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INTEGRATING CELLULAR AND MOLECULAR APPROACHES INTO STUDIES OF DEVELOPMENT AND EVOLUTION: THE ISSUE OF MORPHOLOGICAL HOMOLOGY

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ABSTRACT

Understanding the development and evolution of complex morphological characters requires broad, multidisciplinary approaches. By combining biological imaging, phylogenetic analyses, embryological manipulations and additional modern molecular techniques (e.g., whole mount *in situ* hybridization), we can address fundamental questions, such as the determination of homology. Many of the novel structures that evolved in vertebrates and distinguish them from their ancestors (such as jaws and the mechanosensory lateral line) are derived embryonically from two migratory ectodermal tissues, neural crest and/or various epidermal placodes. In particular, time-lapse cinematography of fluorescently labeled cells in living *Xenopus* and fish embryos has proved a powerful technique, revealing new information on migration and differentiation. These data allow us to reexamine the developmental criterion for morphological homology. There are three criteria for determining morphological homology of a structure between species: position (anatomical location), development (common origin, gene expression and/or cell behavior), and phylogeny (identification of the structure in an outgroup). Currently the phylogenetic criterion is the most regularly employed. Previous problems with the application of the developmental criterion were based on a paucity of detailed comparative developmental data and the overlooked assumption that ontogenies can not change during evolution and remain homologous while morphologies can. Homology has been defined as similarity due to continuity of information. The rejection of developmental data as a homology criterion is based on emphasizing lack of similarity without considering the information on continuity provided by these data. Often missing from previous analyses are the concepts of multiple developmental mechanisms in the formation of a structure (including such issues as developmental redundancies) and that ontogenies can change during evolution. By including these two concepts in a broadly comparative analysis, such problems as seemingly different developmental and genetic bases for homologous structures, can be explained and are even expected. As examples of how such an analysis can be done, we compared the development of the neural tube and lateral line among several vertebrate species. We show that while across species a given structure's ontogeny may differ, a more broadly comparative developmental criterion using modern cell and molecular biological techniques provides a good homology criterion. Also, we find that molecular homology alone is not yet a good basis for morphological homology. While we would argue that a phylogenetic perspective is important (and critical for polarizing evolutionary changes), it is not essential for our usage of the developmental criterion. Thus, a broadly comparative and detailed understanding of developmental mechanisms makes for a robust criterion of homology.

Key words: development, evolution, homology, lateral line, morphology, neural crest, neurulation, placode, *Xenopus*, zebrafish.

INTRODUCTION

Evolutionary biology still awaits integration of developmental concepts on a par with its earlier integration of genetics (Dobzhansky 1982) and systematics (Hennig 1965; Mayr 1982) into the "new synthesis." Even so, the number of studies and interest in the area of development and evolution are high (Akam et al. 1994; Barinaga 1994; Bonner 1982; Goodwin et al. 1983; Gould 1977; Patel 1994; Raff and Kaufman 1983; Raff and Raff 1987) and the routine utilization

of developmental data in evolutionary analysis appears imminent. Current studies of development and evolution can be divided into two types: those of developmental biologists looking at evolution, and those of evolutionary biologists looking at development. Each type offers distinct methodological advantages. Developmental biologists bring modern cellular and molecular biological techniques, honed on a model system, to bear on other species and map these results onto a known cladogram. Evolutionary biologists use phylogenetic approaches and quantitative genetic techniques to place developmental data within a comparative context. Typically the expertise of the individuals involved

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is different. Hanken (1993) offers a good discussion of the limitations and advantages of each approach. The developmental biology approach yields detailed developmental data for only a few species, typically model systems, limiting the phylogenetic scope of the comparison. The evolutionary biology approach is often limited by developmental data collected using older techniques or inferred from nonliving materials.

In this paper we attempt to integrate these two approaches to developmental and evolutionary studies by using modern cellular and molecular biological techniques within a broader phylogenetic context. It is necessary to bring new experimental methods to studies of evolution (Zimmer 1994). This synthesis allows us to address a major goal of developmental and evolutionary studies, to identify and begin understanding the developmental transformations that occurred during evolution to produce the present-day varied morphologies. We emphasize our view of what developmental and evolutionary studies entail because of the large variety of different perspectives and methodologies available. For example, studies of development and evolution encompass work on juvenile to adult morphological growth (e.g., Lessa and Patton 1989) as well as theoretical research using quantitative genetic techniques (e.g., Cowley and Atchley 1992). The differences among these approaches illustrate the breadth of research done under the rubric of development and evolution.

There are two features common to and necessary for all developmental evolutionary studies, that is they must be both comparative and undertaken in a phylogenetic framework. While comparative studies are essential for the study of evolution, not all comparative developmental studies are evolutionary, nor do they need to be (Wake 1990). Comparative developmental studies are important for two nonevolutionary reasons: they reveal the generality of developmental principles, and the amount of variation (including absence) in a given developmental process. An example of functional understanding from comparative studies is the prediction of ribosomal RNA secondary structure (Raff and Raff 1987). A phylogenetic hypothesis on which developmental data are mapped allows one to propose possible evolutionary transformations of development. It also assists one in selecting which taxa might be the most appropriate for further studies and comparisons.

An evolutionary issue that is particularly amenable to analysis with developmental data is the concept of homology (Donoghue 1992). Homology has been called the central concept of biology yet it is difficult to define (Wake 1994). We prefer Van Valen's (1982) definition that homology is similarity due to continuity of information because it is the most inclusive (Haszprunar 1992). Homology is certainly a fundamental concept for all of comparative biology. It is the basis

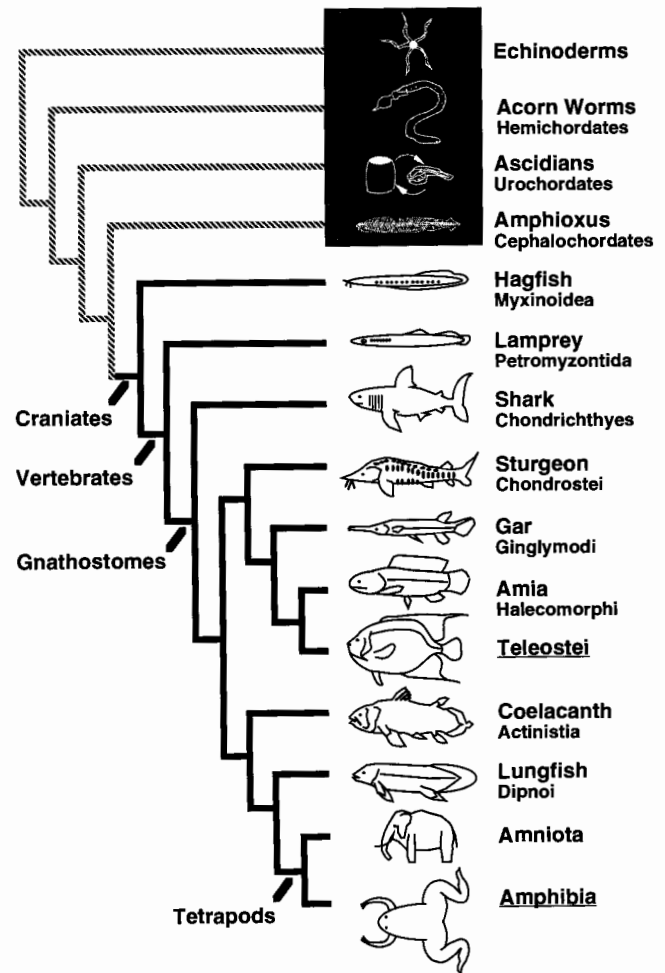


Fig. 1. Phylogeny of deuterostomes. Illustrations of representative species (not to scale) are shown. This phylogeny is derived from several sources (Janvier 1981; Lauder and Liem 1983; Maisey 1986). The group of interest to this study is the monophyletic deuterostome clade Craniata (Janvier 1981; Kingdom Animalia), which is identified with solid lines in the cladogram. Two vertebrate clades, Teleostei and Amphibia, whose names are underlined, will be the focus of our developmental studies. Four major craniate clades are labeled on the phylogeny. (Most craniate illustrations based on Nelson 1984)

for determining characters in cladistic analyses. It is used for determining molecular similarity. It is necessary for deciding which behaviors, structures or molecules found in different organisms are comparable. Determining homology, however, is not a simple problem and will be a major focus of this paper.

The taxonomic group under consideration here is the monophyletic deuterostome clade, Craniata (Janvier 1981). Craniates are by far the largest deuterostome group in terms of species number. The phylogenetic position of craniates among deuterostomes is well accepted (Maisey 1986). Figure 1 shows a well supported phylogeny of extant groups combined from several sources (Janvier 1981; Lauder and Liem 1983; Maisey 1986). The four outgroups to craniates are en-

closed by the shaded square and, together with craniates but excepting echinoderms (sea urchins, sea stars, sea cucumbers, etc.), form the Chordata. Within craniates, the sister taxon to Myxinoidea is the vertebrates, distinguished by such features as more than one semicircular canal and extrinsic eye musculature (Janvier 1981). Gnathostomes are united by approximately 37 unique shared derived characters (synapomorphies), the most obvious of which are jaws (Maisey 1986). Extant jawless craniates (Myxinoidea and Petromyzontida) are considered to be paraphyletic (Janvier 1981). Beyond the jawless craniates, all craniate taxa except for tetrapods are popularly known as "fishes." Four vertebrate groups (Chondrostei, Ginglymodi, Halecomorphi and Teleostei) form a monophyletic clade called Actinopterygii, the ray-finned fishes (Fig. 1; Lauder and Liem 1983). Most species of tetrapods are terrestrial and have four limbs; within tetrapods the Amniota includes mammals, birds and reptiles. Two vertebrate clades will be the focus of our developmental studies, the actinopterygian group Teleostei, which contains almost half of all craniate species (Nelson 1984), and the Amphibia, time-honored experimental animals.

Most craniate synapomorphies are derived from two embryonic tissues, the neural crest and epidermal placodes (Janvier 1981; Maisey 1986). Both these tissues are derived from the ectodermal germ layer and are unique to craniates (Northcutt and Gans 1983; Selleck et al. 1993). Neural crest and placodal cells are highly migratory and invade many embryonic sites before and during their differentiation (Hall and Horstadius 1988; Le Douarin 1982; Webb and Noden 1993). Together these two sources generate the peripheral nervous system (PNS) and contribute to a wide variety of structures (Hall and Horstadius 1988; Le Douarin 1982; Webb and Noden 1993). The nose, lens of the eye, ear, lateral line system (a mechanosensory structure found in almost all aquatic craniates) and electroreceptors form from epidermal placodes, which also contribute to cranial ganglia (part of the PNS) (Webb and Noden 1993). The neural crest contributes to ganglia of the PNS, adrenal medulla, most of the craniofacial skeleton, teeth, head dermis and all the body pigmentation. It forms such cell types as neurons, glia, chromaffin cells, pigment cells, cartilage and bone cells (Couly et al. 1992; Hall and Horstadius 1988; Le Douarin 1982). Many definitive synapomorphies among the craniates are also neural crest derivatives, for example jaws (the definitive gnathostome feature) are formed by the neural crest.

The contribution of neural crest and placodes to such evolutionary and systematically important characters as jaws, other head bones, gill arches, body coloration and paired sensory structures illustrates the need for their developmental study. As we will show,

there is still much to be discovered about their development. We will concentrate on members of two animal clades but we hope to show that these methodologies have broader applicability, even in plants though their cells do not migrate. Ours is a two part analysis. First we will provide new insights to embryonic processes using modern cellular and molecular biological techniques. Second the implication of these new findings for evolutionary biology will be explored, particularly relating to the issue of morphological homology.

MATERIALS AND METHODS

Embryos

Eggs of the African clawed frog *Xenopus laevis* Daudin, eggs fertilized *in vitro* were acquired as previously described (Krotoski et al. 1988). Embryos were staged according to the normal table of Nieuwkoop and Faber (1967). Embryos were either dejellied mechanically with forceps or chemically using a standard protocol (Kay and Peng 1991) and were maintained in a standard saline solution (Collazo et al. 1993). *Xenopus* embryos at the appropriate stage were prepared for injection and low-light-level imaging as previously described (Collazo et al. 1993).

Eggs were collected from wild-type zebrafish (*Danio rerio* Hamilton) adults by natural spawnings and dechorinated using standard protocols (Westerfield 1993). Homozygous mutant zebrafish embryos were acquired from natural matings of heterozygous adults and staged according to the standard normal table (Westerfield 1993). Zebrafish embryos were raised to appropriate stages in 10% Hank's solution (Westerfield 1993).

Labeling Lateral Line Hair Cells

Anesthetized *Xenopus* and zebrafish embryos were dipped in a solution of their respective salines saturated with 4-Di-2-Asp (Molecular Probes, Eugene, OR) for 5 minutes. Embryos were then rinsed by transferring through two petri dishes of clean saline and imaged with low-light-level microscopy (Collazo et al. 1993, 1994).

Microinjection of Fluorescent Dyes

Microinjection of DiI (1,1-dioctadecyl-3,3,3',3'-teramethylindocarbocyanine perchlorate; Molecular Probes, Eugene, OR) into *Xenopus* embryos was done as previously described (Collazo et al. 1993). Two different DiI's were used (C3 and C5), differing only in the wavelengths they emit. Small groups of cranial neural crest cells (20–100) were labeled by injections at stages 14 to 18. The posterior lateral line placode was labeled by injections at stage 35. These stages were chosen because cell migration had not yet begun.

Microinjection of lysinated rhodamine dextran (LRD, 10 kDa; Molecular Probes, Eugene, OR) into *Xenopus* embryos was done at stages 14–18 (Collazo et al. 1993). Iontophoretic injection allows single cells to be labeled.

Microinjection of lysinated fluorescein dextran (LFD, 10 kDa; Molecular Probes, Eugene, OR) or LRD into early cleavage stages of *Xenopus* embryos was done as described for DiI microinjection (Collazo et al. 1993) with the following modifications. The dyes were made up in distilled water at 100 mg/ml. Single cells were labeled at these early stages. Embryos were raised to a stage between 19 and 41, and preserved for whole-mount *in situ* hybridization as described below.

Time-lapse Videomicroscopy

After DiI or LRD microinjection, an individual embryo was chosen for time-lapse, low-light-level videomicroscopy (Collazo et al. 1993; Krull et al. 1995). Intact *Xenopus* embryos were anesthetized and placed in agar grooves for the duration of the time-lapse.

Whole-mount RNA in-situ Hybridization

Xenopus embryos were preserved and processed for *in situ* hybridization (Harland 1991; Hemmati-Brivanlou et al. 1990) with modifications described in (Zimmerman et al. 1993). The probes used were a β subunit of the *Xenopus* acetylcholine receptor (unpublished probe cloned by Bill Todd) and, as a positive control, a *Xenopus* muscle actin (pAC100 used in Hemmati-Brivanlou et al. 1990). Some embryos processed for *in situ* hybridization had been labeled with a fluorescent dextran.

RESULTS AND DISCUSSION

The first half of the remainder of the paper presents specific examples of the types of modern methodologies that can be used to provide a better understanding of developmental processes. Our examples mainly involve biological imaging because this is the focus of our laboratory. These examples illustrate the new insights possible from using these methodologies. The second half relates the significance of these and other modern developmental data to the issue of determining homology.

Developmental Data

Fluorescent labeling of specific cells in living embryos allows us to observe directly important developmental events such as cell migration and differentiation. Low-light-level videomicroscopy allows the same cells to be observed daily because phototoxic effects are minimized. Fluorescently labeled cells may

still be visible after one month, more than sufficient time to see most developmental events in *Xenopus* and zebrafish (in fact, one week suffices for most of the questions we ask). A potential problem with these techniques is that cell division during development can dilute the dye beyond the threshold of detection. Therefore, one can not be certain if the observed cells are all the descendants of the originally labeled cells. This is a trade-off for the resolution provided by these methodologies but the problem is not so serious if one takes a conservative approach to interpreting the data by assuming the variability observed is a minimum estimate. These imaging techniques have confirmed many earlier results from other methods, including histology and scanning electron microscopy (Sadaghiani and Thiebaud 1987); tissue ablation (Seufert and Hall 1990); retroviral labeling (Frank and Sanes 1991); and labeled tissue grafts (Krotoski et al. 1988; Le Douarin 1982).

