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What Salamanders have Taught Us about Evolution

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Abstract

Taxon-based research in evolution permits the development of a multidimensional approach, illustrated here with lessons learned from research on salamanders. The clade is widespread and diverse, yet sufficiently small that one can keep all of the species in mind. This facilitates research from diverse perspectives: systematics and phylogenetics, morphology, development, ecology, neurobiology, behavior, and physiology. Different avenues of research offer unique perspectives on how a relatively old vertebrate clade has diversified. An integrated, hierarchically organized, multidimensional program of research on a taxon illuminates many general principles and processes. Among these are the nature of species and homology, adaptation and adaptive radiations, size and shape in relation to issues in organismal integration, ontogeny and development in relation to phylogeny, the ubiquity of homoplasy, ecological niche conservation, species formation, biodiversity, and conservation. Opportunities for future research and threats to the continued existence of salamanders are briefly outlined.

INTRODUCTION

In January, 1864, the French herpetologist Auguste Duméril received some strange animals from Mexico. These organisms, axolotls, possibly huge salamander larvae, did not resemble familiar species. After months in captivity they attained sexual maturity and bred, still in the larval morphological state with large external gills. Eggs were produced, they developed, and larvae formed. But then something unexpected happened—several of the larvae successfully metamorphosed and were recognized as typical members of the salamander genus *Ambystoma*. The original axolotls could not be induced to metamorphose but remained giant larvae. These observations had two outcomes. First, the conjecture by several herpetologists (e.g., Gray 1850) that axolotls were salamanders rather than a separate taxon intermediate between fish and tetrapods was validated. This reflects a major theme of this review—phylogeny matters. Second, as Gould (1977) has recounted, these events revealed that highly developed forms (e.g., fully metamorphosed salamanders) could revert to simpler stages by becoming sexually mature while still larvae, thus showing that orthodox ideas of recapitulation were not universal. The phenomenon displayed by axolotls, subsequently termed neoteny (a category of paedomorphosis), marks the first great impact of the study of salamanders on evolutionary biology. These observations set a course that ultimately led to the currently dynamic field of evolutionary developmental biology—evodevo—and the literature on development in axolotls, alone, is voluminous.

After studying salamanders for 50 years, lessons of general evolutionary interest have been learned, and challenges for future researchers developed. Examples dealing with important issues in evolution are presented in this review, which shows how a multidimensional approach to taxon-focused research has led to an understanding of general principles that are broadly applicable.

Salamanders, Order Caudata, together with frogs (Order Anura) and caecilians (Order Gymnophiona) (all clades), constitute the Class Amphibia. The number of salamander species is about 10% that of frogs, but they are three times more abundant than caecilians. Most taxonomies place salamanders in ten families: Ambystomatidae (1 genus, 32 species; North America), Amphiumidae (1 genus, 3 species; southeastern North America), Cryptobranchidae (2 genera, 3 species; eastern Asia, eastern North America), Dicamptodontidae (1 genus, 4 species; northwestern North America), Hynobiidae (9 or 10 genera, 52 species; Asia, entering eastern Europe), Plethodontidae (27 genera, 394 species; North, Middle, and South America, Korea, southern Europe), Proteidae (2 genera, 6 species; eastern North America, southern Europe), Rhyacotritonidae (1 genus, 4 species; northwestern North America), Salamandridae (20 or 21 genera, 81 species; Europe, Asia, North Africa, North America), Sirenidae (2 genera, 3 species; southeastern North America). The nearly 600 species of salamanders occur mainly in the Northern Hemisphere, but the Plethodontidae has successfully occupied the New World tropics, where nearly 40% of all salamander species are found. Several clades within Plethodontidae include the spelerpines (free-tongued species with larvae from eastern to central North America), desmognathines (eastern North American species with unique mouth-opening mechanisms), and New World tropical bolitoglossines (free-tongued species lacking larvae). In the Old World a few members of the most widely distributed family, Salamandridae, reach the tropics in southern China and northern Vietnam, Laos, and Thailand. All but one of the families (Hynobiidae) live in North America, which has five endemic families (AmphibiaWeb 2009).

