

REVIEW

Zebrafish in Context: Uses of a Laboratory Model in Comparative Studies

Brian D. Metscher and Per Erik Ahlberg

Department of Palaeontology, The Natural History Museum, London SW7 5BD, United Kingdom

With the recent interest in the reintegration of evolutionary and developmental biology has come a growing need for understanding the phylogenetic relations and degree of generality of the model organisms upon which we rely so heavily. In vertebrate biology the zebrafish *Danio rerio* has become a paradigmatic system for studies at levels of organization from molecular to interspecific. Studies of model systems in development are often techniques-driven rather than questions-based; however, informative hypotheses for developmental research can be derived from phylogenetic distributions of characters. With some understanding of how general the characters of interest are, a thoughtful comparison of the requirements of the questions with the lists of available embryos, reagents, and protocols can guide choices of new vertebrate models. We describe here the phylogenetic placement of zebrafish within the vertebrate world and discuss how generally observations on zebrafish can be taken to apply. We outline a practical protocol for investigating development in a comparative context, illustrated with an example from an ongoing study of teleost tail fin evolution. The principles and procedures presented here apply equally well to any comparative study with an interest in evolution, at any level of phylogeny from intraspecific studies to comparisons across phyla. © 1999 Academic Press

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INTRODUCTION: THE IMPORTANCE OF PHYLOGENY

In the past decade or so the zebrafish, *Danio rerio*, has become a vertebrate of choice in developmental biology laboratories. It is easy to keep, has a short generation time, breeds readily, and produces conveniently transparent embryos; for these and other reasons it has gradually acquired the prestigious rank of Model Organism. But, as with any model animal in basic research, the use of zebrafish carries with it the problem of generalization of results: what in fact is it a model of?

A “model organism” in basic research usually refers to one that has been subjected to intensive study and is used for studying multiple aspects of biology; *Drosophila* and *Caenorhabditis* are clear examples. In a looser sense, an organism is a “model” if it is being used as an analog or illustration for something that is not itself under direct study, such as the whole of a group of animals or an irretrievable ancestor. In the latter sense any animal chosen

for study is a model,¹ if it is taken to represent some aspects of a larger group or an inaccessible taxon, unless a number of models have been chosen in order to study diversity. A characteristic of a model organism is general for the group if it is shared by the other members of the group, whether the similarity is due to shared ancestry, convergent evolution, or even similar function.

Thus there are two aspects to this generalization problem, one relating to present-day diversity and one to evolutionary history. First, to what extent can conditions observed in zebrafish be regarded as general for related animals, such as other fishes or vertebrates at large? Second, to what extent can zebrafish be taken to embody the ancestral condition for groups such as bony fishes or vertebrates? Both aspects have important implications for the robustness of any hypotheses that derive from zebrafish studies but are applied to other organisms.

¹ In another important sense, all of these are means of constructing and refining our internal cognitive “models” of the “actual” biological world and its processes.

In the following discussion we show how both aspects of the generalization problem relate directly to—and can be resolved only by reference to—the phylogenetic context of the model animal. We describe the positions of the most popular laboratory vertebrates on the vertebrate phylogenetic tree and examine the specific extent of *Danio's* usefulness in comparative studies, with particular emphasis on morphology. We then discuss general considerations and a practical approach for the use of a laboratory developmental model in comparative studies with evolutionary implications, and we illustrate the approach with a morphological example relating to the evolution of caudal fins. However, it applies equally well to any comparison with a phylogenetic dimension, whether morphological, functional, or molecular.

LABORATORY VERTEBRATES IN THEIR PHYLOGENETIC CONTEXT

All research in developmental biology incorporates phylogenetic assumptions. Even in a study with no avowedly evolutionary or even comparative components, the vocabulary used to describe the developmental phenomena will contain numerous terms for structures or genes (“ectoderm,” “rhombomere,” “*Hoxc6*”) that occur in species other than the one being studied. These statements imply the existence of homologies, and thus common ancestry, between taxa; they are themselves rudimentary phylogenetic hypotheses (Abouheif *et al.*, 1997 and references therein). Developmental biology is embedded in phylogeny, and use of an erroneous phylogenetic framework in a developmental investigation may confuse or invalidate its conclusions. Inattention to phylogenetic contexts will also tend to overlook some of the really interesting questions.

This phylogenetic context, always important, becomes crucial in studies with a comparative or generalizing component or when comparing with published results from different taxa. In order to understand the significance of developmental data to vertebrates (or any other group) generally, or to compare two morphologies or ontogenies in any productive way, some assumption about their relationship must be made, whether stated or not. For many questions the most directly useful relationship is ancestry: comparison of a derived form with the ancestral form from which it has undergone an evolutionary change. The comparison between a lab-induced mutant and its “parent” wild type is a simple example. However, when dealing with comparisons between species, this kind of pair is a luxury rarely to be had. Instead, the investigator will most likely be faced with two (or more) organisms, neither of which is the ancestor of the other. For a given trait, it may be that one of the species nevertheless embodies the ancestral condition for both, but it is also possible that both are derived from some third, unknown, ancestral condition. Furthermore, even if a pair of species shows the “ancestral” and “descendant” conditions of some particular trait, it may not be obvious which is which.

These uncertainties are phylogenetic questions: they concern the relationships of the species under investigation to each other and to other species not being studied. Any comparative study is thus by its nature underpinned by a phylogenetic hypothesis, whether stated or unstated.² If this phylogeny is flawed, the directions (polarities) of the character transformations are likely to be misinterpreted and the inferences misleading or fallacious.

In developmental studies with some interest in evolutionary implications, representative living taxa are often chosen from the few available laboratory models without adequate consideration of their actual relationships and the bearing of their comparison on the question being discussed (e.g., Sordino *et al.*, 1995; Arnault *et al.*, 1996). Casual comparison of a new parcel of zebrafish gene expression data with mouse and chick data for the same gene family may have an evolutionary air about it, but such *ad hoc* comparisons between species are fraught with peril. They will sometimes fall within the boundaries of phylogenetic accuracy, but can result in specious and unfounded conclusions. Regardless of the outcome, coincidental alignments between the standing body of knowledge about phylogenetic history and some unstated assumptions about ancestry of the vertebrates can hardly be said to constitute sound methodology.

Before delving deeper into the phylogenetic position of the zebrafish and its utility as a model animal for developmental studies, it is worth taking a brief look at the foundations and practices of modern phylogenetic analysis—a topic which is rarely given adequate coverage in general texts on animal diversity and evolution. This is an important issue, because the discipline has undergone a profound double revolution in recent decades with the development of new methodologies (notably computer-based search algorithms, more of which below) and the emergence of molecular data as a major new source of information. The utility of phylogenetic analysis has been greatly enhanced as a result.

