptions from the literature of larvae in *A. jeffersonianum*, *A. opacum*, *A. cingulatum*, and *A. texanum* (Bishop, 194; Orton, 1942; Brandon, 1961) were used. Phylogenetic data (based on skull morphology and allozymes) on North American ambystomatids reported by Shaffer *et al.* (1991) and reanalyzed by Jones *et al.* (1993) provided the phylogeny, in which *A. mexicanum* was inserted as sister taxon to *A. tigrinum* (Shaffer, 1984). The salamandrids (e.g. *Triturus alpestris*) were placed as outgroups in accordance with recent rRNA based family-level phylogenies of salamanders (Larson, 1991; Larson and Dimmick, 1993).

The pigment patterns were coded as character states: 0= horizontal stripes, 1= vertical bars and horizontal stripes; 2= vertical bars without horizontal stripes. The character tracing resulted in the mapping presented in Figure 12. The primitive state is horizontal stripes, as determined by the outgroup. More derived are vertical bars, which have evolved only once. The pattern seen in the axolotl, which lacks horizontal stripes, has evolved twice (in *A. mexicanum* and *A. barbouri*), and is indicated by double bars in the cladogram. Two reversals have taken place, where vertical bars have been lost (in *A. maculatum* and *A. cingulatum*). This is indicated by crossed bars in the cladogram). Another equally valid interpretation would require that vertical bars had evolved twice and been lost only once. Both these scenarios are rather conservative, indicating that pattern formation mechanisms evolve slowly. The reasons may be both internal and external. Early events in development, such as the formation of aggregates in the NC, are often constrained from changing by the dynamics of the developmental system (see Maynard Smith *et al.*, 1985). Once the aggregate formation mechanism and the

vertical bars that it forms have arisen, they can also be constrained from changing by a stabilising selection mechanism.

In conclusion, the phylogenetic analysis shows that the aggregate formation mechanism giving rise to vertical bars is an evolutionary innovation that arose early in the history of ambystomatids. From these data we can see that changes in pigment patterns, and in patterning mechanisms can take place within a family. Therefore, a broad family-level analysis of the evolution of larval pigmentation would need to be undertaken in detail, and would involve many species from each family. It is therefore tempting to broaden this study in the future to include all the salamander families, a task which seems feasible because in most families only one or very few species exist.

The reasoning behind an evolutionary analysis of developmental phenomenon in pigment pattern formation is that all evolutionary morphological changes are caused by changes in ontogeny, i.e., in the developmental mechanisms that create the adult organism. A complete theory of evolution therefore needs to incorporate developmental data into a new synthesis.

Acknowledgments

HE gratefully acknowledges grants from the DFG (Ep 8/4-1,2) and TU Dresden, and JL financial support by the Swedish Natural Science Research Council (B-BU 3810-308). LO wishes to thank the Sweden-America Foundation for supporting his postdoctoral research at the University of Colorado, USA. We are grateful to Jens Burlefinger for the drawings.