These advanced imaging techniques have expanded our knowledge of *Xenopus* trunk neural crest migration. Two previously undescribed migration pathways into the ventral fin were discovered (Collazo et al. 1993), where cell migration into the transparent fins is particularly easy to observe. By looking at the same embryo at three different stages, the often great distances neural crest cells migrate can be appreciated (Fig. 2). The initial injection site which is quite small, appears brighter at later stages as the embryo becomes more transparent. Interesting differences in the migration pathways of prospective pigment cells occur within amphibians (Collazo et al. 1993; Epperlein and Löfberg 1993; Krotoski et al. 1988). In the frog, *Xenopus*, pigment cells migrate along a deep pathway between the somite and neural tube while those of salamanders, such as *Triturus* and *Ambystoma*, migrate just under the skin, the pattern seen in avian embryos (Bronner-Fraser 1993).

More detailed observations of cell migration and its timing are obtained by time-lapse videomicroscopy, using low-light-level imaging to acquire images every 2 or 4 minutes for up to 48 hours. Neural crest cells begin migrating from all or most of the dorsal neural tube's rostrocaudal extent (depending on species; see Osumi-Yamashita et al. 1994), just after it forms, except in the head regions of frogs and rodents where migration begins earlier (Sadaghiani and Thiebaud 1987; Tan and Morriss-Kay 1985; but see following). Our cranial neural crest migration time-lapse films provide dynamic confirmation of previous studies (e.g., Stone 1922) that could only infer movements from static views of different embryos. We find that cranial neural crest migration begins later than previously thought (Sadaghiani and Thiebaud 1987), not much before the neural tube has closed, making *Xenopus* more similar to chicken than rodents for this trait.

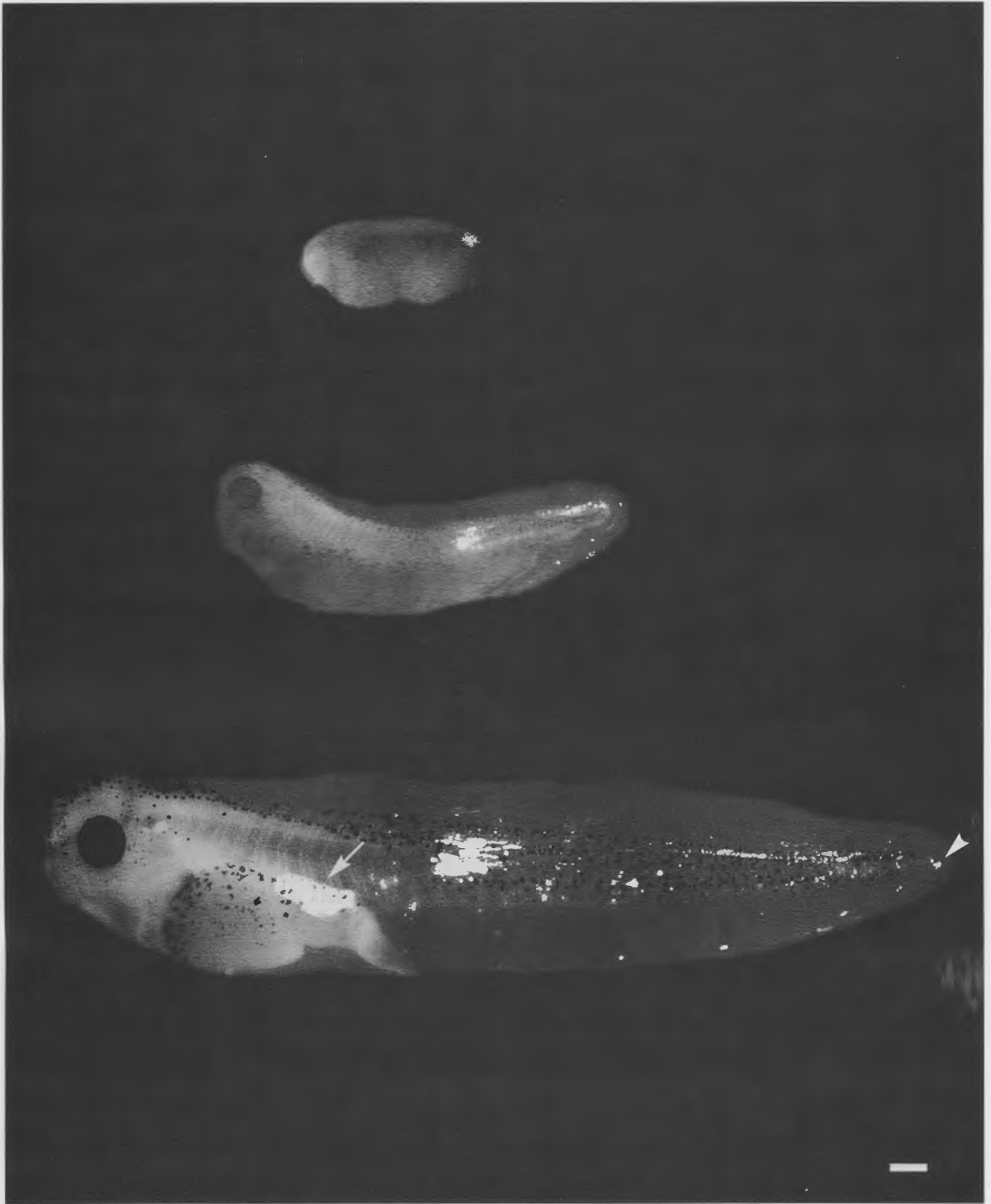


Fig. 2. Neural crest migration in a living frog. The illustration is of the same *Xenopus laevis* embryo at three different stages (from top to bottom the stages are 21, 34, 41), showing DiI-labeled neural crest cells migrating into the ventral fin and enteric region (arrow). The embryo was injected in the caudal neural crest, initially located in the dorsal region of the neural tube. The caudal limit of neural crest migration is indicated with an arrowhead. The injection site is lined up and all three stages are to scale. Scale bar equals 500 μm .

From the films it is easy to see why cells from the first three visceral arches (mandibular, hyoid and first branchial) can be mapped to specific regions of the brain while cells into the remaining, more posterior arches (three in *Xenopus*; four in zebrafish) are intermixed (Sadaghiani and Thiebaud 1987; Schilling and Kimmel 1994). Cells from the same neural tube region migrating into one of the more posterior arches often move either forward or backward into another arch. Observations on cranial and trunk neural crest migration (Collazo et al. 1993, 1994) reveal that, while migrating individual neural crest cells have a net movement in a given direction, this net movement is composed of forward, backward and lateral components.

Such dynamic analyses of cell migration can be used in examining tetrapod development. A novel chicken explant culture system has been developed which allows time-lapse videomicroscopy on labeled neural crest cells, without perturbing normal development (Krull et al. 1995). The complex migration of these individually labeled cells is very similar to that observed in *Xenopus* and two species of teleost fishes. This is surprising because previous work had suggested that *Xenopus* neural crest cells migrate in looser groups than those of chickens (Collazo et al. 1993; Krotoski et al. 1988). The analysis of the migration of chick neural crest cells has revealed that they often move in groups that may disperse and reaggregate at several points during their migration, and that at least some of these groups may be clonally related (Krull et al. 1995). Even when dispersed, these cells may still be in contact by thin processes. The advantage of explant/whole embryo systems over *in vitro* analyses is that experimental perturbations to assay the role of a candidate molecule on neural crest migration are possible. The study in chick embryos demonstrates this well because it uses dynamic data, such as rate of migration and a cell's trajectory, to reveal an effect on migration after removal of an inhibitory molecule (Krull et al. 1995).

Molecular techniques offer powerful tools for addressing questions in the area of development and evolution, yet their potential remains unfulfilled. Molecular studies have concentrated on either developmental or evolutionary questions, not those questions resulting from the integration of these two areas. Most of the few developmental and evolutionary studies using molecular techniques have concentrated on heterochronic shifts in the expression of one or more molecules (Raff and Wray 1989). Almost all these studies have used antibodies to a specific epitope that cross-reacts with several species, for their comparisons (e.g., Collazo 1994; Wray and McClay 1989). Antibodies, when combined with whole-mount protocols, provide a means for visualizing the positions of proteins and resolving fine embryonic structures, often at earlier

stages than those provided by previous histological methods (Dent et al. 1989; Hanken et al. 1992). If an antibody to the protein product is not available for a gene of interest, its nucleotide sequence may be known. RNA *in situ* hybridization techniques then may allow for the visualization of the activities of the gene being studied, even in whole-mounts (Harland 1991); also transcripts usually are expressed earlier than the protein product. Although few comparative *in situ* hybridization studies exist (because of difficulty in adapting a protocol to a new species or the inability of a probe to hybridize in a different species), these nucleic acid studies are potentially more widely useful.

Neural crest derivatives.—Although a wide range of neural crest (Hall and Horstadius 1988; Le Douarin 1982) and placodal (Webb and Noden 1993) derivatives have been identified, the list is still expanding. Neural crest cells from different axial levels do not necessarily contribute to the same range of derivatives (for example, only cranial neural crest cells can form cartilage). Determining if a given cell is in a particular structure is relatively straightforward when these cells are fluorescently labeled. For example, neural crest contribution to fins and portions of the enteric nervous system can be observed *in vivo* (Fig. 2). Once the embryo reaches a stage where most of the neural crest cells have differentiated, it can be fixed and sectioned for confirmation. Cell type can be assessed by phenotype, position and/or antibody labeling (Bronner-Fraser and Fraser 1988; 1989). We, along with others, have described new derivatives to which neural crest cells contribute (Collazo et al. 1993, 1994): pronephric duct, posterior portion of the dorsal aorta (also observed in avian embryos, J. Sechrist personal communication), and lateral line neuromasts. The pronephric duct is part of the embryonic excretory system that forms by cell migration and was thought to be exclusively derived from intermediate mesoderm (Lynch and Fraser 1990). The posterior portion of the dorsal aorta, like the remainder of the aorta, was thought to be exclusively derived from splanchnic mesoderm (Gilbert 1991, p. 202). While neural crest derived ganglia closely juxtaposed to the aorta have been identified (Le Douarin 1982), the position and amount of labeled cells we observed can not be accounted for solely by these ganglia. The lateral line was thought to be exclusively derived from placodes (Landacre and Conger 1913; Webb and Noden 1993). The three primary germ layers of vertebrates (ectoderm, mesoderm and endoderm) provide a useful method of categorizing organ origins (Gilbert 1991). The neural crest, however, has always been problematic because this ectodermal tissue contributes to cell types and structures across all three layers (Le Douarin 1982). The contribution to the same structure from multiple tissues and

different germ layers has important implications for the issue of developmental and morphological homology.

Lateral line development.—Segmentation of vertebrate neural structures provides a means to simplify the complexity of neural development (Fraser 1993). One way to characterize this segmentation is by lineage restriction, as has been demonstrated in the chicken hindbrain (Fraser et al. 1990). The developing lateral line offers another system for studying segmentation by cell lineage restriction. The clusters of cells dropping off the migrating primordium, called neuromast primordia, are potential lineage compartments. While this has been inferred by several studies (Metcalf 1989; Winklbauer 1989), data we have gathered using time-lapse videomicroscopy in *Xenopus* embryos reveal that neuromast primordia are not lineage compartments. Cells may migrate from one neuromast primordium to another, even bypassing two neuromasts, by moving along the lateral line nerves. This movement is not necessarily in the same direction as that of the original primordium. The ability of regenerated neuromasts in the salamander tail to form from support cells of adjacent mature neuromasts (Jones and Corwin 1993) demonstrates that cells can migrate even out of mature neuromasts.

A recent and surprising result has been the observation in three species (the Siamese fighting fish, *Betta splendens* Regan, the zebrafish, and *Xenopus*) that neural crest cells also contribute to the development of the lateral line (Collazo et al. 1994). This study also labeled placodes and confirmed their contribution to the development of the lateral line. The lateral line of living embryos can be visualized by fluorescently labeling hair cells with the vital dye 4-Di-2-Asp (Fig. 3). Each white dot on an embryo can consist of up to two dozen hair cells. The patterns on the embryo formed by the lateral lines can be quite elaborate and are usually species specific, providing an important taxonomic character. All these lines are thought to originate from five ancestral placodes (Northcutt et al. 1994) which are shown in Fig. 4. The two teleost species have more complex lateral lines, particularly in the tail, than the *Xenopus* tadpole which has a single line running along the base of the dorsal fin (Fig. 3). The derivation of the lateral line from placodes has been studied extensively (Metcalf 1989; Winklbauer 1989). Shortly after a lateral line placode forms it splits into two halves, one to form the ganglion, the other to migrate. The migrating portion of the placode is now called a primordium and moves through the epidermis. As a primordium migrates, it drops off clusters of cells that will differentiate into neuromasts. As the embryo grows, these original neuromasts divide and form more neuro-

masts, eventually generating the adult pattern. *Xenopus* lateral line placodes were labeled with a different lipophilic dye (DiI C5) whose emission wavelength we can distinguish from that of 4-Di-2-Asp so double labeling experiments can be done in the same embryo. The results clearly show that neuromast hair cells form from the placode. The same experiment needs to be done labeling the neural crest instead of the placode. By labeling neural crest and placodes with DiI's whose emission wavelengths we can distinguish (C3 and C5) the relative contributions of neural crest and placodes to the lateral line can be determined. Some teleost species have thousands of neuromasts (Puzdrowski 1989). The possibility that some of these neuromasts are neural crest derived does not seem unreasonable. A dual embryonic origin for vertebrate neuromasts should not be surprising given that both tissues also form the cranial ganglia (Hall and Horstadius 1988; Le Douarin 1982) and that placodes can compensate for ablated neural crest (Kirby 1988a, b). Later in this paper, we will explore the significance of this dual embryonic origin to the issue of lateral line homologies among different species.

Multipotency of individual neural crest cells.—A major issue in the study of neural crest development is determining the potential of an individual cell (Selleck et al. 1993). A given neural crest cell may be unipotent, contributing to one cell type in a single derivative, or multipotent, contributing to two or more cell types and/or derivatives. Labeling single trunk neural crest cells in chicken, *Xenopus*, zebrafish, and mouse embryos have demonstrated that many, if not most, neural crest cells are multipotent (Bronner-Fraser and Fraser 1988, 1989; Collazo et al. 1993; Raible and Eisen 1994; Serbedzija et al. 1994). In fact, the data for chicken, *Xenopus* and mouse show that neural tube and neural crest cells can share a common progenitor up to the point of neural crest migration. A majority of migrating neural crest cells are still multipotent (Fraser and Bronner-Fraser 1991). All these results suggest that external cues could be responsible for determining the cell fate of many neural crest cells (Selleck et al. 1993). This has been observed in culture experiments, where a self-renewing neural crest stem cell has been identified (Anderson 1989, 1993; Stemple and Anderson 1992, 1993). It is important to note that all these experiments also revealed some neural crest cells were unipotent. This suggests that the neural tube consists of a mixed population of cells with different potentials or a single multipotent population whose fates are sequentially restricted (Selleck et al. 1993).