Modern phylogenetic analysis does *not* consist of a search for ancestors in the fossil record and does *not* involve the reconstruction of hypothetical archetypes. Rather, it is the recognition of groups of organisms on the basis of shared morphological or molecular characteristics. The underlying assumptions can be summarized as follows:

(1) Evolution is dominated by linear descent and branching events, while hybridization is rare (particularly among animals). As a result, the phylogenetic relationships between organisms are generally tree-like branching patterns, not anastomosing networks.

(2) An evolutionary novelty (morphological or molecular) will tend to be retained in the descendants of the organism in which it first evolved.

(3) As a consequence of 1 and 2, a group comprising *all*

² This does not, of course, include functional, behavioral, or ecological comparisons that may be based on analogies rather than homologies.

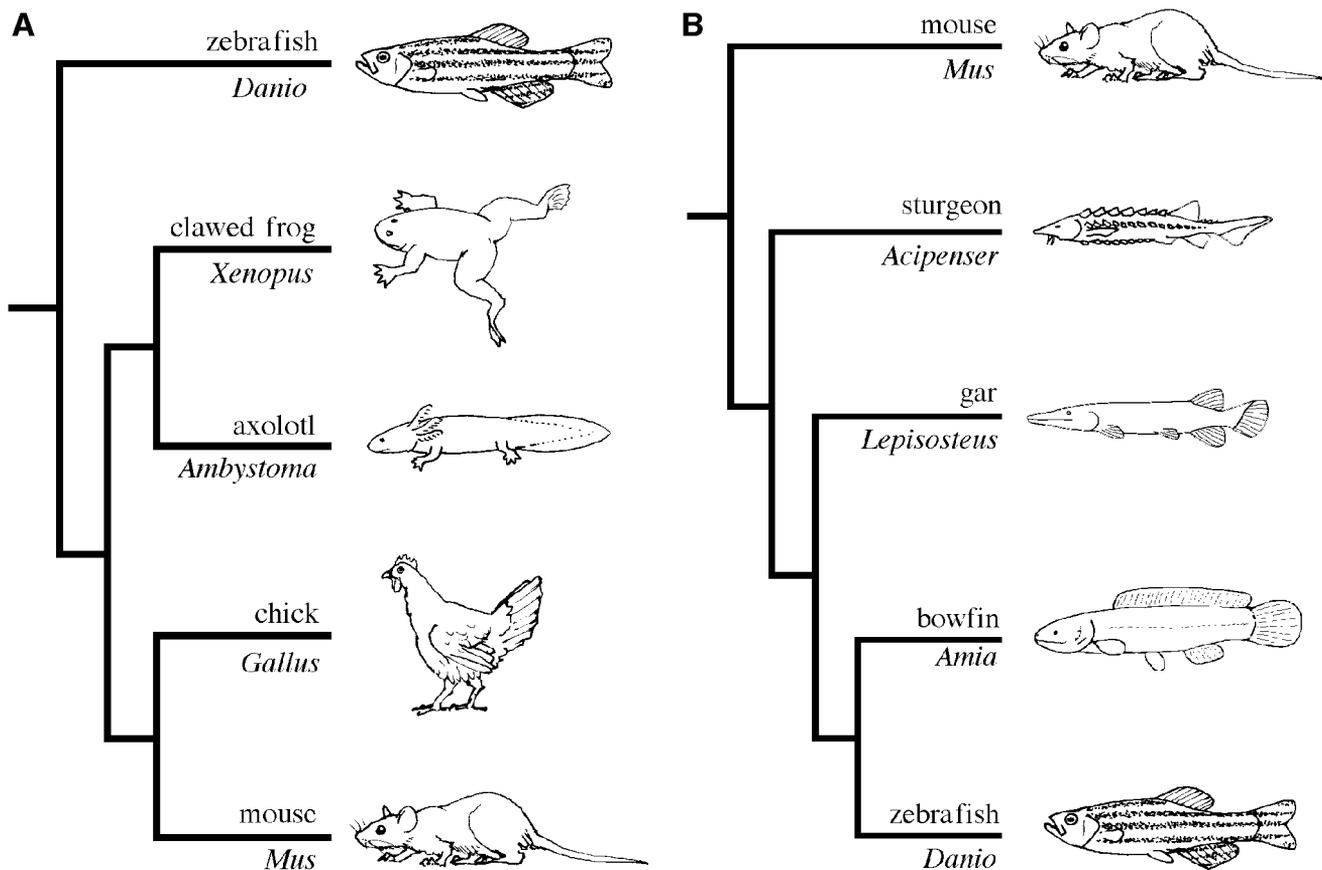


FIG. 1. (A) A simple phylogeny of some common laboratory vertebrates. The relative closeness of evolutionary relationships is depicted correctly, but the scant information offered by this representation can be misleading due to the bias toward our own kind. (B) A Daniocentric view of vertebrate relationships. This tree would appear to show the mammal as the basal and possibly ancestral form to the fishes. Biased as these two trees are, they are still better than the *scala naturae* of laboratory animal evolution from *Drosophila* to fish to amphibian to mouse (see Hanken, 1993, Fig. 1), as sometimes seems to be assumed.

the descendants of a *single* common ancestor (known as a “natural group,” “monophyletic group,” or “clade”) will be recognizable because the members all possess one or more characteristics which first appeared in that common ancestor. Organisms that are not descendants of this ancestor, and thus not members of the group, will not possess the characteristic(s). Characteristics that delineate a natural group in this way are known as “synapomorphies,” or “shared derived characters” of the group. (Some workers regard “synapomorphy” and “homology” as equivalent concepts; see Patterson, 1988.)

(4) Natural groups never overlap, but always nest hierarchically, one inside another. (This is a necessary consequence of a branching pattern of relationships.) As a result, synapomorphies are also distributed hierarchically.

To see what these points mean in practice, consider the simple phylogeny of some popular laboratory vertebrates shown in Fig. 1A. This phylogeny (a type known as a “cladogram”), which represents the consensus of the ma-

jority of recent molecular and morphological analyses, has no real time scale and contains no ancestors. The branching pattern simply indicates the arrangement of natural groups. [All branches that spring from one node belong to one natural group: the node.] Four natural groups can be identified in this diagram. They are (with formal name and sample synapomorphies in parentheses):

(I) Chick and mouse (Amniota: synapomorphies include the possession of extraembryonic membranes—amnion, chorion, and allantois).

(II) Clawed frog and axolotl (Amphibia: synapomorphies include granular glands in the skin and a “papilla amphibi-orum” in the inner ear).