Most salamanders have a generalized vertebrate morphology. Exceptions are the sirenids and amphiumids, which have greatly elongated trunks, but relatively short tails; amphiumids have ridiculously small limbs, and sirenids lack hind limbs entirely, whereas some plethodontids have tails that are twice head-plus-body length. The typical life history of a short (few weeks to months)

aquatic larval stage and a long (several years) terrestrial adult stage occurs in most of the families, but some salamandrids give birth either to larvae or to fully metamorphosed juveniles, and most plethodontids lack the larval stage and young are hatched from terrestrial eggs (Wells 2007). The typical life history is truncated in some taxa, which may be permanently gilled (proteids, sirenids) or incompletely metamorphosed and permanently aquatic (amphiumids, cryptobranchids). A few species in all taxa except rhyacotritonids are capable of achieving sexual maturity as larvae. The stream-dwelling cryptobranchids exceed 1.8 m in total length and 64 kg in weight. Amphiumids and sirenids, pond-dwelling aquatic forms, are as large as about a meter, and the aquatic proteids reach 50 cm. The largest metamorphosing salamanders are ambystomatids, dicamptodontids, and plethodontids, some reaching 30 cm or more. The largest fully terrestrial species are tropical plethodontids (*Pseudoeurycea*), which reach 29 cm. Many metamorphosing species are very small, and among plethodontids some species of the Mexican genus *Thorius* can be sexually mature at 4 cm or less (AmphibiaWeb 2009).

PHYLOGENY MATTERS

The history of the axolotl hints at how important it is to have a robust phylogenetic hypothesis. Until the axolotl metamorphosed and revealed itself to be a species of *Ambystoma*, taxonomic speculation was rampant. Even with the example of the axolotl in mind, when the famous Texas Blind Salamander (now *Eurycea rathbuni*), a depigmented, blind, permanently gilled species, was discovered in subterranean waters near San Marcos, Texas, it differed so much that it was variously called a proteid or placed in its own family. Dunn (1926) showed it was a neotenic plethodontid, but only in this century was its very close relationship to geographically nearby species of *Eurycea* conclusively demonstrated (Hillis et al. 2001).

Because of the aberrant morphologies and life histories of some species, it took a long time to determine that salamanders form a clade, and even recently a paleontologist, making an assumption about the strength of a phylogenetic signal in a single morphological character, raised questions about monophyly (Boisvert 2009), despite strong molecular evidence for it (Roelants et al. 2007). Only with the advent of DNA sequencing has it been conclusively determined that the incompletely metamorphosed taxa, which share many morphological features, have evolved independently (Wiens et al. 2005). When paedomorphic characters were excluded it was not possible to sort the groups appropriately using morphology alone because there were insufficient synapomorphies among nonpaedomorphic clades, and there has been extensive parallel adaptation to aquatic life by paedomorphic taxa. Homoplasy, which can be resolved only with a robust phylogenetic hypothesis, is rampant in salamander evolution.

Soon a robust phylogenetic hypothesis for all salamanders will exist. DNA sequence data are available for all species of most families except the Plethodontidae, for which all genera and 90+% of species are represented. At present, nuclear datasets exist for some species of all families (Roelants et al. 2007); and for many species in the Plethodontidae (Vieites et al. 2007, Wiens et al. 2007), complete mitochondrial genomes exist for most families and many species of the largest families such as Plethodontidae (Mueller et al. 2004), Hynobiidae (Zhang et al. 2006), and Salamandridae (Zhang et al. 2008), and combined morphological and molecular datasets exist for subsets of taxa (e.g., Frost et al. 2006, Wiens et al. 2005) as well. Extensive morphological datasets exist (e.g., Larson & Dimmick 1993, Wiens et al. 2005). We are unlikely to be misled in the future by characters that are thought to be so fundamental that they could not evolve independently. Phylogenies are the critical backbone for all evolutionary studies.

GENERAL EVOLUTIONARY MESSAGES FROM STUDIES OF SALAMANDERS

The literature dealing with salamander evolution is enormous (Petranka 1998, Wells 2007). The focus here is on a few broad generalizations of great personal interest.

Old Words, New Problems

Words matter because in science they often represent concepts. Two old words have persistently caused problems and also stimulated research: species and homology. Both words have critical conceptual relationship to the evolutionary issues discussed.