References

- ABERCROMBIE, M. (1961). The bases of the locomotory behaviour of fibroblasts. *Exp. Cell Res. (Suppl.)* 8: 188-198.
- BAGNARA, J.T. (1976). Color change. In *Physiology of the Amphibia*, Vol. 3 (Ed. B. Lofts). Academic Press, New York, pp. 1-52.
- BAGNARA, J.T. (1987). The neural crest as a source of stem cells. In *Developmental and Evolutionary Aspects of the Neural Crest* (Ed. P.F.A. Maderson). Wiley, New York, pp. 57-87.
- BISHOP, S.C. (1941). The salamanders of New York. *NY State Mus. Bull.* 324: 1-365.
- BORDZILOVSKAYA, N.P., DETTLAFF, T.A., DUHON, S.T. and MALACINSKI, G.M. (1989). Developmental-stage series of axolotl embryos. In *The Developmental Biology of the Axolotl* (Eds. J.B. Armstrong and G.M. Malacinski). Oxford University Press, New York, pp. 201-219.
- BRANDON, R.A. (1961). A comparison of the larvae of five north-eastern species of *Ambystoma* (Amphibia, Caudata). *Copeia*: 377-383.
- BRONNER-FRASER, M. and FRASER, S. (1988). Cell lineage analysis reveals multipotency of some avian neural crest cells. *Nature* 335: 161-164.
- CHIBON, P. (1967). Marquage nucléaire par la thymidine tritiée des dérivés de la crête neurale chez L'amphibien urodèle *Pleurodeles waltlii* Michah. *J. Embryol. Exp. Morphol.* 18: 343-358.
- CORNING, H.K. (1899). Über einige Entwicklungsvorgänge am Kopfe der Anuren. *Morphol. Jb.* 27: 173-241.
- CORNISH, J.A., KLOC, M., DECKER, G.L., REDDY, B.A. and ETKIN, L.D. (1992). Xlcaax-1 is localized to the basolateral membrane of kidney tubule and other polarized epithelia during *Xenopus* development. *Dev. Biol.* 150: 108-120.
- DALTON, H.C. (1950a). Inhibition of chromatoblast migration as a factor in the development of genetic differences in pigmentation in white and black axolotls. *J. Exp. Zool.* 115: 151-173.
- DALTON, H.C. (1950b). Comparison of white and black axolotl chromatophores *in vitro*. *J. Exp. Zool.* 115: 17-33.
- DALTON, H.C. (1953). Relations between developing melanophores and embryonic tissues in the Mexican axolotl. In *Pigment Cell Growth* (Ed. M. Gordon). Academic Press, New York, pp. 17-27.