(III) Chick + mouse + clawed frog + axolotl (Tetrapoda: synapomorphies include paired limbs with digits).

(IV) Chick + mouse + clawed frog + axolotl + zebrafish (Osteichthyes: synapomorphies include the possession of certain skull bones such as maxilla and premaxilla).

Note that the Amniota and Amphibia nest *within* the Tetrapoda, which in turn nests within the Osteichthyes. One natural group cannot be “ancestral” to another, nor “give rise” to it.

Modern phylogenetic analysis tries to retrieve the true branching pattern of relationships from the distribution of morphological and molecular characters among organisms. Points 3 and 4 above might suggest that this is rather easy, but in practice the pattern of character distribution is seriously disrupted by parallel and convergent evolution between lineages and by secondary character loss (e.g., snakes are tetrapods but have lost their limbs and digits); it is often very difficult to distinguish genuine synapomorphies from parallelisms. In order to get around this problem, mathematical algorithms are used to choose the branching pattern that best fits the data. The most commonly used approach is “parsimony,” which simply looks for the phylogeny that requires the fewest instances of parallelism, convergence, and evolutionary reversal; this can be applied to both morphology and molecular data. However, some molecular phylogeneticists prefer to use “maximum likelihood” methods, which incorporate assumptions about the probability of different kinds of change. Whichever approach is used, the phylogeny thus produced is a proper hypothesis based on a strictly formulated analysis of an explicit data set; it can be challenged by demonstrating that there are flaws in the data set or that the method of analysis is inappropriate. This represents an enormous advance over past approaches to phylogenetic reconstruction, which generally used intuitive *ad hoc* judgments about the importance of particular characters, often tried to construct ancestor–descendant chains from very inadequate data, and produced phylogenies that were unfalsifiable expressions of personal opinion. An excellent summary of modern phylogenetic thought is provided by Forey *et al.* (1992).

Returning to Fig. 1A, we have noted that it contains no ancestors or absolute times of evolutionary divergence. It correctly shows that mouse and domestic chicken are more closely related to each other than either is to zebrafish (that is, mouse and chicken diverged from their last common ancestor more recently than that ancestor diverged from its common ancestor with zebrafish), but it does not say when either event happened or what those ancestors were like.

Divergence dates can be readily mapped onto a phylogeny by reference to the fossil record, invocation of “molecular clock” assumptions, or a combination of the two. The absence of explicit ancestors, on the other hand, is a fundamental feature of all modern phylogenies: all species

in a phylogenetic analysis, whether living or fossil, are treated as terminal branch ends during the algorithmic search for the most parsimonious tree (that is, the one that requires the fewest evolutionary steps). The incompleteness of the fossil record makes it very unlikely that actual ancestor–descendant chains will be discovered among fossils, and palaeontologists usually make no attempt to search for such chains.

It is tempting to look at a phylogeny like Fig. 1A and carry away an inference that the zebrafish is the most “primitive” of the species listed. That inference is erroneous, even if it is shielded with terms like “lower vertebrate” or “simpler organism.” The phylogeny does imply that the tetrapods (clawed frog, axolotl, chicken, and mouse) share derived character states which are not present in zebrafish, but it does not imply that zebrafish retains the ancestral condition for taxa depicted. The zebrafish may have—and in fact has, as we shall see—acquired different derived character states of its own during the evolution of its lineage. The importance of this point cannot be overstressed.

An instructive contrast is provided by the less terrestrial perspective in Fig. 1B: this depiction of relationships focuses attention on the zebrafish lineage, but it might tend to point one toward odd notions of fur and whiskers as ancestral characters of fishes. In fact it is perfectly compatible with Fig. 1A: both are, to the best of our knowledge, accurate statements of relationship, and the bottom node in each phylogeny is identical.

Just as important, the terms “primitive” and “derived” properly apply to individual characters, not to species or lineages. Thus the zebrafish may be primitive relative to the tetrapods with respect to some character states but not others. Indeed, the tetrapods may well be primitive relative to the zebrafish in some respects, by retaining certain ancestral features of anatomy, development, or genomic structure that were lost or altered in the lineage to which zebrafish belong. A final point to note here is that primitive and derived are not universal terms but relate to the before/after conditions of a single transformation. Thus, in the transformation from fin to limb, fin is primitive and limb is derived, but in the transformation from limb to bird’s wing, limb is primitive and wing is derived.

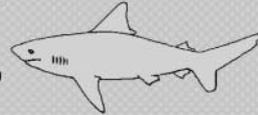
To formulate informative hypotheses and to interpret meaningfully any comparative results, a more detailed picture of the relationships of vertebrate animals is necessary. Figure 2 is an abridged phylogeny of jawed vertebrates, showing the positions of the most common laboratory

FIG. 2. A more complete (though still abridged) phylogeny of vertebrate lineages, showing the relative positions of the laboratory models in Fig. 1. Here the evolutionary relationships are shown in more detail, with approximate divergence times and some fossil forms (†) represented. The shaded regions correspond with those in Fig. 3 and the taxonomic levels in Tables 1 and 2. Note that *Osteolepis* is not itself included in or ancestral to the clade Tetrapoda, but that a slightly more inclusive clade comprises all tetrapods + *Osteolepis* (along with other fossil taxa; Ahlberg and Johanson, 1998). Any phylogeny is a representation of a historical reconstruction and is therefore formally hypothetical; this one is sufficiently well corroborated (Janvier, 1996, and references therein) that we may use it to investigate the distribution of morphological and developmental characters.

Divergence dates (millions of years ago)

500 | 400 | 300 | 200 | 100 | 0

Chondrichthyes (sharks, rays, chimaeras)