Studies on cranial neural crest cells in zebrafish suggest these cells are unipotent (Schilling and Kimmel 1994) and, when taken together with the trunk data,

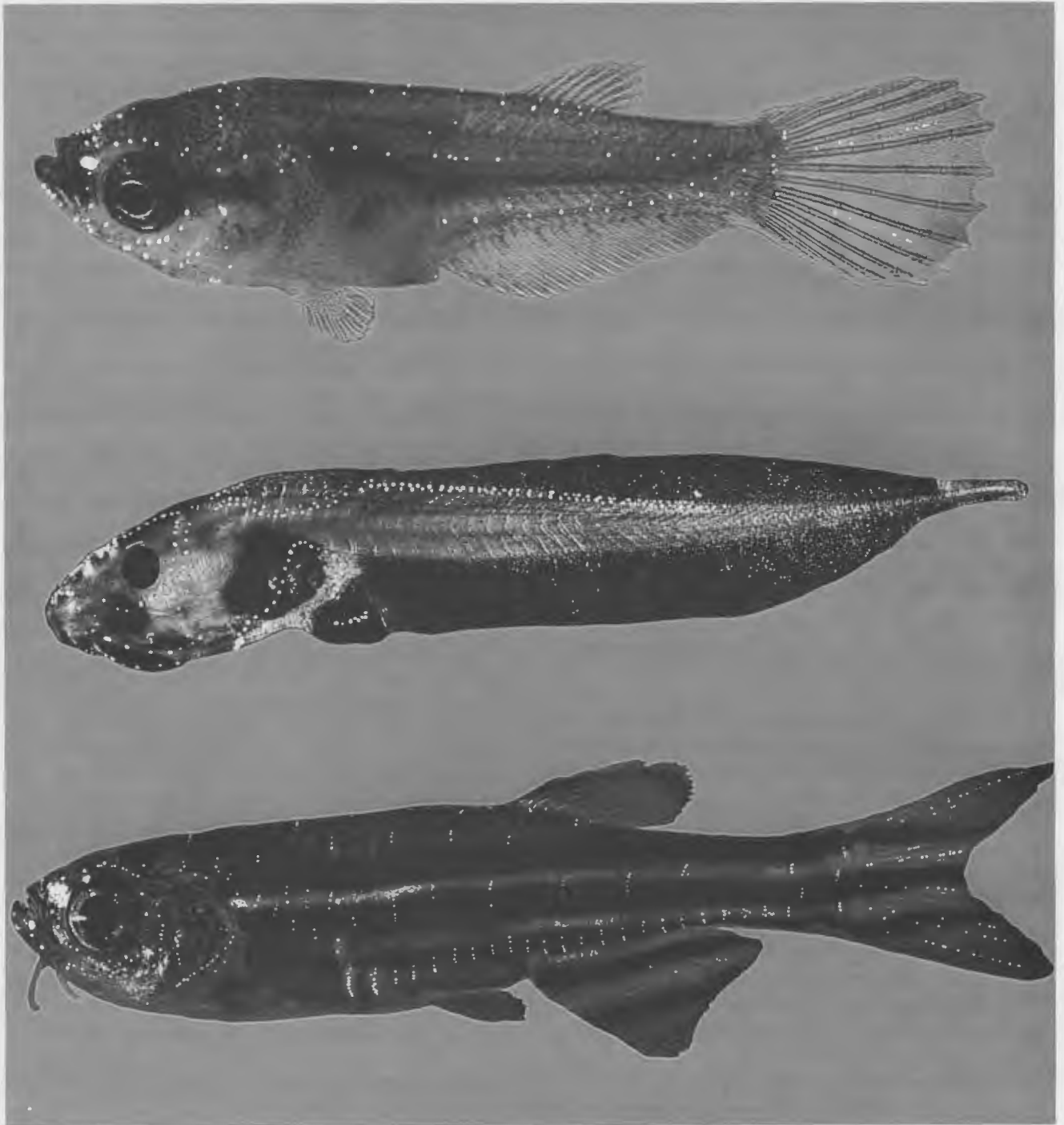


Fig. 3. Lateral line labeling in three vertebrate species with 4-Di-2-Asp. The dye labels the neuromast hair cells. From top to bottom: *Betta splendens*, *Xenopus laevis*, and *Danio rerio*. Not to scale. Their total lengths in mm, respectively, are 8.2, 10.4, and 14.2. The image combines fluorescent and bright field views. The vital dye also labels a subgroup of olfactory sensory cells.

raise the possibility that there are rostrocaudal differences in the potential of zebrafish neural crest cells. Since our prior work on *Xenopus* was done in the trunk we decided to label single cranial neural crest cells to see if rostrocaudal differences also exist in amphibians. As in the trunk, most cranial neural crest cells are multipotent and some share a common pro-

genitor with the brain. In fact, one clone had cells located in a cranial ganglion, along a lateral line nerve and in a neuromast; confirming previous work showing that groups of labeled neural crest cells contribute to the lateral line (Collazo et al. 1994). Unlike fish, frog neural crest cells are multipotent along their whole rostrocaudal extent. The variety of fates gen-

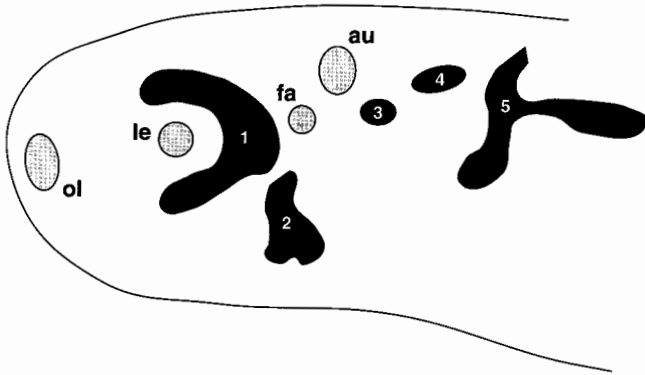


Fig. 4. Positions of the lateral line placodes early in their development. Lateral view of the head with anterior towards the left and dorsal towards the top. Ancestrally the lateral line is thought to be derived from five placodes (numbered and in black): 1, anterodorsal lateral line placode; 2, anteroventral lateral line placode; 3, middle lateral line placode; 4, supratemporal lateral line placode; 5, posterior lateral line placode. Some of the adjacent cephalic placodes are shown in gray: *ol*, olfactory placode; *le*, lens placode; *fa*, facial ganglion placode; *au*, auditory placode. (Adapted from Northcutt et al. 1995).

erated by different neural crest cells indicate to us that any definition of homology needs to take into account the issue of developmental redundancies.

Lateral line in mutant zebrafish.—Genetic approaches provide a powerful means to analyze development (Nüsslein-Volhard and Wieschaus 1980). The zebrafish has quickly become a model system for studies of vertebrate genetics because large-scale screenings for embryonic phenotypes can be done relatively inexpensively, and the mutations isolated complement studies of homologous genes in other model systems (Driever et al. 1994). We are interested in how lateral line development might be perturbed in mutant zebrafish. We have discussed above how the lateral line can be visualized fluorescently in living embryos. By labeling wild type and mutant embryos we can visualize how a given mutated gene effects lateral line development (Fig. 5). At these stages the number of neuromasts is still relatively small, even though neuromasts on both sides are visible through the transparent embryo. The mutants chosen affect different regions of the developing embryo and potentially different regions of the lateral line. The effects of the mutant gene *floating head* (*flh*) are restricted to the trunk and tail, the head being essentially normal (Talbot et al. 1995; Bill Trevarrow at University of Oregon, personal communication). No neuromasts form in the tail, while the head lateral lines appear normal, suggesting that the posterior lateral line primordium has either stopped migrating, migrates but does not drop off neuromast primordia, or that these primordia do not differentiate (Fig. 5A,B). We currently favor the first possibility because the defects in the mutant suggest a possible mechanism. The *floating head* mutant has no notochord and

no horizontal septum between dorsal and ventral somites (Talbot et al. 1995; Bill Trevarrow personal communication). The horizontal septum (or midbody line) is the pathway along which the posterior lateral line primordium migrates (Metcalf 1985, 1989). It is thought that the notochord is required for the muscle pioneer cells to form which are required for the formation of the horizontal septum (Talbot et al. 1995; B. Trevarrow personal communication). Without this pathway the migration of the primordium may be disrupted.

A second mutant, *cyclops*, most strongly effects the head, in extreme cases having a single anteriorly located eye (Hatta 1992; Hatta et al. 1991). The lateral line defects in this mutant are more subtle (Fig. 5C,E). The posterior lateral line appears normal, as expected, and the only effect on anterior lines visible at 48 hours is a posterior shift of the line ventral to the eye (Fig. 5A,C). This change does not seem to disrupt primordial differentiation as neuromast numbers are similar to those in wild type. By 96 hours the changes in the position of the anterior lateral line relative to wild type are minimal (Fig. 5D,E). The number of neuromasts and their positions relative to the eye are comparable, even given the severe defects in the head. Unlike the *floating head* gene, *cyclops* does not appear to have a strong effect on lateral line development. Combining genetics with the lineage techniques we discussed above is an ongoing project in many laboratories (Eisen and Weston 1993). This combined approach will allow one to study the effects of one or relatively few genes on complex developmental processes such as lateral line formation and to begin understanding the number of genes that may be involved in the ontogeny of a given structure.

Gene expression studies.—Whole mount *in situ* hybridization techniques applied to *Xenopus* provide a good system for addressing gene expression patterns during development after an evolutionary duplication event. *Xenopus laevis* is one of the few species of vertebrates that is polyploid (Kobel and Pasquier 1986). Its genome underwent a polyploidization event within the last 30 million years to produce a tetraploid (4N) condition (Bisbee et al. 1977). Therefore the haploid genome contains two or more copies of each gene. We have studied the muscle acetylcholine receptor (AChR), a glycoprotein constructed from four evolutionarily related proteins with the following stoichiometry: alpha(α)₂, beta(β), gamma(γ), and delta(δ) (Hucho 1993 and Fig. 6). The AChR is found in the neuromuscular junctions of embryos and adults, although in the adult animal the gamma subunit is replaced by epsilon (ϵ) (Hucho 1993). This stoichiometry has been conserved during the evolution of such diverse taxa as rays and mammals, indicating an an-

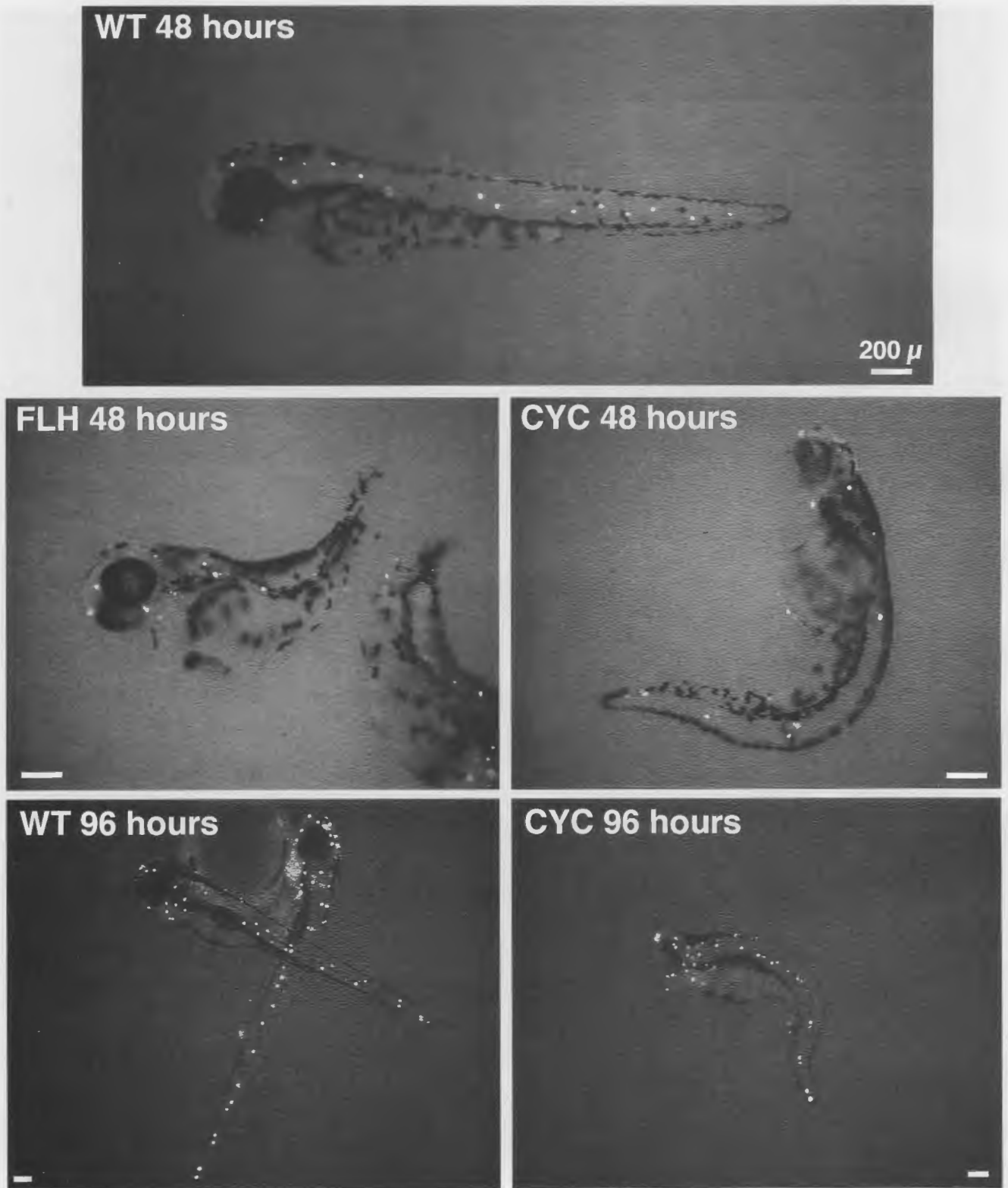


Fig. 5. Lateral line development in wild-type and mutant zebrafish. Of the two mutations analyzed, *floating head* disrupts lateral line development while *cyclops* does not. The hair cells of the lateral line neuromasts are labeled with 4-Di-2-Asp. All images are fluorescent views.—A. Wild-type zebrafish at 48 hours.—B. *floating head* mutant at 48 hours.—C. *Cyclops* mutant at 48 hours.—D. Two wild-type zebrafish at 96 hours.—E. *Cyclops* mutant at 96 hours. All scale bars equal 200 μm .

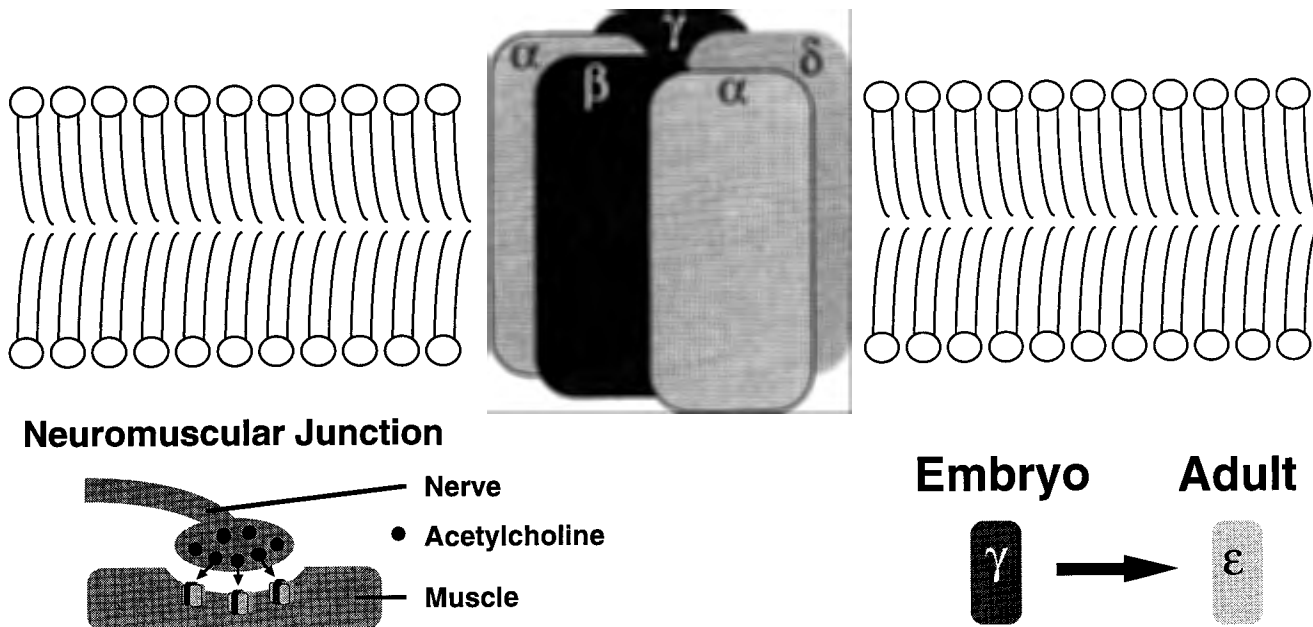


Fig. 6. The muscle acetylcholine receptor. The five subunits are shown diagrammatically in a muscle cell membrane, their relative positions is one of the more likely arrangements (Hucho 1993). The positions of the receptors in a neuromuscular junction are shown at bottom left: arrows in synapse show route of acetylcholine to its receptor. In the adult animal the gamma subunit is replaced by epsilon (ϵ).

cient divergence of these subunit genes. The genome duplication, in contrast, provides copies of these genes that are relatively recent in terms of evolutionary time. The possible composition of the embryonic AChR from a pool of eight genes of four types (α , α' , β , β' , γ , γ' , δ , and δ') provides a complex context for the study of developmental redundancies.