Species. In many areas of biology, species are treated as natural “kinds” in an almost biblical sense. For many studies in ecology, behavior, physiology, morphology, and other areas species are treated as nondimensional units (“what you see is what you get”), and there is little concern with what is generally understood as the species problem. Among my first publications were descriptions of new species of tropical salamanders, represented in collections by a handful of specimens, sometimes only one. It was relatively straightforward to sort specimens by morphology and determine, by comparisons with museum specimens and the literature, that some represented unnamed taxa. There were problems with some plethodontid genera—*Plethodon*, *Batrachoseps*, and *Ensatina*, for example—for which abundant specimens existed. The advent of molecular methods, initially starch-gel electrophoresis of allozymes, led to an era of splitting. Vastly more differentiation than suggested by morphology was encountered, and taxa proliferated, largely by subdivision (*Plethodon*: Highton 1989, Highton & Peabody 2000; *Desmognathus*: Tilley & Mahoney 1996; *Batrachoseps*: Jockusch & Wake 2002). The ring-species *Ensatina* (Stebbins 1949) seemed like low-hanging fruit when my molecular systematic studies began. Stebbins worked in the era of polytypic species (Mayr 1942). He reduced several species to subspecies within a single species *Ensatina eschscholtzii*, ranging in western North America from British Columbia to northern Baja California, west of the Cascade-Sierra Nevada mountain ranges. I expected *Ensatina* to be a multispecies complex. Indeed, initial studies showed that genetic variation within the complex was great (Wake & Yanev 1986). However, decades of on-going research have failed to yield well-defined, conventional species (see below).

Sharp debates over species and criteria for their recognition (Mayden 1997) culminated in the recognition that different species definitions are variations on the same general species concept—species are but temporal stages, or segments, of a lineage within the general phylogeny (de Queiroz 1998). The various so-called concepts reflect differences in the criteria used to identify species borders and recognize species taxa. Our modern species problem can be summarized as, “what do you want species to be?” We have the tools (mainly molecular, but also behavioral and genetic) to make fine distinctions; if the goal of modern species-level systematics is to name every possible lineage segment, we will have vastly more species than at present. *Ensatina*, which exemplifies a Darwinian mode of species formation involving range expansion and contractions, adaptive differentiation, and vagaries of geological and ecological history, might be broken up into many species, thereby obscuring the ring-species pattern, as has occurred in other complex biogeographic examples. For example, the ring-like *Plethodon jordani* complex is now many species, despite substantial discordance of morphology, allozymes, and mtDNA (Highton & Peabody 2000; cf. Weisrock et al. 2005, Weisrock & Larson 2006).

By recognizing that species are pieces of history, reified by extinction of intergrading populations, it might seem that the job of a systematist is mainly that of a kind of historian, but species

are hypotheses and, by naming them, systematists are making predictions, e.g., of independence. A resulting implication is that many of the generalizations made about species have only an incidental relation to their status as species. Several fallacies severely bias research. Following the lead of Williams (1992), some of these are:

1. **Unique niche occupancy.** The idea of one species to a niche, or even the idea that each species has a niche, is partly an artifact of niche and species definitions. We know that because of phylogenetic niche conservatism several species can have the same niche (perhaps geographically adjacent) (Wake 2006, Wiens 2004), and subdivisions of the same species can have different niche requirements (Rissler et al. 2006). In a perceptive analysis arguing that whatever they are, species must have ecologies, not necessarily unique, Van Valen (1976) asked why, other than for names, must we have species?
2. **Species individuality.** Ghiselin (1966) argued that as taxa, species are individuals. Because the origins of most species are only vaguely determined, the notion of species as individuals holds, at best, only in an instantaneous or in a philosophical sense and has little practical value.
3. **Adaptive uniformity.** Adaptive polymorphism is well documented within species of salamanders (cannibalistic morphs of *Ambystoma tigrinum*, Larson et al. 1999), and geographic variation in adaptation is nearly ubiquitous. The *Eurycea* salamanders of the Ozark and Ouachita regions (Bonett & Chippindale 2006), where populations vary geographically with respect to neoteny, is a relevant example.
4. **Species selection.** Species selection is nothing more than a special case of clade selection (Williams 1992).
5. **Species integration.** The notion of species integration is rather romantic. What Darwin termed “unity of type” applies more to clades than species (Wake 2006, Wake et al. 1983a, Wiens 2004).