Osteolepis

†

clawed frog

Xenopus



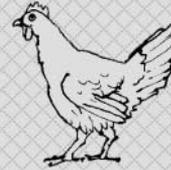
axolotl

Ambystoma



chick

Gallus



mouse

Mus



Tetrapoda + *Osteolepis*

lungfish

Neoceratodus



coelacanth

Latimeria



Sarcopterygii

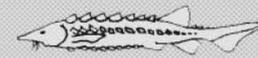


Cheirolepis

†

sturgeon

Acipenser



gar

Lepisosteus



zebrafish

Danio



Teleostei

Actinopterygii

Osteichthyes

Gnathostomata

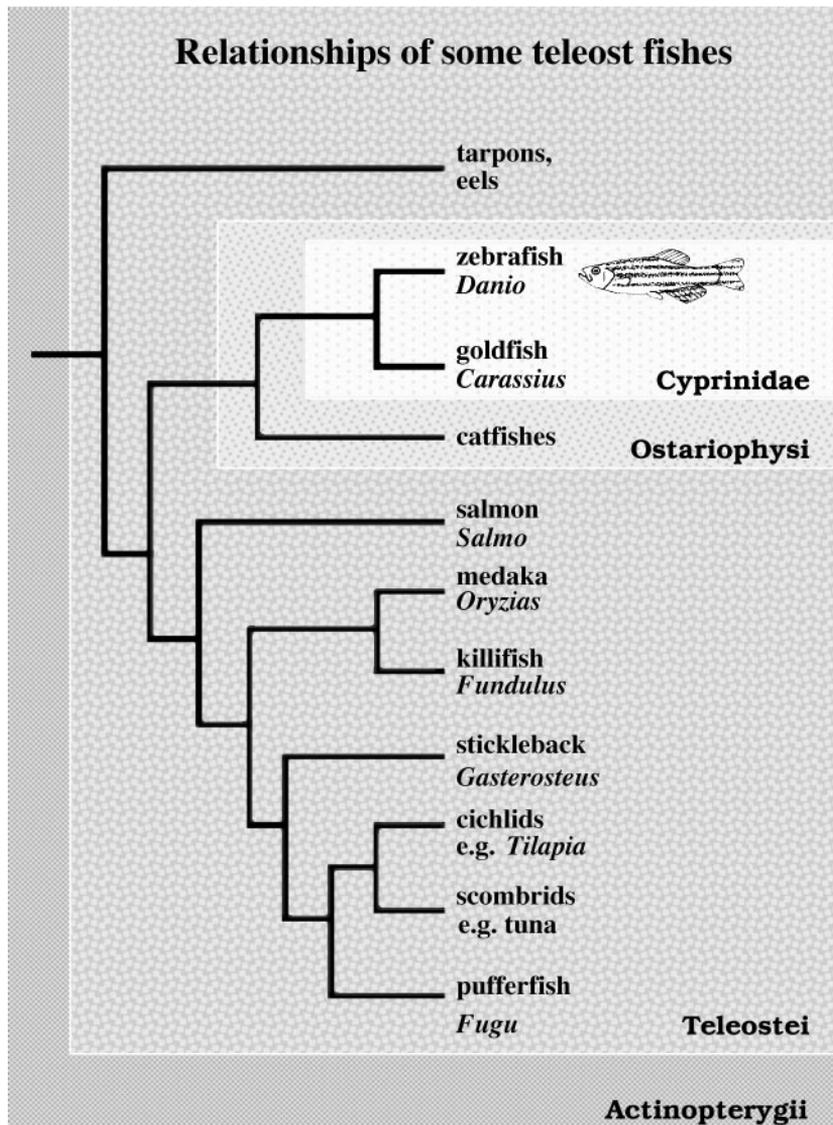


FIG. 3. Teleost relationships, showing the more commonly studied fishes (Lauder and Liem, 1983; Nelson, 1994). More basal lineages such as the Ostariophysi have fewer shared derived characters in common with "higher" lineages like scombrids. For any given character, however, they may or may not represent the ancestral condition: that determination requires outgroup analysis. Divergence times are not represented here. For a detailed analysis of relationships among cyprinids closely related to zebrafish, see Meyer *et al.* (1993, 1995).

models among some less famous living relatives. It also includes two fossil fishes, *Osteolepis* and *Cheirolepis*, which come from the Devonian period and are about 380 million years old (Janvier, 1996). Several points about this phylogeny are worth noting in detail.

(1) The laboratory models are concentrated in two narrow clusters among vertebrate lineages, with disproportionate emphasis on tetrapods. The reasons for this nonuniform distribution are clear enough: we have devoted significantly more attention to our own ecological and taxonomic neighborhood of the vertebrate world than to regions farther from home.

(2) The divergence times of the laboratory models are all

deep in the past; none is especially recent compared with the others.

(3) All of the major laboratory vertebrates are strikingly dissimilar to each other, and all have numerous unique specializations. None can be regarded outright as more primitive than another.

(4) The two fossil fishes are quite similar, even though one belongs to the zebrafish lineage and the other to the tetrapod lineage. This resemblance is due to shared primitive characters, that is, characters which were present in the common ancestor of the two lineages. Many of these characters have been lost in modern tetrapods and zebrafish, and their existence could not be inferred from a

straightforward comparison of the laboratory models alone. However, some of them persist in other living species; for example, the shared primitive asymmetrical tail of *Osteolepis* and *Cheirolepis* is also present in living sturgeon and shark.

A relatively complex phylogeny such as this contains numerous nodes, each of which can be taken to represent the undiscovered common ancestor of two lineages. It is possible to say quite a lot about these common ancestors even though they cannot be observed directly. Any characters present in both descendant lineages must either have been there in the ancestor also or have been derived twice (which is generally less likely). For example, all vertebrates have a backbone, and this character is thus present in the entire vertebrate phylogeny including all the unknown common ancestors of different vertebrate lineages. If on the other hand the two descendant lineages show different character states, but one of these states is shared with more distantly related animals, that state is more likely to have been present in the common ancestor. For example, *Osteolepis* has fins but all tetrapods have limbs; what was the condition in their common ancestor? The answer is that fins are also found in lungfishes, coelacanth, actinopterygians, and sharks and thus seem to be the ancestral condition for the *Osteolepis* + tetrapod lineage. This indicates that fins were present in the common ancestor of *Osteolepis* and tetrapods. Only after the two had separated did tetrapods modify their fins into limbs. This analytical approach to establishing character polarities and node conditions is called “outgroup comparison.”

Using this approach, we can draw up a thumbnail sketch of the common ancestors of the main laboratory vertebrates and examine how they differ from their descendants. The last common ancestor of all the vertebrate laboratory models was the common ancestor of the Actinopterygii (ray-finned fishes) and Sarcopterygii (lobe-finned fishes and tetrapods). It probably lived during the Silurian period, approximately 420 million years ago, and it was a fish resembling *Osteolepis* or *Cheirolepis* (Janvier, 1996). Within the ray-finned fishes, the dominant group today is the Teleostei with over 20,000 species. The zebrafish is a teleost, and so are all the paradigmatic laboratory fishes such as medaka, goldfish, stickleback, tilapia, and pufferfish. More specifically, the zebrafish belongs to the family Cyprinidae within the large teleost subgroup Ostariophysi, along with goldfish, carp, and many other freshwater species. The teleosts began a major evolutionary radiation in Triassic times, about 200 million years ago (Lauder and Liem, 1983). As a group they are characterized by many derived characters which are absent in primitive ray-fins like *Cheirolepis*. Teleosts are thus morphologically remote from the common actinopterygian/sarcopterygian ancestor. However, within the Teleostei, the Ostariophysi (and thus zebrafish) retain many primitive characters and occupy a relatively basal position (Lauder and Liem, 1983).