The sequences of one copy of each *Xenopus* subunit except beta are known (Baldwin et al. 1988) and for the alpha subunit a second copy has been identified (Hartman and Claudio 1990). Interestingly, these two alpha subunits differ in their timing of expression. One alpha gene is expressed in both the oocyte and developing muscle while the other is expressed only in muscle development (Hartman and Claudio 1990). These temporal differences in expression must have evolved within the last 30 million years. The beta AChR subunit has been cloned and partially sequenced (Bill Todd, unpublished data from University of California at Irvine). *In situ* hybridizations using this probe found expression in the developing muscles (Fig. 7). Future goals are: 1) to clone copies of the other *Xenopus laevis* subunits as well as multiple subunits in other species of Pipidae (the family containing *Xenopus*), preferably with differing numbers of ploidy events (Kobel and Pasquier 1986); and 2) to study their expression patterns in more detail. Good phylogenies, both morphological and molecular, are available for the family (Cannatella and Trueb 1988; Carr et al. 1987) so the analyses can be done within an evolutionary context.

What about the possibility of combining whole-

mount *in situ* hybridizations with lineage analysis? We have been collaborating with several people (Nancy Papalopulu, Chris Kitner, and Gail Burd, the first two at the Salk Institute, the last at University of Arizona at Tucson) and the answer appears to be that it is possible (Fig. 8). After labeling a single cell in the 8–16-cell-stage *Xenopus* embryo (stages 4–5) with a fluorescent dextran and raising the embryo to a late neurula, early pharyngula stage (stage 25); we found that most of the labeled cells were restricted to the neural tube (Fig. 8C). New chromogenic substrates for the alkaline phosphatase enzyme used in most *in situ* hybridization protocols allow for double labeling analyses (Hauptmann and Gerster 1994). One of these substrates, Fast Red, emits at a wavelength distinct from that of fluorescein, allowing these two labels to be distinguished (Fig. 8B,C). By using a probe specific to neural tissue (β -tubulin), the neural tube was Fast Red stained (Fig. 8A). Not only did the fluorescein labeling survive the *in situ* protocol (we scored the embryo before and after) but double labeling could be distinguished in regions of the anterior neural tube (Fig. 8). Microinjection at these early cleavage stages deposits far more dye than the single-cell microinjection protocol used at later stages because the cells are much larger. A more challenging test would be *in situ* hybridization on embryos in which a single cell had been labeled using iontophoretic microinjection at later stages. In approximately half these embryos, the label could still be seen after having undergone the *in situ* hybridization procedure.



Fig. 7. In situ hybridization with an antisense probe to the β subunit of the acetylcholine receptor in a stage 20 *Xenopus* embryo; anterior is right. The label is restricted mainly to the prospective muscles. Scale bar equals 100 μm .

These results represent a sample of the data collected and questions addressed using current techniques. Their potential insights for developmental biology are great. These techniques have revealed previously undescribed migratory pathways and derivatives of the neural crest. The need to explore the issue of developmental redundancy and its ramification for evolution becomes more pressing. Combining different techniques such as lineage analysis and *in situ* hybridizations may provide further developmental insights. Understanding the number of genes involved in the development of given structure is an important step in studying the relationships between genes and morphology. All these examples reveal that it is critical to understand normal development so that experimental perturbations can be interpreted in the correct context. The next section will more explicitly relate these developmental data and methodologies to systematics and evolutionary biology.

Homology

We have discussed how new methods can better define developmental mechanisms. What does this un-

derstanding mean to evolutionary studies? The issue of morphological homology is central to evolution and should be strongly influenced by developmental concepts and results. The concept of homology is fundamental to comparative biology and systematics (Donoghue 1992; Hall 1994; Patterson 1982; Wagner 1989a); without it, one would not be able to determine which characters are comparable. Morphological homology can be distinguished from molecular homology, which presents some similar issues (Patterson 1988). We will not discuss molecular homology except as it relates to morphological homology. We use the following definition of homology, similarity due to continuity of information, because it is the most inclusive (Haszprunar 1992; Van Valen 1982). The definition of homology has changed through time, from its origin in pre-Darwinian times to its current evolutionary usage. Although its importance is not doubted, morphological homology has been difficult to define (de Beer 1971) resulting in several definitions which are not mutually exclusive (Patterson 1982; Roth 1988; Wagner 1989a): 1) classical or idealistic homology—this type is pre-Darwinian in origin and typ-

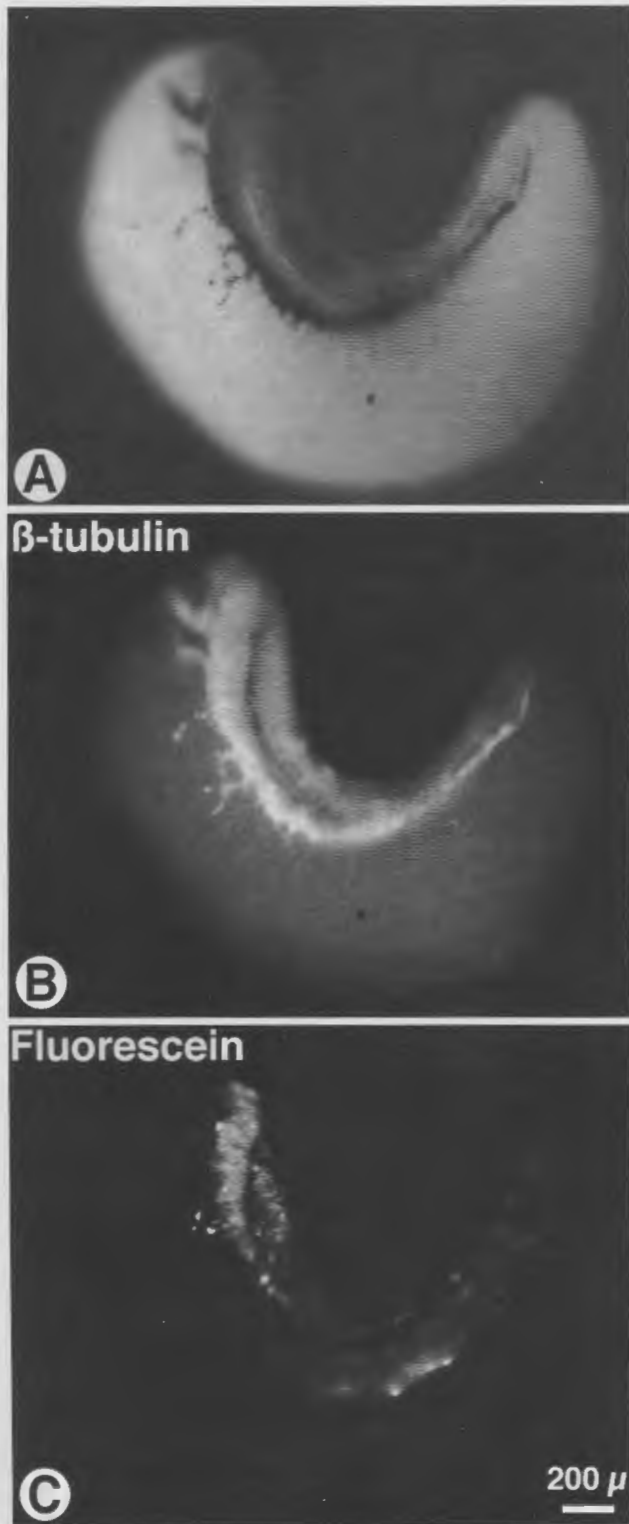


Fig. 8. Whole-mount *in situ* hybridizations can be combined with lineage analysis. The stage 28 embryo is double labeled with a lineage dye and a probe to β -tubulin.—A. Bright-field image of embryo in which the Fast Red labeling appears dark.—B. Fluorescent image of the Fast Red β -tubulin staining in the neural tube.—C. A single blastomere was injected with fluorescein at an early cleavage stage. The resulting progeny are restricted to the anterior neural tube. The *in situ* hybridization with β -tubulin was done by Nancy Papalopulu.

ically consists of essentialist concepts (similarity as implied by a Bauplan or related concept, Jones 1996); 2) evolutionary or historical homology—this is the most generally used and involves the origin of a structure from a common ancestor; 3) Phenetic homology, based on overall similarity and operationally derived from phenetic systematic techniques though some definitions harken back to those of classical homology (Patterson 1982); 4) Cladistic homology, which equates homology with synapomorphy, sometimes grouped as a subcategory of historical (Wagner 1989a); 5) Biological homology which uses common developmental mechanism as the basis for identifying homologies. The following treatment applies to at least three of these definitions: evolutionary, cladistic and biological homologies. These three definitions also are subsumed under one more inclusive definition by Roth (1988), who termed it biological homology. While definitions are important, the critical issue for our discussion is the practical identification of homologies. For this, one needs to know what criteria need to be met to identify homologous characters in two different organisms.

The most important issue when using the homology concept, often repeated by researchers (see Patterson 1982), is the necessity for a conditional phrase (but see Nelson 1994, pp. 128–129 for a contrary view). Two structures are not just homologous, they are homologous as something. One example, discussed below, is the homology of insect and vertebrate eyes (Barinaga 1995). They may be homologous as sensory organs but they are not homologous as image-forming organs. Another example is the homology of bird and bat wings which are homologous as forelimbs but not homologous as wings. Often in discussions of homology the conditional phrase is understood but it is important for researchers to be unambiguous.

Criteria for homology.—There are three primary criteria (called tests by Patterson 1988) for morphological homology: positional, phylogenetic, and developmental. In order to define and illustrate these criteria we will use the classical example of the vertebrate forelimb, specifically its bones (Coates 1994; de Beer 1964; Hinchliffe and Johnson 1981; Hinchliffe 1994; Shubin 1994). The forelimb is a complex structure in most vertebrates consisting of numerous tissue types (bone, cartilage, muscle, tendon, nerve, fat, dermis and epidermis). The evolutionary history of forelimbs offers a good example of the bewildering array of forms producible by natural selection (Fig. 9). Forelimbs may function as flippers or wings as well as terrestrial limbs. The forelimbs of tetrapods (group defined in Fig. 1) consist, from proximal to distal, of the following bones or bone groups (Fig. 9): humerus (from shoulder to elbow in humans), radius and ulna (parallel

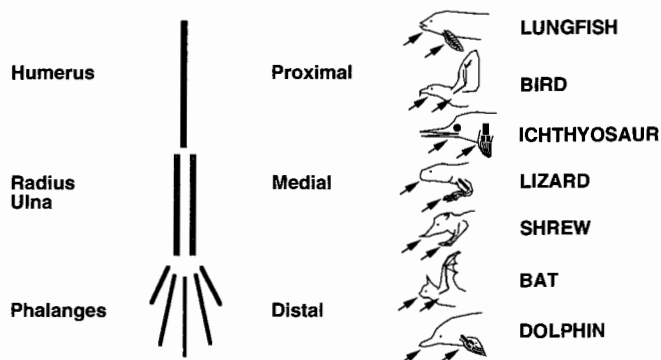


Fig. 9. Positional criterion for homology of foreappendage and some bones in seven species. The bones of the forelimb and their proximal to distal relationships shown to the left; medial bone(s) indicated with shaded lines: two arrows point to presumed homologous positions. All of the taxa are Amniota but lungfish (see Fig. 1). (Not all of the podial elements are shown).

from elbow to wrist), mesopodium (carpals, a group of wrist bones), metapodium (metacarpals, a group of bones in the palm of hand), and phalanges (the many bones of the fingers).

The positional criterion (called topographic by Patterson 1982) requires that two structures in two organisms occur in the same relative positions to be considered homologous. To identify the homology of foreappendages using the positional criteria, common landmarks, such as the eye or mouth (Fig. 9, left arrows), must be found, and the positions of the proposed homologues relative to the landmark must be the same (Fig. 9, right arrows). From this we could say that the flipper of an ichthyosaur is homologous to the pectoral fin of a lungfish and wing of a bat. The compared structures do not have to appear similar, although it makes comparisons easier if they do, such as in the case of the lungfish, ichthyosaur and dolphin flippers. Homology is a hierarchical concept; that is, homologues can be nested under other homologues. The forelimb example illustrates this nicely. We can compare whole forelimbs across these seven species or we could compare individual forelimb bones such as the humerus. Again using the positional criterion, this time within the forelimb, we say that the most proximal single bones are homologous. Such positional comparisons can also be made for the medial paired bones and the distal phalangeal elements. Whereas comparisons are relatively easy to make among the six tetrapod species, determining which if any bone in the lungfish pectoral fin is homologous to the humerus or any of these other forelimb bones is much more problematic. Even within tetrapods, problems arise in determining these homologies because of fusions of the paired medial bones in bats, phalange reduction in birds, and the phalangeal proliferation in ichthyosaurs. Numerous examples exist where the positional criterion alone can be misleading or difficult to apply: the

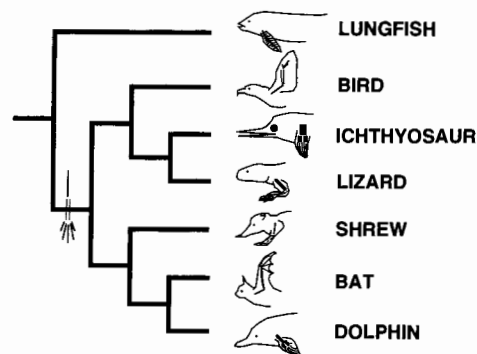


Fig. 10. Phylogenetic criterion for homology of foreappendage and some bones forming in seven species. The phylogenetic hypothesis shown is from Maisey (1986), Novacek et al. (1988), and Pough et al. (1996). Relationships of the three mammals (shrew, bat, dolphin) are tentative (Novacek et al. 1988). The medial bone(s) indicated by shaded lines.

eyes of the derived teleost order pleuronectiformes (over 500 flatfish species) that form bilaterally only to migrate to the same side of the body (Policansky 1982; Nelson 1984); the anus of the teleost fish *Aphredoderus sayanus* Gilliams (pirate perch) which ends up between the gill membranes, anterior to all its fins (Nelson 1984); and the relative positions of inflorescences and leafy branches in different plant species (Coen and Nugent 1994). Although the positional criterion has its problems, it is the practical basis for a large number of defined homologies, is often the starting point for the other criteria, and is interesting when contradicted.

The phylogenetic criterion (congruence test of Patterson 1982) is currently the most used criterion for homology. This criterion equates homology with synapomorphies (shared derived characters) and uses the methodologies of cladistics such as parsimony and outgroup comparisons to determine homology (Patterson 1982). Going back to our limb example, the specific bones (humerus, radius, etc.) of tetrapod forelimbs are homologous because the ancestor of the six tetrapod groups shown had them and these bones are difficult or impossible to identify in extant outgroups (Fig. 10). The cladogram illustrates a conservative hypothesis for the phylogenetic relationships of these seven species (Maisey 1986; Novacek et al. 1988; Pough et al. 1996). Whereas forelimb characters may have been used in its construction, tautology can be avoided by independent corroboration with molecular data (Akam et al. 1994; but see Kluge 1989 about the necessity of combining all data sets). Patterson (1982) considers the phylogenetic criterion the most powerful because it is the only criterion that distinguishes the relations useful to systematics (see discussion following on Patterson's proposals for homology criteria). The biggest problem in using the phylogenetic criterion for homology is identifying convergence and par-

allelism (homoplasy or nonhomology as interpreted by Patterson 1982). The degree of homoplasy varies among taxa and its detection by phylogenetic methods becomes more difficult as homoplasy increases (Wake 1991). The phylogenetic criterion, unlike the positional, restricts comparisons to monophyletic groups, such that the features of a dolphin are compared to those of the bat or shrew (other mammals) before those of the superficially similar ichthyosaur (a diapsid reptile) for determining homologous bones.

Perhaps the most powerful feature of the phylogenetic criterion is that it allows one to propose transformations from a hypothesized ancestral condition. Three examples of vertebrate transformations are reduction of phalangeal elements in birds, increase of these elements in ichthyosaurs, and transformation of two medial elements to one in bats. However, neither the positional nor the phylogenetic criterion provides a mechanism for the hypothesized transformations, for this we must look at the data used in the final criterion (Roth 1991; Wagner 1989a).