Naming species is a way of keeping track of biodiversity, but species are not equal and we should recognize that they are simply segments of phylogenetic lineages, viewed at varying points along evolutionary trajectories. Echoing de Queiroz (2007), there are no necessary attributes of species other than being separately evolving metapopulation lineages. It is desirable to have an accounting system to keep track of just what parts of the broad genealogical nexus of life exist at any given time. Care must be taken to be sure that we are not endowing species with qualities they do not have.

Homology. Homology is another old word that predates Darwin. Ghiselin (1966) argued that homologues, like species, are individuals from a philosophical perspective. To say that two structures are homologous, one has individuated the structures and made a statement that if the structures are indeed homologues, they have had a common phylogenetic origin. Differences between them are likely the result of evolution (although they could be simply the result of phenotypic plasticity, mutation, etc.). Because homology is an inevitable outcome of phylogenesis, Wake (1999) advocated abandoning largely fruitless discussions on what homology is, how it should be defined, etc. Homology is a practical issue: Specific hypotheses of homology are tested by phylogenetic analysis of a larger dataset. Latent homology, the appearance of “lost” homologues in taxa whose ancestors lacked them, is a fascinating topic (Hall 2007). An example is a lost tarsal element in the limbs of salamanders that is referred to as “m” or Schmalhausen’s “m”. This element, present in Paleozoic amphibians, is still “present” although usually not expressed in the ontogeny of living salamanders. It appears as a rare variant, sometimes symmetrical in the two hind limbs (Shubin et al. 1995), but in a given species it combines with one of two distal tarsal elements forming two

or three alternative adult morphologies. Element “m” is a latent homolog of a bone in ancient amphibians; it can be expressed in new ways in modern relatives (see below).

Cherished Ideas about Evolution Die Hard

It can be difficult to give up cherished ideas that have been treated as certainties, as in the following examples from salamanders. Desmognathines are distinctive in many ways and long were classified as a separate family or subfamily (Wake 1966). Most species of *Desmognathus*, especially those long considered to most closely resemble ancestral forms, are aquatic to semiaquatic with larvae that may live for several years. However, some small semiterrestrial to terrestrial species have no larval stage, but experience direct development from encapsulated embryos to hatchlings that are miniatures of adults. The morphologically divergent, fossorial *Phaeognathus hubrichti*, which also develops directly, is the sister taxon of *Desmognathus*. Early DNA studies recovered the direct developing *Desmognathus* in relatively basal phylogenetic positions (Titus & Larson 1996). Perhaps larvae had been lost as many as three times in desmognathines. Molecular evidence accumulated to the point that the most parsimonious explanation, contrary to expectations, was that larvae, present in all families of salamanders and considered ancestral, had been lost in the ancestors of desmognathines and had re-evolved in a crown clade within *Desmognathus* (Mueller et al. 2004, Wiens et al. 2005). However, this result has been challenged by Bruce (2005), the foremost student of desmognathine life histories, and it has been very difficult for many workers to accept; the result has now been strongly reinforced by new nuclear DNA data (Vieites et al. 2007) and seems unassailable. The possibility that larvae, once lost, re-evolved remains hard to accept.

Some researchers still rely on single character phylogenies rather than using total evidence. Boisvert (2009), a paleontologist, argued that a particular pattern of salamander vertebral development, centra developing before neural arches, was unique. In order for this event to be true Hynobiidae must be paraphyletic, because two genera (*Ranodon* and *Hynobius*) retain the ancestral trait, whereas *Salamandrella* (also a hynobiid) and all other salamanders studied share the supposedly unique trait. Her argument for paraphyly is supported solely by the trait in question! Monophyly of the family is strongly supported (Zhang et al. 2006), so the trait is homoplastic.