On the tetrapod side, the deepest node is that between amphibians and amniotes. The common ancestor at this

point lived near the Devonian/Carboniferous boundary, about 365 million years ago, and was a type of animal known as an “early tetrapod.” (“Amphibians” refers to the extant group of frogs, salamanders, and caecilians; Trueb and Cloutier, 1991.) We can infer that it had amphibian-like reproductive physiology, with aquatic eggs and gilled larvae. Fossil species close to this node (Coates, 1996; Jarvik, 1996) are roughly the size and shape of small crocodiles, with complex heavily ossified skulls and rather short limbs. Generally speaking, amphibians have diverged from this ancestry by becoming much smaller and simplifying their skeletons. Their dependence on cutaneous respiration is probably also an evolutionary innovation (Duellman and Trueb, 1986). Amniotes have dramatically modified their reproductive physiology, but their skeletal characteristics remain closer to the ancestral condition.

The last common ancestor of chicken and mouse was probably also the last common ancestor of all amniotes: all known reptiles (living turtles, lizards, snakes, tuataras, and crocodiles, along with fossil groups like dinosaurs) seem to be more closely related to birds than to mammals and thus belong to the chicken lineage in this simple phylogeny (Benton, 1991). The common ancestor lived during the Carboniferous period, probably about 340 million years ago, and was a small, cold-blooded, superficially lizard-like animal. Birds and mammals have both diverged dramatically from their common ancestry and have evolved endothermy independently.

This overview can be summarized by stating that none of the model laboratory vertebrates approximates closely the ancestral condition of any other. The zebrafish is not a model primitive vertebrate, and the axolotl and clawed frog are not model primitive tetrapods. However, each laboratory vertebrate has some character states that are primitive relative to those in one or more of its fellows. The trick is identifying these character states.

THE GENERALITY OF ZEBRAFISH

Table 1 shows some of the morphological characters that make up the zebrafish body plan and the phylogenetic/taxonomic level at which each character is generally present. The group names correspond to increasingly inclusive groups within which zebrafish can be placed:

Cyprinidae—carps and minnows (includes zebrafish and goldfish),

Ostariophysi—Cyprinids, catfish, and some others,

Teleostei—advanced ray-finned fishes with symmetrical tails (a very large group which includes most living fishes),

Actinopterygii—all ray-finned fishes (includes bichirs, sturgeons and paddlefishes, bowfins, and gars, as well as teleosts),

Osteichthyes—bony fishes (includes all ray-fins, lobe-fins, and tetrapods),

Gnathostomata—all jawed vertebrates,

Vertebrata/Craniata—gnathostomes + lampreys, hagfishes, and many fossil jawless fishes, and

TABLE 1
Some Zebrafish Characters, Showing the Taxonomic Level for Which Each is a General Feature

Character	Taxonomic level
Absence of jaw teeth.	Cyprinidae
Presence of Weberian ossicles (modified processes of the anterior vertebrae which transmit vibrations from the swim bladder to the inner ear).	Ostariophysi
Well-developed pharyngeal dentition and pharyngeal jaws (convergent with some other teleosts).	
Construction of the tail fin—a symmetrical and apparently terminal field of dermal fin rays (lepidotrichia) attached to an asymmetrical axial skeleton. (The general condition for the Actinopterygii, Osteichthyes, and Gnathostomata is an asymmetrical hypochordal tail.)	Teleostei
Very short, unbranched endoskeletons in the paired fins. A pectoral fin pattern of four proximal radials articulating with scapulocoracoid. (General for Actinopterygii and Osteichthyes is a set of such radials plus a series articulating with the posterior element, the metapterygium.)	
An enclosed swim bladder which functions as a buoyancy aid rather than a lung. (General for the Actinopterygii and Osteichthyes—as in <i>Polypterus</i> and the lungfishes—is a functional lung. Nonosteichthyan gnathostomes such as sharks lack a lung/swim bladder altogether.)	
An intestine without a spiral valve. (General for the Actinopterygii, Osteichthyes, and Gnathostomata—exemplified by sturgeons, lungfishes, and sharks—is an intestine with a spiral valve. The spiral valve has been lost independently in teleosts and tetrapods.)	
Well-ossified vertebrae. (General for the Actinopterygii and Osteichthyes is an unconstricted notochord surrounded by weakly developed, incomplete vertebral arches. Solid vertebrae have evolved independently in teleosts and tetrapods.)	
Presence of a propterygium—an enlarged and morphologically distinctive anterior endoskeletal element in the pectoral fin.	Actinopterygii
Scales composed of laminar bone.	
Cellular bone. (Acellular bone is derived in “higher” teleosts.)	Osteichthyes
Suite of dermal skull bones including maxilla and premaxilla.	
Fins supported by dermal lepidotrichia which articulate with endoskeletal radials.	
Presence of a lung/swim bladder.	
Separate paired pectoral and pelvic fins.	Gnathostomata
Presence of anal fin.	
Presence of jaws.	
Presence of ectodermal gills.	
Three semicircular canals	
Presence of tail fin and dorsal fin.	Vertebrata,
Presence of vertebral arches.	Craniata, or
Presence of a placode-derived octavolateralis system (lateral lines and inner ear).	Chordata
Presence of brain and paired sense organs.	
Presence of notochord, pharyngeal slits, and a postanal tail.	

Note. These are not necessarily the characters that *define* the clades within which they lie (Nelson, 1994, and references therein; Lauder and Liem, 1983; Winfield and Nelson, 1991; Roberts, 1972; Parenti, 1986).

Chordata—vertebrates + nonvertebrate chordates, including amphioxus and ascidians.

The zebrafish characteristics listed under each group name are general for that taxonomic level and for lower levels (= groups higher up in the list). For example, the tail morphology of zebrafish is general for the Teleostei, and thus also for teleost subgroups such as Ostariophysi, but it is not general for the Actinopterygii as a whole. “General for . . .” in this context equals “present in the last common

ancestor of . . .,” so zebrafish shows the ancestral tail morphology for teleosts, but not for all actinopterygians. This particular example will be considered further below.

Characteristics unique to zebrafish or its close relatives, such as its distinctive color banding, are unlikely to cause problems in a comparative analysis as they are easily recognized as specializations. A possible exception is polyploidy, which is currently under study (Postlethwait *et al.*, 1998; Vogel, 1998; Wittbrodt *et al.*, 1998). The lineage

that produced zebrafish appears to have duplicated its genome at some point in its history, but further analysis will be needed to determine when that event occurred and what clades may have inherited a polyploid genome.