The developmental criterion has been used classically for identifying homologies (Patterson 1982), although more recently its popularity has waxed (Roth 1984; Wagner 1994) and waned (Hall 1995; Striedter and Northcutt 1991). Two structures are homologous if their development is similar. This similarity can be based on the genes expressed, tissue type(s) contributing to the structure, inductive events, relative timing of developmental events, cell lineages and/or morphogenetic processes. However, the aspect of development typically compared has been classical descriptive embryology using histological sections or whole-mount preparations. Returning to the forelimb example, development becomes a particularly appropriate criterion because it may provide a simpler context for understanding the great complexity and variety found in adult limb morphologies and because of the many studies of limb development. The amount of information on the role of specific molecules in limb development is expanding though integrating these results with the data on embryological manipulations and models of limb development remains difficult (Javois 1984; Johnson and Tabin 1995; Morgan and Tabin 1994). The development of the six species fore-appendages supports the homology of these structures given their common embryological origin from limb buds at the first stage shown (Fig. 11, left). Differences between them based on external features become apparent later in development (Fig. 11, third column). Whereas the developmental sequence of forelimb bone appearance in most of these species has not been characterized it should be possible using several whole-mount techniques (Hanken and Wassersug 1981; Hanken et al. 1992). The absence of the ichthyosaur from the comparison in Fig. 11 highlights a difficulty with

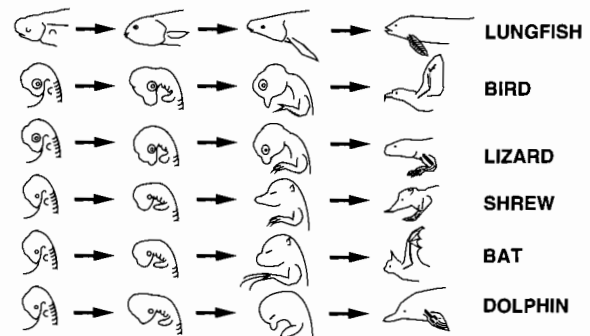


Fig. 11. Developmental criterion for homology of foreappendage and some bones in six species. Four comparable stages for each species shown from left to right, separated by arrows. Earliest stage to the left represents early limb/fin bud stage; fourth stage is the adult condition. The three mammals have relatively reduced eyes during first two developmental stages compared to bird and lizard. Lungfish stages from Kemp (1982) and some remaining stages derived from Gilbert (1991). The medial bone(s) indicated with shaded lines.

the developmental criterion. Its use is limited to extant species because the development of fossils can only be the subject of speculation.

Patterson (1982) also lists three criteria of homology. His differ in that he lumps positional and developmental under the criteria similarity, uses the term congruence for phylogenetic and identifies a third criterion, conjunction (homologous structures are not found in the same organism). The reason we do not consider further the conjunction criterion is that it only separates iterative homology (repetition of the same structure within an individual, also called homonymy and, if segmentally arranged, serial homology) from the rest of homology and nonhomology. We believe, as others (Roth 1984, 1988; Wagner 1989a), that iterative homology should not be separated from discussions of homology as is often done (Striedter and Northcutt 1991). Many structures in animals that are not discussed as iterative homologues are bilaterally (e.g., sensory structures of vertebrates) or pentamerically (e.g., echinoderm arms or plates) repeated. Iterative homology still provides useful characters for systematic analyses (Patterson 1982). A better way to deal with the difference between iterative homology and homology is a hierarchical framework for the comparisons. In this way iterative homologues are compared as whole sets or members of sets to other iterative homologues (Hall 1995). Examples of such comparisons are all the leaves of a plant with all those of another, all somites in an animal with all those of another or tail somites with those of another. Trying to determine the homology of individual units of an iterative series may not be a productive endeavor (Hall 1995). Wagner (1989a, b) has stated that it is necessary to find the correct developmental unit for the successful implementation of these comparisons (one that

has undergone individualization, see conclusion). Patterson (1982) states that structures are homologous only if they pass all three of his criteria (conjunction, similarity and phylogenetic) and distinguishes between eight different named relations based upon passing and/or failing these three criteria (Patterson, 1982, p. 47 and Table II). Though appealing, such homology definitions suffer from the problem that structures known to be homologous can fail one of these criteria. This raises the issue of how can structures be "known" to be homologous if they do not meet reasonable criteria. In these cases homology determination involves a weighting of criteria, placing more value on one over another, typically phylogenetic over developmental.

Many workers have pointed out that homologous structures may have different developmental mechanisms (Alberch et al. 1985; Roth 1988; Striedter and Northcutt 1991; Wagner 1989*a, b*) as expected, given the hierarchical nature of biological processes (Eldredge 1985; Roth 1991; Striedter and Northcutt 1991). This is not a universal opinion as some workers do not consider structures homologous if their development differs (Alberch et al. 1985; Goodwin 1994). The end result of this confusion has been a reluctance to use developmental data for determining homologies (except, of course, by those who consider development the sole criterion or when it is congruent with other criteria) and a greater dependence on the phylogenetic criterion. However, in the next section we will explore the developmental data available for one example (primary versus secondary neurulation) in more detail with some surprising results.

Reexamining the developmental criterion for homology.—In contrast to the somewhat downbeat note regarding the utility of the developmental criterion above, we propose that the developmental criterion can be a powerful determinant of homologies, in many cases equal to the utility provided by the phylogenetic criterion. Two problems contribute to the decline in use of the developmental criterion: 1) a paucity of detailed comparative developmental data; and 2) the overlooked assumption that ontogenies can not change during evolution and remain homologous while morphologies can. We use the term ontogeny to mean the set of developmental mechanisms, which can be quite numerous, that form a particular structure. The first problem can be addressed by collecting more developmental data in more species, in particular using the modern methodologies described previously. More developmental data collected with modern and experimental methodologies are crucial for developmental and evolutionary studies (Müller 1991). This is where a phylogenetic perspective becomes critical as it is unlikely for one scientist or laboratory to be able to study more than a handful of the extant species available. A

phylogeny allows one to choose those species most likely to provide important and relevant developmental data based on their proposed evolutionary relationships. A common assumption of many homology discussions is that homologous structures can have different and therefore nonhomologous ontogenies (Hall 1995; Striedter and Northcutt 1991; Wagner 1989*a, b*). The corollary to this assumption is that different ontogenies can not be homologous—a statement that seems to weigh one meaning of homology, similarity, more than another, continuity of information. This dual nature of homology is an important point that is rarely emphasized (Donoghue 1992 and Roth 1994 provide notable exceptions). Since neither morphological nor functional similarity is necessary to call a structure homologous (compare the flipper and wing from our forelimb example, Fig. 9–11), it seems inconsistent to require extreme similarity in homologous ontogenies. A broadly comparative view of developmental data could reveal overlaps in one or more of the many developmental mechanisms that make up an ontogeny, allowing different ontogenies to be called homologous. Workers in the field recognize that ontogenies change during evolution (Roth 1988; Striedter and Northcutt 1991; Wagner 1989*a*). The problem in seeing the common basis of these ontogenies is that the comparisons typically are made between very few species, often across great evolutionary distances. We, as Roth (1984, 1988) and Wagner (1994), believe that ontogenies consisting of different developmental mechanisms can still be homologous and will expand on their writings.

By incorporating more comparative developmental data and the ramifications of modern developmental biology into discussions of homology, a more robust developmental criterion can emerge. Development that seems superficially different using classical embryological techniques may actually be revealed as much more similar using modern cellular and molecular biological techniques (see neurulation example, and section on molecular data following). For our purposes the most important consequence of developmental biology for evolution is the notion of developmental redundancies, multiple mechanisms that may underly a given process or structure's generation. Typically such redundancies are discussed at the molecular level (Chadwick and Marsh 1992, pp. 298–300; Tautz 1992). However, they also can exist at the cellular level. For example, the source for a tissue can be redundant as portions of the neural tube normally restricted to form central nervous system can be recruited to form neural crest after crest ablations (Scherson et al. 1993). In mice targeted mutagenesis, producing gene "knockouts," provides a powerful tool for analyzing development (Bedington 1992). Knockout studies have revealed that molecules thought to be important

for a given developmental event are not essential for that event (i.e., the role of activins in mesoderm induction; Matzuk et al. 1995*a, b, c*; Smith 1995). Developmental redundancy is typically invoked to explain such conundrums in two ways: 1) another gene product normally expressed in the embryo compensates for the missing gene product; and, 2) eliminating a gene may cause the upregulation and/or downregulation of one or more gene product(s) which compensate(s) for the defect (such as in the upregulation of the βA activin subunit in the ovary when βB is eliminated; Vassalli et al. 1994). The absence of a gross phenotype from a knockout is not proof that the targeted gene is uninvolved in normal development (Routtenberg 1995). Possibly, the phenotype observed is too subtle to be detected by the most commonly used diagnostic tools or the occurrence of weak or absent phenotypes may indicate developmental redundancies. This explains the need to generate double and triple mutants to observe dramatic phenotypes (e.g., Wurst et al. 1994). The redundancies revealed in knockout experiments and the regulative ability of the neural tube (Scherson et al. 1993) indicate that there can be multiple developmental mechanisms, each sufficient for the formation of a given structure.

Probably the strongest argument against developmental processes being homologous comes from regeneration studies (Hall 1995). For example, regeneration of amphibian limbs seems to differ from the original development in terms of the role retinoic acid plays (important in regeneration but unnecessary during development; Brockes 1994) and the need for innervation (required for regeneration but unnecessary during development; Wagner 1994). This could lead one using a restrictive developmental criterion to conclude that a regenerated limb or tail is not homologous to the original, an untenable position. Although regeneration is often thought of as a model for studying development there is at least one major difference: a developing limb bud is much smaller than the regenerating limb's blastema (Brockes 1994). This dramatic geometric difference may confound comparisons because mechanisms that operate at one scale may not be able to function similarly at another. Also the size differences may obscure similar mechanisms, such as the need for the same molecular factor in limb patterning, by requiring different cellular sources—a hypothetical example would be mesodermal cells for the developing limb bud and innervating neurons for regeneration. Where else might similarities between developmental and regenerative processes be revealed? Wagner (1994) has distinguished between morphogenetic mechanisms, those involved in the generation of a structure, and morphostatic mechanisms, those involved in the maintenance of a structure. Both mechanisms are important for the development of a struc-

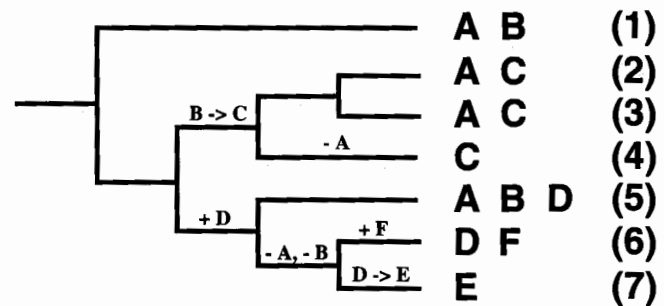


Fig. 12. Hypothetical example of developmental changes during evolution. Seven taxa and their known evolutionary relationships are indicated by numbers on right. Possible developmental mechanisms involved in ontogeny of a given homologous structure indicated by A–F. Homologous structures can be formed by several different mechanisms and combinations thereof. The evolutionary transformations that lead to this pattern are indicated on the phylogeny. Looking at just the developmental mechanisms (letters A–F) listed for each taxon one notes that at least one mechanism is shared with another taxon for six of the seven taxa.

ture and should be considered when using the developmental criterion. While most developmental studies concentrate on morphogenetic mechanisms it may be the morphostatic mechanisms that are more important for understanding regeneration. A more robust developmental criterion should include the role of morphostatic mechanisms on development, revealing the commonalities between development and regeneration.

To better understand how different sets of developmental mechanisms can still be homologous, we use a hypothetical example with seven taxa whose relationships are known (Fig. 12). For each taxon we describe the ontogeny of one structure, homologous to those of the other taxa, which may consist of up to three different mechanisms, indicated by letters (A–F). These developmental mechanisms can represent many things: specific molecules, different tissue types, or distinct morphogenetic processes. Taxon 1 represents the ancestral condition which results from mechanisms A and B. If developmental data were only available for taxa 1, 4, and 7 the conclusion that the homologous structure is formed by nonhomologous ontogenies would appear reasonable, because the different developmental mechanisms (A, B, C, E) are quite distinct. This is where the illustrative power of more comparative data becomes clear. With data from more taxa, the overlap in developmental mechanisms and the possible transformations that occurred during the evolution of the structure's ontogeny, are revealed. During evolution of the hypothetical structure, developmental mechanisms producing the ontogeny can change in three ways (Striedter and Northcutt 1991): by a transformation to another mechanism (A+B to A+C), deletion of a mechanism (A+C to C), and addition of a mechanism (A+B to A+B+D). Striedter and Northcutt (1991) use such differences in developmental

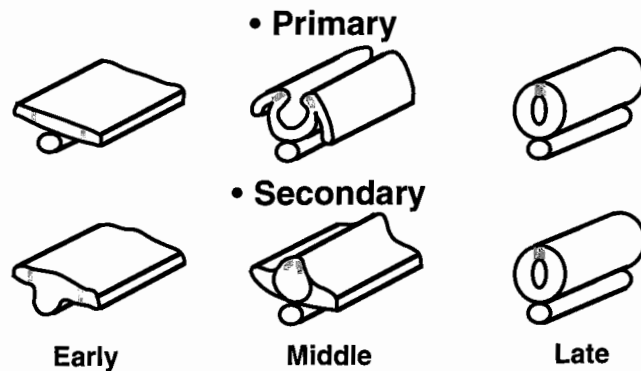


Fig. 13. Neurulation. Primary versus secondary: three stages in development of a neural tube showing proposed morphogenetic events. Note that during primary neurulation a groove is formed (middle stage). This is not true for secondary neurulation. Prospective neural crest cells are indicated with shaded lines. These cells are located at the border between neural plate and epidermis.

mechanisms to argue that different ontogenies are not homologous, the opposite of what we would conclude. Given that during evolution ontogenies can change (Alberch 1985; De Queiroz 1985; Striedter and Northcutt 1991), and that quite different looking structures (Fig. 9–11) can still be called homologous, it seems logical that it should still be possible to consider the different sets of developmental mechanisms that generate those structures to be homologous. With sufficient developmental data from intermediate species, the overlap in or similarity between mechanisms should become clearer. A major problem is in deciding the appropriate unit or level for developmental comparisons (Wagner 1989a, b) an issue to which we will return. To clarify these hypothetical examples, we discuss two actual examples.

Primary versus secondary neurulation.—A classic example of a homologous structure that forms by different developmental mechanisms is the vertebrate neural tube (Striedter and Northcutt 1991). Vertebrate neural tube homology is based on criteria other than development. After gastrulation is completed, the prospective neural tube is a superficial sheet or keel underlain by the prospective notochord (Fig. 13), ready to begin neurulation. In many vertebrate groups, including tetrapods, neurulation occurs by the rolling up of the neural plate until the lateral edges meet, forming the dorsal neural tube, in a process called primary neurulation (Fig. 13). The cellular mechanisms by which primary neurulation occurs have been extensively studied (reviewed by Jacobson 1991). Teleosts, in contrast, form a neural tube directly from a superficially amorphous mass of cells by cavitation in which no groove or fold is visible. This process is called secondary neurulation (Fig. 13). Secondary neurulation also occurs in the tail bud of all vertebrate species so far examined (Griffith

et al. 1992) including those that undergo primary neurulation anterior to the tail bud.

The cellular processes by which secondary neurulation occur are not well known (Nieuvestein et al. 1993; Tucker and Slack 1995) but they are presumed to be quite different from those of primary neurulation. However, recent work on the teleost zebrafish using single-cell labeling suggests that the anterior neural tube forms by a mechanism similar to that of primary neurulation (Papan and Campos-Ortega 1994). If cavitation were occurring, the expectation would be that a cell labeled shallow and medial in the neural keel would contribute to the dorsal portion of the neural tube, while more lateral cells should end up in the lateral neural tube. Surprisingly, labeled cells in the medial neural keel contribute to the ventral tube, while those located laterally contribute to the dorsal tube; labelled cells in between contribute to the appropriate intermediate region of the neural tube. In Fig. 13 the positions of the prospective neural crest (initially located at the lateral edges of the neural plate) are indicated by shaded lines, with the positions in the secondary neurulation diagram being based on the results of Papan and Campos-Ortega (1994). The neural crest offers a good landmark for orienting their results because their subsequent migration from the neural tube makes them easy to score and their initial induction appears to result from the apposition of epidermis and lateral neural plate (Selleck and Bronner-Fraser 1995). These data offer persuasive evidence that cell movements similar to rolling occur in the anterior neural tube of at least one teleost species. If these results prove to be general for teleosts, the ramifications for the homology of neurulation are obvious: a homologous structure would be shown to develop by homologous rather than nonhomologous developmental mechanisms, vindicating the developmental criterion.