Body elongation is common in salamanders, but only in *Lineatriton* has it been achieved by elongated vertebrae rather than increases in vertebral numbers (Wake 1991). However unlikely, molecular data suggest that the phenomenon, together with extreme shortening of limbs and reduction in manus and pes, may have occurred twice within *Lineatriton*, making it paraphyletic, and its species are deeply nested within the morphologically conservative *Pseudoeurycea*, suggesting that these large morphological changes occurred rapidly and recently (Parra-Olea & Wake 2001). If the evidence from mitochondrial DNA is bolstered by nuclear data, taxonomic revision will be necessary to place *Lineatriton* in an expanded *Pseudoeurycea*, despite its extraordinary morphology (e.g., Frost et al. 2006). This is a striking example of homoplasy.

Species Formation Is usually Vicariant, but Ecological Species Formation Occurs as Well

Early students of species (notably Mayr 1942, Rensch 1960) envisioned them originating, spreading out geographically and differentiating to form polytypic species (“Rassenkreise” in Rensch’s terminology). He envisioned components of such species representing one another geographically (nonadaptive divergence, see below) or ecologically (i.e., phylogenetic niche conservatism, see below) and thought they could be incipient species. This conception fits *Ensatina* perfectly, but the hardening of the so-called biological species concept (Mallet 2008) and the current trend to split

taxa (Highton 2000) have the effect of obscuring critical questions concerning factors involved in species formation. Most polytypic species of salamanders have been split into many often narrowly distributed species that form hybrids or intergrade zones in a complex pattern: *Plethodon jordani* (Highton & Peabody 2000), *P. glutinosus* (Highton 1989), and *Lyciasalamandra luscbani* (Veith & Steinfartz 2004). The *Triturus* complex ranging over continental Europe has proven difficult taxonomically; seven species are broadly parapatric and a hard polytomy prevents a resolved phylogeny (Arntzen et al. 2007). A stable hybrid zone (or hybrid swarm), which has existed for well over a century in western France (Arntzen & Wallis 1991), led to recognition of *Triturus cristatus* and *Triturus marmoratus* as species, and a subspecies of *T. marmoratus* in Spain was elevated to species status as *T. pygmaeus* when biological differences were detected between the closely parapatric forms with no evidence of hybridization (Espregueira Themudo & Arntzen 2007). The picture is far less clear in eastern Europe, where four nominal forms meet (Arntzen et al. 2007). Widespread polytypic species that have avoided full taxonomic splitting are *Ensatina*, the *Ambystoma tigrinum* complex (Shaffer & McKnight 1996), and *Salamandra salamandra* in Europe and the Middle East (Steinfartz et al. 2000). Hybridization and intergradation suggest that all are in the midst of a long period of lineage differentiation with varying stages of species formation that may, through extinction of intermediates, result in distinct, bounded species. In *S. salamandra* geographically adjacent subspecies display different modes of reproduction, showing that the complex is evolving adaptively (Buckley et al. 2007, García-París et al. 2003). One must be careful not to taxonomize significant evolutionary questions into obscurity.

Ensatina has been a focus of study in my laboratory. Stebbins (1949) envisioned an ancestral stock, from the northern redwood region, spreading out and adaptively diverging to give rise to boldly marked, spotted, and blotched animals ranging throughout the Sierra Nevada and into southern California, and uniformly and aposematically colored mimics of dangerously poisonous newts along the central California coast. These lineages met in southern California, where they occur in sympatry with some hybridization. The more generally upland, blotched animals (*klauberi*) contrasted strongly with the more lowland, uniformly colored animals (*eschscholtzii*). This was a ring-species complex, illustrating stages in a gradual process of adaptively driven, Darwinian species formation. Extensive new data and different methods have failed to reject Stebbins' biogeographic hypotheses, despite adding much that is new (Alexandrino et al. 2005; Jackman & Wake 1994; Kuchta 2005; Kuchta et al. 2009a,b; Moritz et al. 1992; Wake 1997; Wake et al. 1986, 1989). Critics suggest that as many as 11–12 species could be recognized (Highton 1998), yet discordances between datasets and evidence of ongoing genetic exchange argue against breaking the complex into many species (Wake 2006; Wake & Schneider 1998). Although *Ensatina* is not a perfect ring-species (there is no on-going gene flow throughout the ring, and the complex is relatively old), it is perhaps the most convincing of the relatively few well-documented ring-species complexes (Irwin & Irwin 2002). Species formation in *Ensatina* has been “Darwinian” in that the most divergent populations in terms of coloration are vivid examples of divergent selection and isolation leading toward species formation.