By contrast, those characters that are general for some more inclusive group are both informative and problematic: informative because they cast light on relationships and evolution, problematic because a comparison at the wrong level of generality will give a false result. If the zebrafish is to be used (explicitly or implicitly) as a “model ancestor” for comparison with some other animal, only those characters that are general to groups which contain both the zebrafish and the other species can be considered.

For example, if zebrafish is being used as a model ancestor for mouse (i.e., taken explicitly or implicitly to represent the ancestral form for some group that includes the mouse), those of its characters that are general for the Osteichthyes, Gnathostomata, Vertebrata, and more inclusive groups can be considered. However, zebrafish characters that are general for less inclusive groups (say Ostariophysi or Teleostei) cannot be considered as ancestral to the mouse; they arose in the actinopterygian–teleost lineage after it had separated from the sarcopterygian–mammal lineage.

What is the significance of this character distribution to the utility of zebrafish as a paradigm animal? It appears that the overall body plan of zebrafish contains a lot of characters that are general at the osteichthyan or even the gnathostome level; to this extent it is a rather generalized vertebrate and can justifiably be considered a model ancestor for tetrapods such as mouse or axolotl. Particularly significant in this respect is the retention of characters such as functional gills, lateral line organs, median fins, and dermal fin rays, which have been lost or greatly modified in tetrapods. Likewise, its range of tissue types and derivatives (such as dermal and endoskeletal bone) is general at osteichthyan or more inclusive levels. However, the detailed morphology of many of its features has been greatly modified. For example, consider the zebrafish tail fin: the presence of a tail fin is general for the Chordata, and the presence of lepidotrichia in the fin is general for the Osteichthyes, but the morphology of the tail fin is general only for the Teleostei and quite unlike the primitive osteichthyan condition. Sometimes the zebrafish morphology is quite deceptive. Thus, zebrafish and tetrapods have superficially similar gut morphologies, but these have evolved independently; the general condition for Osteichthyes and Gnathostomata (present today in lungfishes, coelacanth, sturgeons, and sharks) is a rather complex gut with a spirally twisted lumen (Goodrich, 1958; Millot *et al.*, 1978). In a few cases, notably the swim bladder/lung, tetrapods show the general osteichthyan condition, whereas zebrafish show a specialized teleost condition (see Table 1).

On the basis of this phylogenetic review, we thus conclude that:

(1) The zebrafish is a rather generalized teleost and can in most cases be used to represent the “primitive” or “ances-

tral” condition in comparisons with more derived lab teleosts such as tilapia, medaka, fugu, and stickleback (Lauder and Liem, 1983). Two principal exceptions to this judgment are the Weberian apparatus, an elaboration of the anterior vertebrae that is a unique specialization of the Ostariophysi, and the absence of jaw teeth, which is characteristic of the family Cyprinidae (Roberts, 1972).

(2) The overall body plan of zebrafish is reasonably close to the general osteichthyan condition. Zebrafish characters such as the main body axis and sensory organs are retained from the common osteichthyan—and indeed gnathostome—ancestor. The zebrafish head, like the heads of other teleosts, has a visceral arch skeleton of a more primitive pattern than any tetrapod. It is not yet clear, however, whether zebrafish is more primitive than the lab tetrapods in such characters as patterning of the central nervous system and branchial skeleton (see, for example, Schilling and Kimmel, 1997; Prince *et al.*, 1998a).

(3) The detailed morphology of zebrafish is in most cases not comparable with the general osteichthyan condition, being instead marked by characters that evolved later within the zebrafish lineage and which are general only for the Actinopterygii, Teleostei, or Ostariophysi. Thus, the detailed skull bone pattern, dentition, vertebral structure, pattern of the fin endoskeletons, tail morphology, and gut morphology of zebrafish are not more primitive than those in lab tetrapods and should not be used to represent the ancestral condition in comparative studies with tetrapods.

(4) The phylogenetic generality of structures tends not to be unitary, but rather to comprise a hierarchy of several different levels—as shown, for instance, by the tail fin example given above. This will also be true for the developmental cascades that generate these structures.

GENERILITY OF ZEBRAFISH DEVELOPMENT

The phylogenetic distribution of morphological characters is at present much better understood than that of developmental or genetic characters, which are more cryptic. Phylogenetic mapping of the latter character types will be an important task for evolutionary developmental biologists in coming years. In the meantime we offer a few general considerations for developmental and genetic results in comparative contexts.

A few characteristics of zebrafish development are listed in Table 2, arranged as in Table 1. Most zebrafish research that uses our favored fish as a model vertebrate is concerned with structures or processes that are common to gnathostomes or vertebrates (or higher groups), such as axial patterning and neural crest migration (e.g., Raible *et al.*, 1992; Prince *et al.*, 1998a,b). It seems likely that the general developmental patterning of major structures such as the body axis, somites, central nervous system and sense organs, and branchial skeleton is similar to that in the common ancestor of all osteichthyans.

How far can we extrapolate the generality of an adult

TABLE 2
A Few Examples of Zebrafish Developmental Characters, Arranged According to Their Probable Levels of Generality, as in Table 1

Character	Taxonomic level
Patterning of the pharyngeal dentition (Stock, 1998; Huysseune <i>et al.</i> , 1997).	Cyprinidae or Ostariophysi
Patterning of the pectoral cartilage into four fin radials. Symmetric patterning of caudal lepidotrichia.	Teleostei
Formation of the propterygial cartilage (Cubbage and Mabee, 1996).	Actinopterygii
Neural crest contribution to median fin rays (Smith <i>et al.</i> , 1994). Involvement of <i>shh</i> , <i>ptc1</i> , and <i>bmp2</i> in fin-ray patterning (Laforest <i>et al.</i> , 1998). Development of an apical ectodermal fold (Thorogood, 1991).	Osteichthyes
Four (or more) <i>Hox</i> clusters (Holland and Garcia-Fernández, 1996). Polarizing activity in the pectoral limb bud (Akimenko and Ekker, 1995).	Gnathostomata
<i>Hox</i> -defined regions of the central nervous system (Holland and Garcia-Fernández, 1996). Migration of dorsolateral placodes and development of sensory organs from them (Northcutt, 1997).	Vertebrata, Craniata, or Chordata

Note. For most zebrafish developmental and genomic observations, data from other taxa will be required before they can be placed at the proper level. Readers interested in the possibility of an interactive, user-updated web page for housing information such as this are encouraged to contact the authors.

character to its ontogeny? If we can tie a particular developmental pathway to a particular morphology we can infer—always with some caution—the presence of those same ontogenetic elements in a fossil form or in an extant one that has not been or cannot be assayed for such developmental details.³ Thus we would expect that the tissue interactions and molecular pathways involved in tooth development in laboratory mice (Thesleff *et al.*, 1995) were present in ancestral amniotes and that they will be found in some form in the zebrafish pharyngeal dentition as well. However, the specific genes involved may not match up as simply as we might hope (see, for example, Akimenko *et al.*, 1994, 1995).