In the preceding section we argued that homologous ontogenies can consist of different sets of developmental mechanisms, so let us suppose that even though secondary neurulation may share some similarities with primary, it is still different from primary neurulation. The neural tube in many vertebrate species (chickens, frogs, salamanders, mouse, rat, opossum, pig) forms by both neurulation types (Griffith et al. 1992) suggesting to us that homologous development can use either or both types. Figure 14 plots the available data on neurulation type for six vertebrate taxa (Griffith et al. 1992; Nelsen 1953) onto a cladogram. No species has only primary neurulation—all species with primary also have secondary neurulation. Three taxa have only secondary neurulation, the Petromyzontida (lampreys), ginglymodi (gars), and teleostei. Even though data are not available for almost half the listed taxa, the distributions of combined primary and secondary versus only secondary neurulation are quite

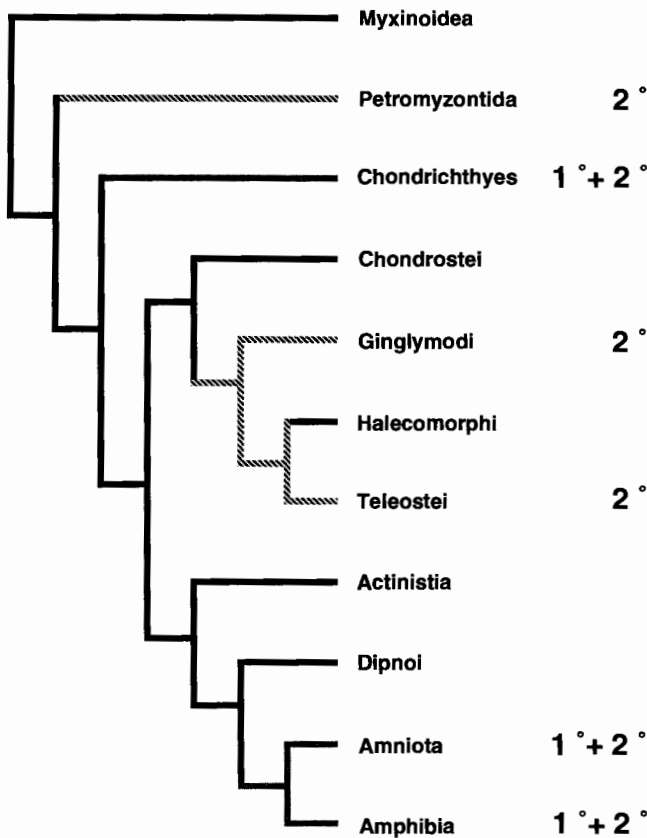


Fig. 14. Phylogenetic distribution of primary and secondary neurulation in craniates. For those species with both types of neurulation, secondary neurulation is occurring in the tail. There is no example of a taxon with just primary neurulation along its whole rostrocaudal axis. While it is known that Dipnoi have primary neurulation (Kemp 1982) it is not known what is happening in the tail. For the common names of all taxa shown see Fig. 1.

mixed. Based on the available data it is difficult to determine which state represents the ancestral condition. Neurulation in the outgroup to craniates, cephalochordates (amphioxus), appears to be primary but the developmental data are not easy to interpret (Nelsen 1953). Given this uncertainty, it appears that anterior neural tube formation changed during craniate evolution from primary to secondary neurulation and/or from secondary to primary neurulation in one or more taxa. While more data are necessary, especially those collected with modern developmental methodologies (e.g., Papan and Campos-Ortega 1994), the available data are sufficient to support the proposition that a broadly comparative developmental criterion can be useful for determining homologies. In this more inclusive definition of the developmental criterion we can see that different sets of developmental mechanisms can be homologous. This is particularly obvious when both mechanisms are used in the same individual.

Embryonic origins of the lateral line.—Earlier we mentioned our rather surprising finding that neural crest contributes to lateral line neuromasts (Collazo et

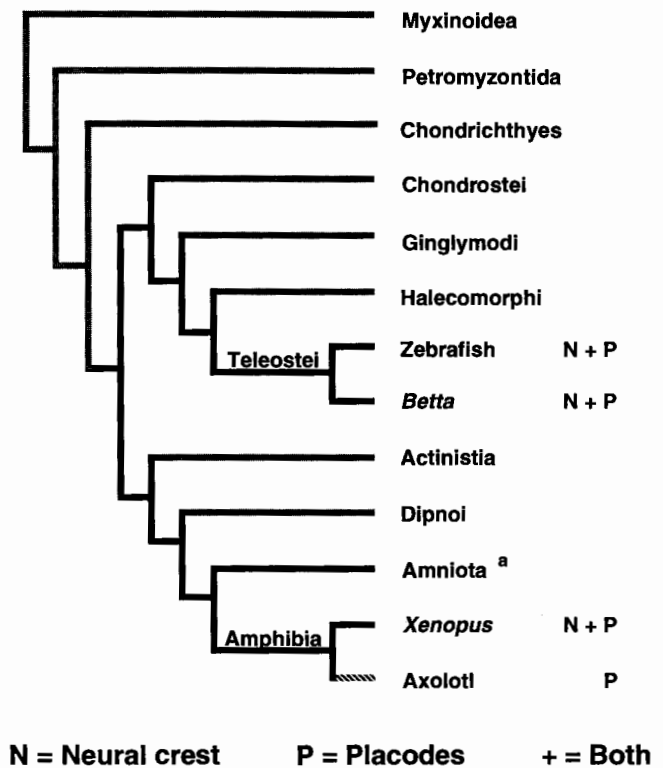


Fig. 15. Phylogenetic distribution of embryonic tissue origins for neuromasts in craniates. Dual embryonic origin for neuromasts has been described in three taxa studied: siamese fighting fish (*Betta splendens*), zebrafish (*Danio rerio*) and african clawed frog (*Xenopus laevis*). In a fourth species, the axolotl (*Ambystoma mexicanum*), only a placodal origin has been seen so far (Northcutt et al. 1994). Amniota do not have a lateral line.

al. 1994). Two tissue types, neural crest and placodes, contribute to the formation of lateral lines though it remains to be determined if these contributions could be to two distinct neuromast populations. Such a segregation is unlikely given that not all of the cells in a given neuromast are always labeled and that both labeled and unlabeled neuromasts are often intermixed (Collazo et al. 1994). Looking at the data in a comparative and phylogenetic context reveals a rather sparse taxonomic representation of three species (two teleosts and one amphibian): *Danio rerio*, *Betta splendens*, and *Xenopus laevis* (Fig. 15). In another amphibian, the axolotl *Ambystoma mexicanum* Shaw, a salamander, it has been clearly demonstrated that the lateral line is formed by placodes (Northcutt et al. 1994). Although the possibility of a neural crest contribution remains (Northcutt et al. 1995) we assume that the axolotl lateral line is derived solely from placodes. Determining whether a dual embryonic origin for neuromasts is ancestral for vertebrates (Fig. 15) will require data from more species. The phylogenetic distribution of lateral line tissue origins for the four species reveals an analogous situation to that seen in the neurulation example (Fig. 14, 15). The most par-

simonious interpretation is a loss of the neural crest contribution to the neuromast in the lineage leading to the axolotl. Phylogenetically we describe these absences as losses but developmentally we do not necessarily mean to infer that there are fewer neural crest cells or neuromasts. Neural crest cells that formerly contributed to neuromasts may now form other structures and/or placodal cells may proliferate to compensate. In looking at this and the previous example on neurulation it is important to remember that phylogenies provide an evolutionary context for comparisons and may suggest possible transformations but they do not tell us the biological mechanisms of these transformations (Roth 1991).

The apparent differences in neuromast embryonic origins provide another example of homologous structures (lateral lines) that appear to develop by different mechanisms, in this case in the form of different tissue contributions. We consider lateral line development homologous because we look across many species to see the total variation in ontogeny produced during evolution. The mechanisms may change during evolution but this is expected and does not change the fact that the different sets of developmental mechanisms utilized in lateral line formation of different species are homologous any more than variation in forelimb morphologies alters the fact they are homologous. This argument for lateral line development homology does not even consider the underlying similarity of neural crest and placodes revealed by their close evolutionary association (Northcutt and Gans 1983), common ectodermal origin, and ability to compensate for each other when one is ablated (Kirby 1988*a, b*). A more broadly comparative developmental criterion, based on a deeper understanding of developmental processes and collected with modern cell and molecular methodologies, provides a powerful criterion for homology. There is the concern that such a broad application of the developmental criterion may lead to everything being called homologous but this is not the case (see the section below on using the developmental criterion to determine if structures are nonhomologous).

Seductiveness of molecular data.—A potentially powerful way to determine morphological homologies is to use underlying molecular homologies that may be involved in the development of a structure (Roth 1984). This approach is subject to misinterpretation, but when the results are congruent with available non-molecular data, they provide further support that the structures are homologous. The involvement of the same gene (*eyeless* in the fly *Drosophila* or *Pax-6* in the mouse) in the development of *Drosophila* and mouse eyes, even the ability of the mouse gene's regulatory region to substitute for that of *Drosophila*, are remarkable examples of functional conservation

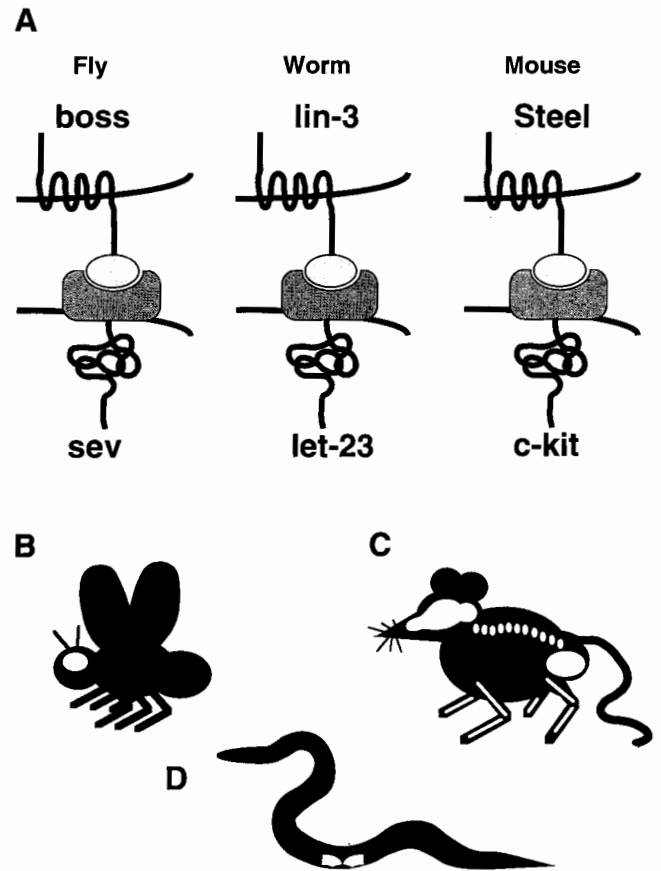


Fig. 16. Homologous genes are not active in homologous tissues.—A. Receptor-ligand pairs of homologous genes in fly, worm and mouse. Ligand on top and receptor on the bottom. In the figure the structure of the molecules are known in fly and somewhat in the worm but not in mouse. The figure shows them all the same because they share sequence homology. Both the receptor and ligand are trans-membrane. See the text for references.—B. In the fly the genes are expressed in the eye.—C. In the mouse the genes are expressed in many different tissues. See text. —D. In the worm the genes are expressed in the vulva.

through evolution (Barinaga 1995; Halder et al. 1995; Quiring et al. 1994; Zuker 1994). Ironically, the *eyeless* gene of *Drosophila* was used by de Beer (1971) as an example of a gene that does not support eye homologies because fly lines with a complete loss of function mutation in their *eyeless* gene could regain their eyes by selection for mutations in modifier genes, meaning that flies without the *eyeless* gene product could still have eyes.

Another example of homologous molecules acting during development provides reasons to be cautious. It involves two proteins, a tyrosine kinase receptor and the receptor's ligand, a transmembrane protein. The homologous genes (Fig. 16) in four species are (ligand first): 1) *boss* and *sev* in two *Drosophila* species (Cagan 1993; Cagan et al. 1992; Hart et al. 1993), 2) *lin-3* and *let-23* in the worm *Caenorhabditis elegans* (Hill and Sternberg 1992), and 3) *Steel* and *c-kit* in mouse (Huizinga et al. 1995; Reith

and Bernstein 1991a; Rottapel et al. 1991). These genes act as receptor/ligands, are involved in intracellular signal transduction of specific structures, and are functionally relevant during development. However, these genes are not expressed in homologous structures (Fig. 16B–D). They are expressed in the fly eye, in the vulva of the worm, and in many tissues in the mouse (Motro et al. 1991) including parts of the peripheral nervous system, brain, germ cells, melanocytes and hematopoietic stem cells. The function of *Steel* and *c-kit* in the latter three structures in mouse is particularly well documented (Geissler et al. 1988; Reith and Bernstein 1991b). Thus, although across these species these genes are from homologous families, they are not active in homologous tissues. In fact, it is not clear that they are the most closely related of such genes in each species. During evolution these genes may have been co-opted for use in different structures, because they provide a ready-made system for intracellular signal transduction. Roth (1988) terms this recruitment of genes to a novel developmental pathway “genetic piracy,” providing a strong argument for not reducing homology to a simple correspondence between gene expression patterns and sequence conservation. Molecular homology may yet provide a basis for morphological homology but only if the comparisons involve more of the genes involved in the structure of interest’s development.

A possible problem in comparing homology of such evolutionarily distantly related organisms as worms and mice is that molecular or morphological divergence during evolution may make such comparisons difficult. This does not account for the *eyeless/Pax-6* based eye homology proposals (Barinaga 1995; Zuker 1994) where evolutionary sequence divergence has been minimal. In the *eyeless* example, the genes are homologous but the structures formed in *Drosophila* and mouse, while homologous as light sensing structures, are not homologous as image-forming organs, supporting the phylogenetic evidence that these structures are convergent (see Nipam Patel’s comments in Barinaga 1995). Within vertebrates, homologues to *Steel* and *c-kit* may well be used in the development of homologous structures (Lecoin et al. 1995), though data in *Xenopus* suggest that this may not be the case (Baker et al. 1995; Kao and Bernstein 1995). We have argued that development of homologous structures using different sets of genes can still be homologous if broader developmental criteria (using more comparative and developmental data) are utilized, but we are not advocating comparisons among very distantly related taxa where actions of evolution, over such a long time period, make determination of intermediate conditions difficult. Deciding on which taxa are sufficiently related to warrant such comparisons will vary among taxa and with the structures and molecules be-

ing compared. For the relatively limited amount of molecular data available today to be useful for homology determination, it should probably be applied together with other analyses of development (i.e., at the cellular level) as opposed to the initial basis for this determination.

Using the developmental criterion to determine if structures are nonhomologous.—We have discussed how seemingly different developmental mechanisms can still result in homologous ontogenies. When does the developmental criterion fail to indicate homology? The short answer is “when the development of two structures are dissimilar and discontinuous.” Before considering two ontogenies dissimilar one needs as detailed and up-to-date data on as many phylogenetically appropriate taxa as are feasible. These data can reveal similarity where older techniques revealed differences (i.e., the neurulation example above). A lack of similarity among ontogenies is not a sufficient basis for calling them nonhomologous. While continuity of information is more often thought of as a feature of phylogenetic data, developmental data can also be continuous. An ontogeny typically consists of many developmental processes at several hierarchical levels (molecular, cellular, and tissue). During evolution ontogenies may change, but it is unlikely that all the developmental processes change in unison due to the hierarchical nature of biological data (Striedter and Northcutt 1991). This overlap in developmental mechanisms is revealed by the broadly comparative analyses of neurulation and the embryonic origins of the lateral line we discuss. Without this overlap, one cannot see the continuity expected of ontogenies as they evolve. A confounding factor for such analyses is homoplasy (convergence and parallelism) but this is a problem for all homology criteria. The problem of homoplasy may be less severe for the developmental criterion because, unlike the phylogenetic criterion, it does not appear to worsen as more characters are considered. In conclusion, we would consider two structures to be nonhomologous by the developmental criterion if there was no (or very little) overlap in their developmental mechanisms and if data from many intermediate taxa were also included in the analysis.