- DETWILER, S.R. (1937). Observations upon the migration of neural crest cells, and upon the development of the spinal ganglia and vertebral arches in *Ambystoma*. *Am. J. Anat.* 61: 63-94.
- DREWS, U., KOCHER-BECKER, U. and DREWS, U. (1972). Die Induktion von Kiemebogenknorpel aus Kopfnervalleistenmaterial durch präsumptiven Kiemendarm in der Gewebekultur und das Bewegungsverhalten der Zellen während ihrer Entwicklung zu Knorpel. *W. Roux Arch. Entw. Mech. Org.* 171: 17-37.
- DUSHANE, G.P. (1934). The origin of pigment cells in amphibia. *Science* 80: 620-621.
- DUSHANE, G.P. (1935). An experimental study of the origin of pigment cells in amphibia. *J. Exp. Zool.* 72: 1-31.
- EIMER, G.H.T. (1881). *Über das Variieren der Mauereidechse*. Berlin.
- EPPERLEIN, H.H. (1974). The ectomesenchymal-endodermal interaction-system (EEIS) of *Triturus alpestris* in tissue culture. I. Observations on attachment, migration and differentiation of neural crest cells. *Differentiation* 2: 151-168.
- EPPERLEIN, H.H. (1978). The ectomesenchymal-endodermal interaction-system (EEIS) of *Triturus alpestris* in tissue culture. Morphological and histochemical characterization of developing neural derivatives. *Differentiation* 11: 109-123.
- EPPERLEIN, H.H. (1982). Different distribution of melanophores and xanthophores in early tailbud and larval stages of *Triturus alpestris*. *Roux Arch. Dev. Biol.* 191: 19-27.
- EPPERLEIN, H.H. (1988). Die Kontrolle der Wanderung und Differenzierung von Neuralleistezellen durch die Extrazelluläre Matrix. *Naturwissenschaften* 75: 443-450.
- EPPERLEIN, H.H. and CLAVIEZ, M. (1982a). Changes in the distribution of melanophores and xanthophores in *Triturus alpestris* embryos during their transition from the uniform to banded pattern. *Roux Arch. Dev. Biol.* 191: 5-18.
- EPPERLEIN, H.H. and CLAVIEZ, M. (1982b). Formation of pigment cell patterns in *Triturus alpestris* embryos. *Dev. Biol.* 91: 497-502.
- EPPERLEIN, H.H. and JUNGINGER, M. (1982). The normal development of the newt, *Triturus alpestris* (Daudin). *Amphibia-Reptilia* 2: 295-308.
- EPPERLEIN, H.H. and LEHMANN, R. (1975). The ectomesenchymal-endodermal interaction-system (EEIS) of *Triturus alpestris* in tissue culture. 2. Observations on the differentiation of visceral cartilage. *Differentiation* 4: 159-174.
- EPPERLEIN, H.H. and LÖFBERG, J. (1984). Xanthophores in chromatophore groups of the premigratory neural crest initiate the pigment pattern of the axolotl larva. *Roux Arch. Dev. Biol.* 193: 357-369.
- EPPERLEIN, H.H. and LÖFBERG, J. (1990). The development of the larval pigment patterns in *Triturus alpestris* and *Ambystoma mexicanum*. *Adv. Anat. Embryol. Cell Biol.* 118: 1-101.
- EPPERLEIN, H.H. and LÖFBERG, J. (1993). The development of the neural crest in amphibians. *Ann. Anat.* 175, 483-499.
- EPPERLEIN, H.H., HALFTER, W. and TUCKER, R. (1988). The distribution of fibronectin and tenascin along migratory pathways of the neural crest in the trunk of amphibian embryos. *Development* 103: 743-756.
- EPPERLEIN, H.H., KROTOSKI, D.M., FREY, A. and HALFTER, W. (1990). Development and distribution of enteric neurones in *Xenopus*. *Anat. Embryol.* 182: 53-67.
- EPPERLEIN, H.H., PERRIS, R. and LÖFBERG, J. (1986). Environmental control in pigment pattern formation of the axolotl larva. In *Progress in Developmental Biology*, Part B (Ed. H.C. Slavkin). Alan R. Liss, New York, pp. 191-194.
- FRANK, E. and SANES, J.R. (1991). Lineage of neurons and glia in chick dorsal root ganglia: analysis *in vitro* with a recombinant retrovirus. *Development* 111: 895-908.
- HALL, B.K. and HÖRSTADIUS, S. (1988). *The Neural Crest*. Oxford Univ. Press, London.
- HAUSEN, P. and RIEBESELL, M. (1991). *The Early Development of Xenopus laevis*. Verlag d. Zeitschr. f. Naturforsch., Tübingen; Springer, Berlin.
- HOLTFRETER, J. (1968). Mesenchyme and epithelia in inductive and morphogenetic processes. In *Epithelial-Mesenchymal Interactions*. 18th Hahnemann Symposium. Williams and Wilkins, Baltimore, pp. 1-30.
- HORAN, P.K. and SLEZAK, S.E. (1989). Stable cell membrane labelling. *Nature* 340: 167-168.
- HÖRSTADIUS, S. and SELLMAN, S. (1946). Experimentelle Untersuchungen über die Determination des knorpeligen Kopfskelettes bei Urodelen. *Nov. Act. R. Soc. Sci. Ups. Ser. IV*, 13: 1-170.