That last example brings up the rather Gordian problem of using hypotheses of homology to unravel various intertwined components of developmental systems in different taxa (Abouheif *et al.*, 1997). Developing methods for comparisons of ontogenetic pathways—not just discrete developmental stages—is another important area for future research in evolutionary ontogeny.

³ This is a parsimonious inference, that is, one that conforms to the available data with the fewest additional assumptions. However, instances of alterations to developmental or genetic pathways at one level *without* concomitant changes at another are not in short supply (see Hall, 1992, and references therein). Future investigations are likely to shed much more light on the coherence of ontogenetic pathways during evolution.

COMPARATIVE DEVELOPMENT: A PRACTICAL APPROACH

Late 20th century biology requires a reincorporation of developmental, morphological, and phylogenetic levels of description if it is to continue to develop our understanding of organismic systems. When confronted with a new set of embryological or gene expression data, how does one gauge its generality for any taxonomic level or go about interpreting some potentially comparable data from another organism? Or when designing a study to address an evolutionary or comparative question, how does one establish the context of the inquiry and choose appropriate model taxa? Here we describe a practical procedure for designing and carrying out a comparative study to investigate evolutionary changes in development. We will illustrate the approach with examples of current problems at morphological and molecular levels of developmental study.

This procedure follows and complements that of Northcutt (1992, 1997) for analyzing multiple ontogenetic sequences. His procedure is based on the comparison of known sequences of developmental stages for a given organ system. However, comparative investigations more frequently emerge from scattered morphological (or other static/scalar) observations, so we present here a protocol for using such observations to frame tractable evolutionary developmental questions. It should be emphasized that the two protocols are in no way incompatible; they are simply approaching the problem from different angles.

In brief, the steps are as follows:

(1) Recognize similarities and interesting differences between a number of adult morphologies, gene expression associations, etc.

(2) Consult an appropriate phylogeny for levels of generality: map the phylogenetic position of changes and determine which conditions are ancestral and which are derived, in order to pin down the differences as specific character transformations in phylogeny. (The precision attainable at this point is dependent on the phylogenetic resolution of the group in question. If the group is poorly resolved, it may not be possible to polarize all characters.)

(3) Identify suitable model taxa for study or comparison, delineating for each what information it is to provide. Taxa along the whole spectrum of laboratory utility should be considered: taxa useful for much and various laboratory work, ones of more limited utility (e.g., for morphology but not genetics, like *Amia*), living taxa that are not suitable for laboratory study (for example because they are very rare, difficult to keep, or excessively large and fierce), and fossil taxa. The latter categories afford less information, but they may provide examples of morphologies that are not available among the laboratory taxa. Actual choices of species for study will involve practical criteria such as availability of embryos.

(4) Erect hypotheses about developmental changes underlying repatterning/transformation. How was the ancestral ontogeny restructured to develop into the derived form?

(5) Design laboratory tests of the hypotheses.

This is an iterative and continually refining process. Information gained at any step can inform other steps as well, especially from steps 4 and 5 back to step 2: new developmental information can be placed in its proper evolutionary context and refine the overall investigation. Once sufficient developmental information is available for enough taxa, the protocol will come to resemble Northcutt's (1997) outgroup analysis of multiple ontogenies.

This procedure should, of course, lead to questions of significant developmental interest regardless of their evolutionary relevance, and some developmental researchers may wish to depart from the evolutionary course at this juncture, knowing that their results can ultimately be carried back to this context to exert their influence on larger scale problems.

As an example of this methodology, we present here a brief account of the history of caudal fin morphologies in the actinopterygian fishes, with particular emphasis on the origin of the homocercal (dorsoventrally symmetrical) tail fin characteristic of the teleosts. This is abstracted from a study of osteichthyan fin evolution we are currently undertaking.

Step 1: We note that a dorsoventrally symmetric field of caudal fin rays is a feature of the teleost tail (termed "homocercal"), but not of other actinopterygian fishes (whose asymmetric tails are termed "heterocercal"). It is known that the teleost caudal fin develops ventral to the notochord, which flexes dorsally to direct the fin rays caudally (Huxley, 1859; Geraudie *et al.*, 1995; P. Sordino,

unpublished data). Gar, *Amia*, and sturgeon caudal fins also develop below the notochord (Agassiz, 1878; Dean, 1895), but the result is a distinctly asymmetric structure. Some sarcopterygians (for example coelacanth and living lungfishes) develop a symmetric tail, but the rays develop both ventral and dorsal to the notochord, which remains straight and lies along the line of tail symmetry. The teleost tail's axis of symmetry is independent of the body axis.⁴

Step 2: The teleost fishes have been shown to be descended from a single lineage (i.e., they form a clade; Lauder and Liem, 1983), and a number of caudal fin characters are shared derived features of this group (synapomorphies). The symmetric pattern of the dermoskeletal portion of the caudal fin, the lepidotrichia or fin rays, is one shared derived feature of the teleosts. The range of morphologies in the teleost tail endoskeleton is also derived for the clade, but differs less drastically from the ancestral form. The hypurals do not form a distinctly symmetric array, especially early in development (e.g., Omori *et al.*, 1996; Kohno, 1997), though in some groups such as scombrids they do develop later into a very symmetric structure—a derived feature for those taxa (Potthoff, 1975). The phylogenetically more basal fishes—living gars and sturgeons, for example—have an asymmetric field of caudal fin rays.

Examination of the fossil members of this group of fishes shows that the teleost homocercal morphology is in fact derived from a heterocercal form quite similar to that found in the living gar *Lepisosteus*. Tails of still more basal groups of actinopterygians resembled the tails of living sturgeons and paddlefishes (Acipenseriformes). In each case, the similarities between the living and the fossil taxa are shared primitive features, not mere convergences. The living bichir *Polypterus* has a tail morphology (Bartsch and Gemballa, 1992) that is derived for its lineage (i.e., different from the primitive form but shared among polypterids: an autapomorphy) and is therefore very dissimilar to the primitive actinopt tail morphology (although the same may not be true for other characters, such as cranial morphology or dentition).