Distinguishing between the phylogenetic and developmental criteria.—We believe that a phylogenetic perspective is an essential component of any analysis of development and evolution. However, we do not want to leave the impression that the developmental criterion as we restated it is the same as the phylogenetic criterion. There are several differences, mostly analogous to the argument made by transformational cladists that evolutionary assumptions are not necessary for cladistic theory (Patterson 1980). While technically correct, this is not a desirable goal for many

systematists (Charig 1981). Using Fig. 12 as an example, we illustrate how a developmental criterion could be used without phylogenetic information. In previous sections we have discussed how ontogenies consisting of different developmental mechanisms can still be homologous. That different ontogenies share one of up to five developmental mechanisms (indicated by letters in Fig. 12), is sufficient to indicate either some similarity and, therefore, the possibility that they originate from a common source, or that different mechanisms have been recruited by "piracy." This similarity allows one to determine that the development of six of seven taxa are homologous. While the developmental criterion divorced of systematic relations can not determine the polarity of the changes it may still be useful in determining homologies. Taxon 7 (Fig. 12) does present a problem for the use of this criterion that needs to be addressed by either more developmental studies (to determine if this developmental mechanism really is distinct) or the use of another criterion, such as phylogeny. The purpose of this exercise is not to advocate using the developmental over the phylogenetic criteria (although some workers have, see Goodwin 1994), since the evolutionary context provided by phylogenetic data allows for more than just the determination of homology, but rather to advocate that the developmental criterion not be ignored.

Conclusions.—The developmental criterion for homology must be broadly comparative and attempt to integrate results of modern cellular and molecular approaches. By looking at more and better-chosen species, possible intermediate states of developmental processes can be described. Current developmental methodologies may show similarities between developmental processes that were previously thought to be nonhomologous (such as neurulation or eye development), or allow for a more inclusive definition of homologous ontogenies by considering their underlying developmental processes in sets. Wagner (1989*b*) has stated that for homology to be meaningful the characters compared must have a minimal degree of complexity, differentiation and genetic/epigenetic autonomy; three properties of what he calls "individuality." Individualization is required no matter which criterion for homology is utilized (Wagner 1989*a, b*). It is not sensible to determine the homologies of structures that are not individualized, which is especially obvious in serially homologous structures (Wagner 1989*b*). Some examples of unproductive comparisons are determining homologies of specific foliage leaves among tree species (Wagner 1989*a*), individual bone Haversian systems (Patterson 1982, p. 47), tooth cusps in mammals (Van Valen 1982), and individual phalangeal elements in the forelimbs (Wagner 1989*a* and our fore-

limb example). Wagner (1989*a, b*) provides good explanations of and methods for determining individuality and we refer the reader to his work. Genetic and molecular approaches to developmental biology will help in deciding which structures are individualized. Conflicts between criteria for determining homologies (Wagner 1989*a*) and the more detailed understanding of development resulting from our use of the developmental criterion may help further in determining individuality. However, given a broader comparative approach and more detailed developmental data, we would expect the developmental and phylogenetic criteria to almost always be congruent, a testable proposal. Homology remains a challenging concept for morphologists, but by incorporating more developmental data, the concept's utility will be strengthened.

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LITERATURE CITED

- AKAM, M., P. HOLLAND, P. INGHAM, AND G. WRAY. (eds.). 1994. The evolution of developmental mechanisms. The Company of Biologists Limited, Cambridge, United Kingdom 241p.
- ALBERCH, P. 1985. Problems with the interpretation of developmental sequences. *Syst. Zool.* **34**: 46–58.
- , G. A. LEWBART, AND E. A. GALE. 1985. The fate of larval chondrocytes during the metamorphosis of the epibranchial in the salamander, *Eurycea bislineata*. *J. Embryol. Exp. Morph.* **88**: 71–83.
- ANDERSON, D. J. 1989. The neural crest cell lineage problem: Neurogenesis? *Neuron* **3**: 1–12.
- . 1993. Molecular control of cell fate in the neural crest: the sympathoadrenal lineage. *Annual Rev. Neurosci.* **16**: 129–158.
- BAKER, C. V. H., C. R. SHARPE, N. P. TORPEY, J. HEASMAN, AND C.

- C. WYLIE. 1995. A *Xenopus c-kit*-related receptor tyrosine kinase expressed in migrating stem cells of the lateral line system. *Mechanisms Developm.* **50**: 217–228.
- BALDWIN, T. J., C. M. YOSHIHARA, K. BLACKMER, C. R. KINTER, AND S. J. BURDEN. 1988. Regulation of acetylcholine receptor transcript expression during development in *Xenopus laevis*. *J. Cell Biol.* **106**: 469–478.
- BARINAGA, M. 1994. Looking to development's future. *Science* **266**: 561–564.
- . 1995. Focusing on the *eyeless* gene. *Science* **267**: 1766–1767.
- BEDDINGTON, R. 1992. Transgenic mutagenesis in the mouse. *Trends Genet.* **8**: 345.
- BISBEE, C. A., M. A. BAKER, A. C. WILSON, I. HADJI-AZIMI, AND M. FISCHBERG. 1977. Albumin phylogeny for clawed frogs (*Xenopus*). *Science* **195**: 785–787.
- BONNER, J. T. 1982. Evolution and development. Springer-Verlag, Berlin, Germany. 356 p.
- BROCKES, J. P. 1994. New approaches to amphibian limb regeneration. *Trends Genet.* **10**: 169–173.
- BRONNER-FRASER, M. 1993. Neural crest cell migration in the developing embryo. *Trends Cell Biol.* **3**: 392–397.
- , AND S. E. FRASER. 1988. Cell lineage analysis reveals multipotency of some avian neural crest cells. *Nature* **335**: 161–164.
- , AND ———. 1989. Developmental potential of avian trunk neural crest cells in situ. *Neuron* **3**: 755–766.
- CAGAN, R. L. 1993. Cell fate specification in the developing *Drosophila* retina. *Development* **1993 Supplement**: 19–28.
- , H. KRÄMER, A. C. HART, AND S. L. ZIPURSKY. 1992. The bride of sevenless and sevenless interaction: Internalization of a transmembrane ligand. *Cell* **69**: 393–399.
- CANNATELLA, D. C., AND L. TRUEB. 1988. Evolution of pipoid frogs: intergeneric relationships of the aquatic frog family Pipidae (Anura). *Zool. J. Linnean Soc.* **94**: 1–38.
- CARR, S. M., A. J. BROTHERS, AND A. C. WILSON. 1987. Evolutionary inferences from restriction maps of mitochondrial DNA from nine taxa of *Xenopus* frogs. *Evolution* **41**: 176–188.
- CHADWICK, D. J., AND J. MARSH. 1992. Post implantation development in the mouse. John Wiley & Sons, Chichester, United Kingdom. 315 p.
- CHARIG, A. 1981. Cladistics: a different point of view. *Biologist* **28**: 19–20.
- COATES, M. I. 1994. The origin of vertebrate limbs. *Development* **1994 Supplement**: 169–180.
- COEN, E. S., AND J. M. NUGENT. 1994. Evolution of flowers and inflorescences, pp. 107–116. In M. Akam, P. Holland, P. Ingham and G. Wray [eds.], The evolution of developmental mechanisms. The Company of Biologists Limited, Cambridge, United Kingdom.
- COLLAZO, A. 1994. Molecular heterochrony in the pattern of fibronectin expression during gastrulation in amphibians. *Evolution* **48**: 2037–2045.
- , M. BRONNER-FRASER, AND S. E. FRASER. 1993. Vital dye labelling of *Xenopus laevis* trunk neural crest reveals multipotency and novel pathways of migration. *Development* **118**: 363–376.
- , S. E. FRASER, AND P. M. MABEE. 1994. A dual embryonic origin for vertebrate mechanoreceptors. *Science* **264**: 426–430.
- COULY, G. F., P. M. COLTEY, AND N. M. LE DOUARIN. 1992. The developmental fate of the cephalic mesoderm in quail-chick chimeras. *Development* **114**: 1–15.
- COWLEY, D. E., AND W. R. ATCHLEY. 1992. Quantitative genetic models for development, epigenetic selection, and phenotypic evolution. *Evolution* **46**: 495–518.
- DE BEER, G. 1964. Atlas of evolution. Nelson, London, United Kingdom. 202 p.
- . 1971. Homology, an unsolved problem. Oxford University Press, Boulder, Colorado. 16 p.
- DE QUEIROZ, K. 1985. The ontogenetic method for determining character polarity and its relevance to phylogenetic systematics. *Syst. Zool.* **34**: 280–299.
- DENT, J. A., A. G. POLSON, AND M. W. KLYMKOWSKY. 1989. A whole-mount immunocytochemical analysis of the expression of the intermediate filament protein vimentin in *Xenopus*. *Development* **105**: 61–74.
- DOBZHANSKY, T. 1982. Genetics and the origin of species. Columbia University Press, New York, New York. 364 p.
- DONOGHUE, M. J. 1992. Homology, pp. 170–179. In E. F. Keller and E. A. Lloyd [eds.], Keywords in evolutionary biology. Harvard University Press, Cambridge, Massachusetts.
- DRIEVER, W., D. STEMPEL, A. SCHIER, AND L. SOLNICA-KREZEL. 1994. Zebrafish: genetic tools for studying vertebrate development. *Trends Genet.* **10**: 152–159.
- EISEN, J. S., AND J. A. WESTON. 1993. Development of the neural crest in the zebrafish. *Developm. Biol.* **159**: 50–59.
- ELDREDGE, N. 1985. Unfinished synthesis, biological hierarchies and modern evolutionary thought. Oxford University Press, New York, New York. 237 p.
- EPPERLEIN, H.-H., AND J. LÖFBERG. 1993. The development of neural crest in amphibians. *Ann. Anat.* **175**: 483–499.
- FRANK, E., AND J. R. SANES. 1991. Lineage of neurons and glia in chick dorsal root ganglia: analysis in vivo with a recombinant retrovirus. *Development* **111**: 895–908.
- FRASER, S. E. 1993. Segmentation moves to the fore. *Curr. Biol.* **3**: 787–789.
- , AND M. BRONNER-FRASER. 1991. Migrating neural crest cells in the trunk of the avian embryo are multipotent. *Development* **112**: 913–920.
- , R. KEYNES, AND A. LUMSDEN. 1990. Segmentation in the chick embryo hindbrain is defined by cell lineage restrictions. *Nature* **344**: 431–435.
- GEISSLER, E. N., M. A. RYAN, AND D. E. HOUSMAN. 1988. The dominant-white spotting (*W*) locus of the mouse encodes the *c-kit* proto-oncogene. *Cell* **55**: 185–192.
- GILBERT, S. F. 1991. Developmental Biology. 3rd ed. Sinauer Associates, Inc., Sunderland, Massachusetts. 891 p.
- GOODWIN, B. C. 1994. Homology, development, and heredity, pp. 229–247. In B. K. Hall [ed.], Homology: the hierarchical basis of comparative biology. Academic Press, Inc., San Diego, California.
- , N. HOLDER, AND C. C. WYLIE. (eds.). 1983. Development and evolution. Cambridge University Press, Cambridge, United Kingdom.
- GOULD, S. J. 1977. Ontogeny and phylogeny. Belknap Press of Harvard University Press, Cambridge, Massachusetts. 501 p.
- GRIFFITH, C. M., M. J. WILEY, AND E. J. SANDERS. 1992. The vertebrate tail bud: three germ layers form one tissue. *Anat. Embryol.* **185**: 101–113.
- HALDER, G., P. CALLAERTS, AND W. J. GEHRING. 1995. Induction of ectopic eyes by targeted expression of the *eyeless* gene in *Drosophila*. *Science* **267**: 1788–1792.
- HALL, B. K. (ed.). 1994. Homology: the hierarchical basis of comparative biology. Academic Press, San Diego, California.
- . 1995. Homology and embryonic development. *Evol. Biol.* **28**: 1–37.
- , AND S. HORSTADIUS. 1988. The neural crest. Oxford University Press, New York, New York. 304 p.
- HANKEN, J. 1993. Model systems versus outgroups: Alternative approaches to the study of head development and evolution. *Amer. Zool.* **33**: 448–456.
- , M. W. KLYMKOWSKY, C. H. SUMMERS, D. W. SEUFERT, AND N. INGEBRIGTSEN. 1992. Cranial ontogeny in the direct-developing frog, *Euletherodactylus coqui* (Anura: Leptodactylidae), analyzed using whole-mount immunohistochemistry. *J. Morph.* **211**: 95–118.