- JONES, T.R., KLUGE, A.G. and WOLF, J.A. (1993). When theories and methodologies clash: a phylogenetic reanalysis of the North American ambystomatid salamanders (Caudata: Ambystomatidae). *Syst. Biol.* 42: 92-102.
- KELLER, R.E., LÖFBERG, J. and SPIETH, J. (1982). Neural crest cell behaviour in white and dark embryos of *Ambystoma mexicanum*: epidermal inhibition of pigment cell migration in the white axolotl. *Dev. Biol.* 89: 179-195.
- KITAMURA, K., TAKIGUCHI-HAYASHI, K., SAZAKI, M., YAMAMOTO, H. and TAKEUCHI, T. (1992). Avian neural crest cells express a melanogenic trait during early migration from the neural tube: observations with the new monoclonal antibody, MEBL-1". *Development* 114: 367-378.
- KNOUFF, R.A. (1927). The origin of the cranial ganglia of *Rana*. *J. Comp. Neurol.* 44: 259-361.
- KUPFFER, C. v. (1894). *Studien zur vergleichenden Entwicklungsgeschichte des Kopfes der Kranioten*. II. München und Leipzig.
- LARSON, A. (1991). A molecular perspective on the evolutionary relationships of the salamander families. *Evol. Biol.* 25: 211-277.
- LARSON, A. and DIMMICK, W.W. (1993). Phylogenetic relationships of the salamander families: an analysis of congruence among morphological and molecular characters. *Herpetol. Monogr.* 7: 77-93.
- LINDEN, M. v. (1900). Die ontogenetische Entwicklung der Zeichnung unserer einheimischen Molche. *Biol. Zentralbl.* 20: 144-167.
- LÖFBERG, J., EPPERLEIN, H.H., PERRIS, R. and STIGSON, M. (1989b). Neural crest cell migration in the axolotl embryo: A pictorial essay. In *The Developmental Biology of the Axolotl* (Eds. J.B. Armstrong and G.M. Malacinski). Oxford University Press, New York, pp. 83-101.
- LÖFBERG, J., NYNÄS-MCCOY, A., OLSSON, C., JÖNSSON, L. and PERRIS, R. (1985). Stimulation of initial neural crest cell migration in the axolotl embryo by tissue grafts and extracellular matrix transplanted on microcarriers. *Dev. Biol.* 107: 442-459.
- LÖFBERG, J., PERRIS, R. and EPPERLEIN, H.H. (1989a). Timing in the regulation of neural crest cell migration: Retarded "maturation" of regional extracellular matrix inhibits cell migration in embryos of the white axolotl mutant. *Dev. Biol.* 131: 168-181.
- MAYNARD SMITH, J.R., BURIAN, R., KAUFFMAN, S.A., ALBERCH, P., CAMPBELL, J., GOODWIN, B.C., LANDE, R., RAUP, D.M. and WOLPERT, L. (1985). Developmental constraints and evolution. *Q. Rev. Biol.* 60: 265-287.
- OKADA, E.W. (1955). Isolationsversuche zur Analyse der Knorpelbildung beim Urodelenkeim. *Mem. Coll. Sci., Kyoto Univ., Ser. B* 22: 23-28.
- OLSSON, L. (1993). Pigment pattern formation in the larval ambystomatid salamander *Ambystoma maculatum*. *J. Morphol.* 215: 151-163.
- OLSSON, L. (1994). Pigment pattern formation in larval ambystomatid salamanders: *Ambystoma talpoideum*, *Ambystoma barbouri*, and *Ambystoma annulatum*. *J. Morphol.* 220: 123-138.
- OLSSON, L. and LÖFBERG, J. (1992). Pigment pattern formation in larval ambystomatid salamanders: *Ambystoma tigrinum tigrinum*. *J. Morphol.* 211: 73-85.
- ORTON, G.L. (1942). Notes on the larvae of certain species of *Ambystoma*. *Copeia*: 170-172.
- PERRIS, R. and JOHANSSON, S. (1990). Inhibition of neural crest cell migration by aggregating chondroitin sulfate proteoglycans is mediated by their hyaluronan-binding region. *Dev. Biol.* 137: 1-12.
- PERRIS, R. and LÖFBERG, J. (1986). Promotion of chromatophore differentiation in isolated premigratory neural crest cells by extracellular material explanted on microcarriers. *Dev. Biol.* 113: 327-341.
- PERRIS, R., LÖFBERG, J., FÄLLSTRÖM, C., BOXBERG, Y.V., OLSSON, L. and NEWGREEN, D.F. (1990). Structural and compositional divergences in the extracellular matrix encountered by neural crest cells in the white mutant axolotl embryo. *Development* 109: 533-551.
- PETERS-SANDEN v.d., M. (1994). *The Hindbrain Neural Crest and the Development of the Enteric Nervous System*. Thesis, Univ. of Rotterdam, Netherlands.
- RAVEN, C.P. (1931). Zur Entwicklung der Ganglienleiste. Die Kinematik der Ganglienleistenentwicklung bei den Urodelen. *W. Roux Arch. Entw. Mech. Org.* 125: 210-293.
- ROSIN, S. (1943). Experimente zur Entwicklungsphysiologie der Pigmentierung bei Amphibien (Transplantationen zwischen *Triton* und *Amblystoma mexicanum*). *Rev. Suisse Zool.* 50: 485-578.