Step 3: From this analysis, it is clear that a caudal fin with distinct symmetry in the lepidotrichial field and no or late-developing symmetry in the endoskeleton has evolved from one with little or no apparent symmetry in the lepidotrichia and a lesser tendency toward symmetry in the endoskeleton. This has long been recognized as one of the main evolutionary transformations within the Actinopterygii (Lauder and Liem, 1983; Lauder, 1989).

Given this well-demarcated evolutionary change, it is apparent that some insight into the evolutionary changes to caudal fin ontogeny that led to the symmetric teleost tail can be gained by a straightforward comparison of tail development in a teleost and in a more basal fish. Having

⁴ An intriguing ventralized mutant of the medaka *Oryzias* develops a partial duplicate caudal fin on the dorsal side of its notochord (Tamiya, 1997). This mutant could be an interesting system for studying aspects of teleost tail formation.

already established the levels of generality for the relevant characters, we can choose workable representative taxa with confidence: we may exploit the laboratory advantages offered by *Danio* and choose a basal actinopt based on such practical criteria as availability of hatchling larvae. From *Danio* we can expect to learn something about the morphogenetic and molecular basis of the caudal fin symmetry, from experiments such as neural crest tracing and gene expression studies. A survey of teleost caudal skeletons shows that the zebrafish tail is quite typical for a moderately basal teleost, and so, results from such experiments can be taken as general for teleosts, as far as the overall developmental pattern is common to all teleost homocercal tails. From the basal actinopt—say a sturgeon or paddlefish—we can obtain similar information for the general actinopterygian condition.

Step 4: We can now begin to ask what changes to the ancestral ontogeny must have occurred to bring about the observed changes in morphology. The teleost ancestor acquired a symmetric field of morphogenetic influence in the dermal portion of its developing caudal fin, with only minimal influence on the axial skeleton. What exactly is it in the ventral caudal region of the developing teleost finfold that causes symmetrical formation of lepidotrichia, and which is absent in gars and sturgeons?

At this stage of our investigation, before we have committed resources to such lengthy endeavors as making and screening cDNA libraries, we can afford to examine the development of several fishes to fill out our picture of the evolution of tail development and to refine our developmental questions into truly lab-worthy ones. The fossil record of basal teleosts and their relatives indicates that the symmetric caudal fin exoskeleton appeared suddenly in evolutionary terms, rather than being acquired by progressive adjustment of the tail's shape, and with little or no concomitant change to the endoskeleton (Nybelin, 1963, 1977). The hypural skeleton did undergo a gradual alteration to a slightly more symmetric form in teleosts, and it develops to a very symmetric structure indeed in some of the more derived teleost groups, such as the scombrids (Potthoff, 1975).

Macroscopic examination of gar and sturgeon tails shows that both of those lineages develop a single field of lepidotrichia in the anterior half of the ventral caudal finfold, and lepidotrichia do not form in the posterior portion (which flexes upward to become the dorsal half of the tail fin). In *Danio*, the lepidotrichia form in a mirror-image series from their earliest appearance, before the flexion of the notochord. From the literature this appears to be the case for other teleosts as well (e.g., Potthoff, 1975). The caudal fin of *Amia* shows some tendency toward symmetrical lepidotrichial development, with caudal rays calcifying from a center point outward (Grande and Bemis, 1998, Fig. 76A).

Ionoscopiformes and Caturoidea, fossil sister groups of the amiids (Grande and Bemis, 1998), have tails that are more obviously symmetrical than that of *Amia*. The incipi-

ent symmetry in the developing *Amia* caudal fin could thus either represent an intermediate stage in the evolution of a fully symmetrical tail, or it could be an alternate product of evolution from a homocercal ancestor shared by amiids and teleosts. That area of the actinopterygian phylogeny is still under active study, and its further resolution will shed some light on the question of how teleost tail symmetry arose.

Step 5: The developmental investigation of the homocercal tail could take a number of directions. For instance, what is the role of neural crest in determining the pattern of lepidotrichia formation (Smith *et al.*, 1994), and what differences in neural crest migration patterns or other properties are to be found between *Danio* and sturgeon? Does the *Danio* tail show symmetric patterns of gene expression or other developmental factors, such as BMPs or TGFs? And do those symmetries, if present, indicate a saltational origin for the homocercal tail? At this point the direction of the research may well become techniques-driven, but it is still secure in the broader relevance afforded by its phylogenetic context.

Having chosen a particular developmental mechanism to examine in detail, how are we to interpret similarities and difference between our model animals? Since we have sound reasons for considering one model tail to represent the ancestral morphology and the other the derived form, we can interpret similarities in the details of their development to be conserved components of the ancestral ontogeny and differences to be derived (Northcutt, 1990; Holland, 1992). The validity of such a "rule" for any case must be judged with some care: given the many documented cases of modified ontogenies producing similar adult morphologies (Hall, 1992), it is necessary to consider for the case at hand just how likely it is that evolutionary changes to the ontogenetic processes have occurred without changing the morphological outcome of that ontogeny (Hanken, 1992). Only the continuing pursuit of further data from thoughtfully chosen laboratory models will ultimately resolve such issues.

CONCLUSION

The zebrafish has become a key component in the model systems approach to understanding vertebrate development. But as an element of comparative approaches its limitations must be clearly understood. (See Hanken, 1993 and Bolker, 1995, for lucid discussions of the two approaches.) Results of a developmental investigation of any one vertebrate model can be generalized only as far as the generality of the developmental system under study is known.

When we place results from the standard lab models in their proper phylogenetic context and begin to ask evolutionary questions based on those results, the need to expand the set of laboratory models becomes apparent. The investment in studying other vertebrate taxa will yield a high return in expanding our understanding of both development

and evolution, but new models have to be chosen with some forethought.

Phylogenetic relationships exist whether they are considered or not, and an explicit phylogeny provides a framework for formulating hypotheses and for interpreting the results of any comparison between different species. (Smith, 1997 and Lauder and Liem, 1989, provide guiding examples.) The extent and detail of the phylogeny required need to suit the questions under study. The study of systematics is a large and active field, and most groups of vertebrates have been subjected to far more systematic scrutiny than we in the development community are usually aware. It is left to us to inquire of the appropriate researcher or the relevant literature to find the particular framework we need. (The Tree of Life website is one source for general information and references; Lundberg, 1995.)

The two converging fields of development and evolution stand to derive great benefit from useful exchanges of expertise. We have outlined a practical guide to placing zebrafish in the correct context for making informative comparisons at any level of organization, and we hope it will serve as a model for studies of new laboratory vertebrates as the field of evolutionary ontogeny continues to expand.

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