Ensatina exemplifies aspects of both vicariant and ecological species formation. Other examples involve incipient ecological species formation along ecological (Steinfartz et al. 2007) and altitudinal (García-París et al. 2000) gradients.

Not Everything Is Adaptive—but Do Not Underestimate the Power of Selection

Explanations for extreme morphologies evoke scenarios grounded in natural selection and adaptation. No one doubts that the long, fast, powerful projectile tongues of some salamanders are adaptations for feeding (Deban et al. 1997). Sometimes alternative explanations suffice. Most

terrestrial salamanders are five-toed, but four-toed species have evolved independently three times in plethodontids and once in salamandrids, as well as in hynobiids. Most four-toed species are small, even miniaturized. Unique among vertebrates, the salamander fifth toe is the last to form; it develops as a bifurcation of the fourth mesopodial (for background see Shubin & Wake 2003). Perhaps the last toe in four-toed species is 4 plus 5 rather than 4 (Wake 1991). This example of a developmental constraint in evolution was challenged (Reeve & Sherman 1993) on the grounds that everything is ultimately adaptive. Their line of argument is a category error; a constraint on the generation of form is not a constraint on adaptation (Autumn et al. 2002). It remains to be seen whether the explanation that works for plethodontids and salamandrids also applies to hynobiids. The four-toed hynobiid *Salamandrella* (northern Asia) is small, but four toes are also found in relatively large, long-legged *Batrachuperus* (China) and *Paradactylodon* (Iran, Afghanistan). Evidence is strong that four toes in hynobiids became fixed in three independent clades (Zhang et al. 2006). No adaptive explanation for the loss of a digit in the two large-bodied genera exists as yet.

Bolitoglossa (101 of the 582 species of salamanders) is widespread in the New World tropics and the only clade truly successful in the tropical lowlands. Whereas salamanders are classic North Temperate animals, often restricted to cool, moist environments, species of *Bolitoglossa* are exceptional. The majority have extensively webbed hands and feet, and are typically arboreal. The historic explanation for webbing (Wake 1966) is that it evolved as an adaptation for climbing, and Alberch (1981) showed that the fully webbed feet were capable of creating suction. Subsequent analysis showed that such suction would contribute little functionally and, furthermore, that a heterochronic change (paedomorphosis) is responsible for a clade-wide, persistent morphology, which results from a developmental correlation among many traits (Jaekel & Wake 2007). A pattern characteristic of juveniles of related taxa has become the standard among species of *Bolitoglossa*, and no adaptive explanation for the increased webbing is necessary. The simplest explanation is that the ancestral stock of the clade was miniaturized, paedomorphic, and a lowland dweller, which probably inhabited leaf axils and had indistinctly developed digits. Selection for individuated digits has resulted in the repeated evolution of individuated digits, which have high functional value in different ecological settings. If webbing is ancestral in *Bolitoglossa*, digits have experienced repeated homoplastic evolution in different subclades. Interdigital webbing can be directly functional; a member of a related clade, *Chiropoterotriton magnipes*, a cave-adapted species with very large, fully webbed hands and feet, displays a very different growth trajectory than *Bolitoglossa* (all of whom share a common growth pattern). Accordingly, *C. magnipes* is interpreted as having functionally significant, directly adaptive digital webbing used to maneuver on the wet, smooth walls of caves.

Evolutionary ecological studies of salamanders have shown how communities of close relatives have evolved, including detailed studies of species interactions in a guild-based context (Adams 2007). Selection on size and biomechanics of jaws in relation to aggressive interactions (rather than solely prey capture and food processing) has led to clear demonstrations of morphological and ecological character displacement (Adams 2004). In these instances selection alone is adequate to explain the complex patterns of adaptation.