- SADAGHIANI, B. and THIÉBAUD, C.H. (1987). Neural crest development in the *Xenopus laevis* embryo, studied by interspecific transplantation and scanning electron microscopy. *Dev. Biol.* 124: 91-110.
- SHAFFER, H.B. (1984). Evolution in a paedomorphic lineage. I. An electrophoretic analysis of the Mexican ambystomatid salamanders. *Evolution* 38: 1194-1206.
- SHAFFER, H.B., CLARK, J.M. and KRAUS, F. (1991). When molecules and morphology clash: A phylogenetic analysis of the North American ambystomatid salamanders (Caudata: Ambystomatidae). *Syst. Zool.* 40: 284-303.
- SOBOTTA, J. (1935). Beiträge zur Histogenese der sogenannten Ganglienleiste der Wirbeltiere. Nach Untersuchungen an Selachiern und Amphibien. *Z. Mikrosk. Anat. Forsch.* 38: 660-688.
- STARCK, D. (1963). Die Metamerie des Kopfes der Wirbeltiere. *Zool. Anz.* 170: 393-428.
- STEINBERG, M.S. (1957). A non-nutrient culture medium for amphibian embryonic tissue. *Carnegie Inst. Wash. Yearbook* 56: 347-348.
- STONE, L.S. (1922). Experiments on the development of the cranial ganglia and the lateral line sense organs in *Amblystoma punctatum*. *J. exp. Zool.* 35, 421-496.
- THIÉBAUD, C.H. (1983). A reliable new cell marker in *Xenopus*. *Dev. Biol.* 98: 245-249.
- TWITTY, V.C. (1936). Correlated genetic and embryological experiments on *Triturus*. I and II. *J. Exp. Zool.* 74: 239-302.
- TWITTY, V.C. (1945). The developmental analysis of specific pigment patterns. *J. Exp. Zool.* 100: 141-178.
- TWITTY, V.C. (1966). *Of Scientists and Salamanders*. Freeman and Co., San Francisco.
- TWITTY, V.C. and BODENSTEIN, D. (1939). Correlated genetic and embryological experiments on *Triturus*, III and IV. *J. Exp. Zool.* 81: 357-398.
- VINCENT, M., DUBAND, J-L. and THIERY, J-P. (1983). A cell surface determinant expressed early on migrating neural crest cells. *Dev. Brain Res.* 9: 235-238.
- VOGEL, D.L. and MODEL, P.G. (1977). Development of the sympathetic system in the Mexican axolotl, *Ambystoma mexicanum*. *Dev. Biol.* 56: 76-96.
- WESTON, J.A. (1991). Sequential segregation and fate of developmentally restricted intermediate cell populations in the neural crest lineage. *Curr. Top. Dev. Biol.* 25: 133-153.
- YNTEMA, C.L. (1937). An experimental study of the origin of the cells which constitute the VIIth and VIIIth ganglia and nerves in the embryo of *Amblystoma punctatum*. *J. Exp. Zool.* 75: 75-101.
- YNTEMA, C.L. (1943). An experimental study of the origin of the sensory neurons and sheath cells of the IXth and Xth cranial nerves in *Amblystoma punctatum*. *J. Exp. Zool.* 92: 93-120.