- , AND R. WASSERSUG. 1981. The visible skeleton. *Funct. Photogr.* **16**: 22–26, 44.
- HARLAND, R. M. 1991. In situ hybridization: an improved whole mount method for *Xenopus* embryos, pp. 675–685. In B. Kay and B. Peng [eds.], *Methods in cell biology*, Vol. 36: *Xenopus laevis*: Practical uses in cell and molecular biology. Academic Press, New York, New York.
- HART, A. C., S. D. HARRISON, D. L. VAN VACTOR, JR., G. M. RUBIN, AND S. L. ZIPURSKY. 1993. The interaction of *bride of sevenless* with *sevenless* is conserved between *Drosophila virilis* and *Drosophila melanogaster*. *Proc. Natl. Acad. USA* **90**: 5047–5051.
- HARTMAN, D. S., AND T. CLAUDIO. 1990. Coexpression of two distinct muscle acetylcholine receptor α -subunits during development. *Nature* **343**: 372–375.
- HASZPRUNAR, G. 1992. The types of homology and their significance for evolutionary biology and phylogenetics. *J. Evol. Biol.* **5**: 13–24.
- HATTA, K. 1992. Role of the floor plate in axonal patterning in the zebrafish CNS. *Neuron* **9**: 629–642.
- , C. B. KIMMEL, R. K. HO, AND C. WALKER. 1991. The cyclops mutation blocks specification of the floor plate of the zebrafish central nervous system. *Nature* **350**: 339–341.
- HAUPTMANN, G., AND T. GERSTER. 1994. Two-color whole-mount in situ hybridization to vertebrate and *Drosophila* embryos. *TIG* **10**: 266.
- HEMMATI-BRIVANLOU, A., D. FRANK, M. E. BOLCE, B. D. BROWN, H. L. SIVE, AND R. M. HARLAND. 1990. Localization of specific mRNAs in *Xenopus* embryos by whole-mount *in situ* hybridization. *Development* **110**: 325–330.
- HENNIG, W. 1965. Phylogenetic systematics. *Annual Rev. Entomol.* **10**: 97–116.
- HILL, R. J., AND P. W. STERNBERG. 1992. The gene *lin-3* encodes an inductive signal for vulval development in *C. elegans*. *Nature* **358**: 470–476.
- HINCHLIFFE, J. R. 1994. Evolutionary developmental biology of the tetrapod limb. *Development* **1994 Supplement**: 163–168.
- , AND D. JOHNSON. 1981. Development of the vertebrate limb. Oxford University Press, London, United Kingdom. 268 p.
- HUCHO, F. 1993. The nicotinic acetylcholine receptor, pp. 113–135. In F. Hucho [ed.], *Neurotransmitter Receptors*. Elsevier, New York, New York.
- HUIZINGA, J. D., L. THUNEBERG, M. KLÜPPEL, J. MALYSZ, H. B. MIK-KELSEN, AND A. BERNSTEIN. 1995. *W/kit* gene required for interstitial cells of Cajal and for intestinal pacemaker activity. *Nature* **373**: 347–349.
- JACOBSON, A. G. 1991. Experimental analyses of the shaping of the neural plate and tube. *Amer. Zool.* **31**: 628–643.
- JANVIER, P. 1981. The phylogeny of the Craniata, with particular reference to the significance of fossil “agnathans”. *J. Vertebr. Paleontol.* **1**: 121–159.
- JAVOIS, L. C. 1984. Pattern specification in the developing chick limb, pp. 557–579. In G. M. Malacinski and S. V. Bryant [eds.], *Pattern formation a primer in developmental biology*. Macmillan Publishing Company, New York, New York.
- JOHNSON, R. L., AND C. TABIN. 1995. The long and short of *hedgehog* signaling. *Cell* **81**: 313–316.
- JONES, J. E., AND J. T. CORWIN. 1993. Replacement of lateral line sensory organs during tail regeneration in salamanders: identification of progenitor cells and analysis of leukocyte activity. *J. Neurosci.* **13**: 1022–1034.
- KAO, K. R., AND A. BERNSTEIN. 1995. Expression of *Xkl-1*, a *Xenopus* gene related to mammalian *c-kit*, in dorsal embryonic tissue. *Mechanisms Developm.* **50**: 57–69.
- KAY, B., AND B. PENG. (eds.). 1991. *Methods in cell biology*, Vol. 36: *Xenopus laevis*: Practical uses in cell and molecular biology. Academic Press, New York, New York.
- KEMP, A. 1982. The embryological development of the Queensland lungfish, *Neoceratodus forsteri*, (Kreffft). *Mem. Queensland Mus.* **20**: 553–597.
- KIRBY, M. L. 1988a. Nodose placode contributes autonomic neurons to the heart in the absence of cardiac neural crest. *J. Neurosci.* **8**: 1089–1095.
- . 1988b. Nodose placode provides ectomesenchyme to the developing chick heart in the absence of cardiac neural crest. *Cell Tissue Res.* **252**: 17–22.
- KLUGE, A. G. 1989. A concern for evidence and a phylogenetic hypothesis of relationships among *Epicrates* (Boidae, Serpentes). *Syst. Zool.* **38**: 7–25.
- KOBEL, H. R., AND L. D. PASQUIER. 1986. Genetics of polyploid *Xenopus*. *TIG* **2**: 310–315.
- KROTOSKI, D. M., S. E. FRASER, AND M. BRONNER-FRASER. 1988. Mapping of neural crest pathways in *Xenopus laevis* using inter- and intra-specific cell markers. *Developm. Biol.* **127**: 119–132.
- KRULL, C. E., A. COLLAZO, S. E. FRASER, AND M. BRONNER-FRASER. 1995. Segmental migration of trunk neural crest: Time-lapse analysis reveals a role for PNA-binding molecules. *Development* **121**: 3733–3743.
- LANDACRE, F. L., AND A. C. CONGER. 1913. The origin of the lateral line primordia in *Lepidosteus osseus*. *J. Comp. Neurol.* **23**: 576–633.
- LAUDER, G. V., AND K. F. LIEM. 1983. The evolution and interrelationships of the actinopterygian fishes. *Bull. Mus. Comp. Zool.* **150**: 95–197.
- LE DOUARIN, N. M. 1982. *The neural crest*. Cambridge University Press, Cambridge, United Kingdom. 259 p.
- LECOIN, L., R. LAHAV, F. H. MARTIN, M.-A. TEILLET, AND N. M. LE DOUARIN. 1995. *Steel* and *c-kit* in the development of avian melanocytes: A study of normally pigmented birds and of the hyperpigmented mutant silky fowl. *Developm. Dynamics* **203**: 106–118.
- LESSA, E. P., AND J. L. PATTON. 1989. Structural constraints, recurrent shapes, and allometry in pocket gophers (genus *Thomomys*). *Biol. J. Linn. Soc.* **36**: 349–363.
- LYNCH, K., AND S. E. FRASER. 1990. Cell migration in the formation of the pronephric duct in *Xenopus laevis*. *Developm. Biol.* **142**: 283–292.
- MAISEY, J. G. 1986. Heads and tails: A chordate phylogeny. *Cladistics* **2**: 201–256.
- MATZUK, M. M., T. R. KUMAR, AND A. BRADLEY. 1995a. Different phenotypes for mice deficient in either activins or activin receptor type II. *Nature* **374**: 356–360.
- , ———, A. VASSALLI, J. R. BICKENBACH, D. R. ROOP, R. JAENISCH, AND A. BRADLEY. 1995b. Functional analysis of activins during mammalian development. *Nature* **374**: 354–356.
- , N. LU, H. VOGEL, K. SELLHEYER, D. R. ROOP, AND A. BRADLEY. 1995c. Multiple defects and perinatal death in mice deficient in follistatin. *Nature* **374**: 360–363.
- MAYR, E. 1982. *Systematics and the origin of species*. Columbia University Press, New York, New York. 334 p.
- METCALFE, W. K. 1985. Sensory neuron growth cones comigrate with posterior lateral line primordial cells in zebrafish. *J. Comp. Neurol.* **238**: 218–234.
- . 1989. Organization and development of the zebrafish posterior lateral line, pp. 147–159. In S. Coombs, P. Görner and H. Münz [eds.], *The mechanosensory lateral Line: neurobiology and evolution*. Springer-Verlag, New York, New York.
- MORGAN, B. A., AND C. TABIN. 1994. Hox genes and growth: early and late roles in limb bud morphogenesis. *Development* **1994 supplement**: 181–186.
- MOTRO, B., D. VAN DER KOOY, J. ROSSANT, A. REITH, AND A. BERNSTEIN. 1991. Contiguous patterns of *c-kit* and *Steel* expression: analysis of mutations at the *W* and *Sl* loci. *Development* **113**: 1207–1221.

- MÜLLER, G. B. 1991. Experimental strategies in evolutionary embryology. *Amer. Zool.* **31**: 605–615.
- NELSEN, O. E. 1953. Comparative embryology of the vertebrates. Blakiston Company, Inc., New York, New York. 982 p.
- NELSON, G. 1994. Homology and systematics, pp. 101–149. In B. K. Hall [ed.], *Homology: the hierarchical basis of comparative biology*. Academic Press, Inc., San Diego, California.
- NELSON, J. S. 1984. *Fishes of the world*. 2nd ed. John Wiley and Sons, New York, New York. 523 p.
- NIEUWKOOP, P. D., AND J. FABER. 1967. Normal Table of *Xenopus laevis* (Daudin). 2nd ed. North-Holland Publishing Company, Amsterdam, Netherlands. 252 p.
- NIEVELSTEIN, R. A. J., N. G. HARTWIG, C. VERMEIJ-KEERS, AND J. VALK. 1993. Embryonic development of the mammalian caudal neural tube. *Teratology* **48**: 21–31.
- NORTHCUTT, R. G., K. BRÄNDLE, AND B. FRITZSCH. 1995. Electroreceptors and mechanosensory lateral line organs arise from single placodes in axolotls. *Developm. Biol.* **168**: 358–373.
- , K. C. CATANIA, AND B. B. CRILEY. 1994. Development of lateral line organs in the axolotl. *J. Comp. Neurol.* **340**: 480–514.
- , AND C. GANS. 1983. The genesis of neural crest and epidermal placodes: A reinterpretation of vertebrate origins. *Quart. Rev. Biol.* **58**: 1–28.
- NOVACEK, M. J., A. R. WYSS, AND M. C. MCKENNA. 1988. The major groups of eutherian mammals, pp. 31–71. In M. J. Benton [ed.], *The phylogeny and classification of the tetrapods, Volume 2: Mammals*. Clarendon Press, Oxford, United Kingdom.
- NÜSLEIN-VOLHARD, C., AND E. WIESCHAUS. 1980. Mutations affecting segment number and polarity in *Drosophila*. *Nature* **287**: 795–801.
- OSUMI-YAMASHITA, N., Y. NINOMIYA, H. DOI, AND K. ETO. 1994. The contribution of both forebrain and midbrain crest cells to the mesenchyme in the frontonasal mass of mouse embryos. *Development* **164**: 409–419.
- PAPAN, C., AND J. CAMPOS-ORTEGA. 1994. On the formation of the neural keel and neural tube in the zebrafish *Danio (Brachydanio) rerio*. *Roux's Arch. Developm. Biol.* **203**: 178–186.
- PATEL, N. H. 1994. Developmental evolution: Insights from studies of insect segmentation. *Science* **266**: 581–590.
- PATTERSON, C. 1980. Cladistics. *Biologist* **27**: 234–240.
- . 1982. Morphological characters and homology, pp. 21–74. In K. A. Joysey and A. E. Friday [eds.], *Problems of phylogenetic reconstruction*. Academic Press, New York, New York.
- . 1988. Homology in classical and molecular biology. *Molec. Biol. Evol.* **5**: 603–625.
- POLICANSKY, D. 1982. The assymetry of flounders. *Sci. Ameri.* **246**: 116–122.
- POUGH, F. H., J. B. HEISER, AND W. N. MCFARLAND. 1996. *Vertebrate life*. 4th ed. Prentice Hall, Upper Saddle River, New Jersey. 798 p.
- PUZDROWSKI, R. L. 1989. Peripheral distribution and central projections of the lateral-line nerves in goldfish, *Carassius auratus*. *Brain, Behavior Evol.* **34**: 110–131.
- QUIRING, R., U. WALLDORF, U. KLOTTER, AND W. J. GEHRING. 1994. Homology of the eyeless gene of *Drosophila* to the *Small eye* gene in mice and *Aniridia* in humans. *Science* **265**: 785–789.
- RAFF, R. A., AND T. C. KAUFMAN. 1983. *Embryos, genes, and evolution: the developmental-genetic basis of evolutionary change*. Macmillan Publishing Company, New York, New York. 395 p.
- , AND E. C. RAFF. 1987. Development as an evolutionary process. Alan R. Liss, Inc., New York, New York. 329 p.
- , AND G. A. WRAY. 1989. Heterochrony: developmental mechanisms and evolutionary results. *J. Evol. Biol.* **2**: 409–434.
- RAIBLE, D. W., AND J. S. EISEN. 1994. Restriction of neural crest cell fate in the trunk of the embryonic zebrafish. *Development* **120**: 495–503.
- REITH, A. D., AND A. BERNSTEIN. 1991a. Molecular basis of mouse developmental mutants. *Genes Developm.* **5**: 1115–1123.
- , AND ———. 1991b. Molecular biology of the W and Steel loci, pp. 105–133. In K. E. Davies and S. M. Tilghman [eds.], *Genome analysis. Volume 3: Genes and phenotypes*. Cold Spring Harbor Laboratory Press, New York, New York.
- ROTH, V. L. 1984. On homology. *Biological Journal of the Linnean Society* **22**: 13–29.
- . 1988. The biological basis of homology, pp. 1–26. In C. J. Humphries [ed.], *Ontogeny and Systematics*. Columbia University Press, New York.
- . 1991. Homology and hierarchies: problems solved and unresolved. *J. Evol. Biol.* **4**: 167–194.
- . 1994. Within and between organisms: Replicators, lineages, and homologues, pp. 301–337. In B. K. Hall [ed.], *Homology: the hierarchical basis of comparative biology*. Academic Press, Inc., San Diego, California.
- ROTTAPEL, R., M. REEDIJK, D. E. WILLIAMS, S. D. LYMAN, D. M. ANDERSON, T. PAWSON, AND A. BERNSTEIN. 1991. The *Steel/W* transduction pathway: Kit autophosphorylation and its association with a unique subset of cytoplasmic signaling proteins is induced by the Steel factor. *Molec. Cell. Biol.* **11**: 3043–3051.
- ROUTTENBERG, A. 1995. Knockout mouse fault lines. *Nature* **374**: 314–315.
- SADAGHIANI, B., AND C. H. THIEBAUD. 1987. Neural crest development in the *Xenopus laevis* embryo studied by interspecific transplantation and scanning electron microscopy. *Developm. Biol.* **124**: 91–110.
- SCHERSON, T., G. SERBEDZJA, S. FRASER, AND M. BRONNER-FRASER. 1993. Regulative capacity of the cranial neural tube to form neural crest. *Development* **118**: 10491062.
- SCHILLING, T. F., AND C. B. KIMMEL. 1994. Segment and cell type lineage restrictions during pharyngeal arch development in the zebrafish embryo. *Development* **120**: 483–494.
- SELLECK, M. A. J., T. Y. SCHERSON, AND M. BRONNER-FRASER. 1993. Origins of neural crest cell diversity. *Dev Biol* **159**: 1–11.
- , AND M. BRONNER-FRASER. 1995. Origins of the avian neural crest: the role of neural plate-epidermal interactions. *Development* **121**: 525–538.
- SERBEDZJA, G. N., M. BRONNER-FRASER, AND S. E. FRASER. 1994. Developmental potential of trunk neural crest cells in the mouse. *Development* **120**: 1709–1718.
- SEUFERT, D. W., AND B. K. HALL. 1990. Tissue interactions involving cranial neural crest in cartilage formation in *Xenopus laevis* (Daudin). *Cell Different. Developm.* **32**: 153–166.
- SHUBIN, N. H. 1994. History, ontogeny, and evolution of the archetype, pp. 249–271. In B. K. Hall [ed.], *Homology: the hierarchical basis of comparative biology*. Academic Press, Inc., San Diego, California.
- SMITH, J. 1995. Angles on activin's absence. *Nature* **374**: 311–312.
- STEMPLE, D. L., AND D. J. ANDERSON. 1992. Isolation of a stem cell for neurons and glia from the mammalian neural crest. *Cell* **71**: 973–85.
- , AND ———. 1993. Lineage diversification of the neural crest: in vitro investigations. *Developm. Biol.* **159**: 12–23.
- 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.
- STRIEDTER, G. F., AND R. G. NORTHCUTT. 1991. Biological hierarchies and the concept of homology. *Brain, Behavior Evol.* **38**: 177–189.
- TALBOT, W. S., B. TREVARROW, M. E. HALPERN, A. E. MELBY, G. FARR, J. H. POSTLETHWAIT, T. JOWETT, C. B. KIMMEL, AND D. KIMMELMAN. 1995. A homeobox gene essential for zebrafish notochord development. *Nature* **378**: 150–157.
- TAN, S. S., AND G. M. MORRIS-KAY. 1985. The development and distribution of the cranial neural crest in the rat embryo. *Cell Tissue Res.* **240**: 403–416.

- TAUTZ, D. 1992. Redundancies, development and the flow of information. *BioEssays* **14**: 263–266.
- TUCKER, A. S., AND J. M. W. SLACK. 1995. The *Xenopus laevis* tail-forming region. *Development* **121**: 249–262.
- VAN VALEN, L. M. 1982. Homology and causes. *J. Morph.* **173**: 305–312.
- VASSALLI, A., M. M. MATZUK, H. A. R. GARDNER, K.-F. LEE, AND R. JAENISCH. 1994. Activin/inhibin β B subunit gene disruption leads to defects in eyelid development and female reproduction. *Genes Developm.* **8**: 414–427.
- WAGNER, G. P. 1989a. The biological homology concept. *Annual Rev. Ecol. Syst.* **20**: 51–69.
- . 1989b. The origin of morphological characters and the biological basis of homology. *Evolution* **43**: 1157–1171.
- . 1994. Homology and the mechanisms of development, pp. 273–299. In B. K. Hall [ed.], *Homology: the hierarchical basis of comparative biology*. Academic Press, Inc., San Diego, California.
- WAKE, D. B. 1991. Homoplasy: the result of natural selection, or evidence of design limitations? *Amer. Naturalist* **138**: 543–567.
- . 1994. Comparative Terminology. *Science* **265**: 268–269.
- WAKE, M. H. 1990. The evolution of integration of biological systems: An evolutionary perspective through studies on cells, tissues, and organs. *Amer. Zool.* **30**: 897–906.
- WEBB, J. F., AND D. M. NODEN. 1993. Ectodermal placodes: Contributions to the development of the vertebrate head. *Amer. Zool.* **33**: 434–447.
- WESTERFIELD, M. 1993. *The zebrafish book*. University of Oregon Press, Eugene, Oregon.
- WINKLBAUER, R. 1989. Development of the lateral line system in *Xenopus*. *Progr. Neurobiol.* **32**: 181–206.
- WRAY, G. A., AND D. MCCLAY. 1989. Molecular heterochronies and heterotopies in early echinoid development. *Evolution* **43**: 803–813.
- WURST, W., A. B. AUERBACH, AND A. L. JOYNER. 1994. Multiple developmental defects in *Engrailed-1* mutant mice: an early mid-hindbrain deletion and patterning defects in forelimbs and sternum. *Development* **120**: 2065–2075.
- ZIMMER, E. A. 1994. Perspectives on future applications of experimental biology to evolution, pp. 607–616. In B. Schierwater, B. Streit, G. P. Wagner and R. DeSalle [eds.], *Molecular ecology and evolution: approaches and applications*. Birkhäuser Verlag, Basel, Switzerland.
- ZIMMERMAN, K., J. SHIH, J. BARS, A. COLLAZO, AND D. J. ANDERSON. 1993. XASH-3, a novel *Xenopus* achaete-scute homolog, provides an early marker of planar neural induction and position along the mediolateral axis of the neural plate. *Development* **119**: 221–232.
- ZUKER, C. S. 1994. On the evolution of eyes: Would you like it simple or compound? *Science* **265**: 742–743.