Size Matters—but it Needs to Be Scaled

Not all salamanders are small using human standards. Some species exceed a meter in length but few exceed 20 cm. We measure with a metric scale. However, vertebrates differ greatly in cell volume and this has important consequences when thinking of scale. Salamander genomes, and cells, are dramatically larger than those of birds, reptiles, and mammals, and even larger than all but a very few frogs and caecilians (Gregory 2003). Because salamander cell sizes are

so large, metric size fails to capture what might be called “biological size” (Hanken & Wake 1993). Large cells have slow metabolic rates, but also slow rates of cell division, development, and even differentiation (Sessions & Larson 1987). Salamander brains have greatly simplified organization relative to other amphibians and amniotes, including inferred ancestors, and display many signs of paedomorphosis (Roth & Wake 2001). Yet, they have sophisticated vision and are capable of complex behaviors, including biomechanically complicated tongue projection (Deban et al. 1997, 2007) and prey capture, and individual recognition ability (Jaeger 1981). Roth & Wake (2001) refer to brain devolution, a curious reversal of trends in other vertebrates. Despite the negative implications of increased genome size, there is no satisfying adaptive explanation for the repeated evolution of large genomes. Very small salamanders must make do with organs made of many fewer cells, leading to new patterns of brain organization and such unexpected evolutionary events as the enucleation of red blood cells of large-genomed but physically small plethodontids (e.g., *Batrachoseps*; Mueller et al. 2008). It is for these reasons that Hanken and Wake refer to salamanders as being much smaller, biologically, than they appear to be using metrics. If one were to scale salamanders by numbers of brain cells they certainly would be the smallest vertebrates and would be equivalent to small insects. An advantage is that they have very low metabolic rates and make less demand on the environment (for example, for food) than their physical size would suggest. In short, salamanders are much smaller than they appear to be.

An Individual Is an Ontogeny—Evolutionary and Phylogenetic Implications

Individual organisms are often treated as if their instantaneous status is permanent. Salamanders are long-lived, and species with larval stages might spend most or even all of their lives in an “imperfect” state. For example, *Dicamptodon copei*, from western Washington and northern Oregon, undergoes metamorphosis rarely if at all. The sirenids, amphiumids, and cryptobranchids live in ambiguity, neither fully larval nor fully metamorphosed, and this has obscured their phylogenetic relationships. The huge, aggressive, aquatic amphiumids lay eggs out of the water and guard them (Gunzberger 2003), traits characteristic of their sister taxon, the mainly terrestrial plethodontids, most of whom are small and innocuous. Yet, many plethodontids with aquatic larvae are neotenic, especially those associated with caves. Every organism is an ontogeny and has its own ontogenetic trajectory, and members of a population, a species, or a clade share at some level a common trajectory, which may stop, save for reproductive organs, at nearly any point, including extending the ancestral trajectory (Alberch et al. 1979).

Awareness of the existence of an ontogenetic trajectory gives insight into the phylogenetic diversity of salamanders (Alberch et al. 1979). In general, there is a shared, conservative salamander ontogeny, but as we have seen, members of different taxa can progress to different stages of the trajectory, or diverge from it. Heterochronic terminology can be abstruse and confusing, but for salamanders neoteny is generally taken to be a slowdown in rate of morphological development in relation to sexual maturity. Neotenic species can grow to great size without ever fully metamorphosing. Neoteny is a kind of paedomorphosis, the incorporation of juvenile morphological states of ancestors (inferred from out-group phylogenetic analysis) into the terminal adult stages of descendants. Not all paedomorphic taxa are large; many plethodontids remain small, either as permanent larvae (e.g., species of *Eurycea*) or, in the case of direct-developing taxa, as miniatures (e.g., *Tborius*, *Batrachoseps*). In the most extreme cases of paedomorphic minaturization, skull bones fail to complete development and do not articulate, or may even fail to appear (Alberch & Alberch 1981, Hanken 1984). Heterochrony in many guises has been a major theme in salamander evolution (Alberch & Blanco 1996).

The necessity to “think ontogenetically” is well illustrated by the complex pattern of different closely related species occupying different points along a general ontogenetic trajectory. Within the spelerpines, a great range of end points are achieved by different closely related taxa, and homoplasy is rampant (Bonett & Chippindale 2006). An absolute necessity for an analysis of the relation of ontogeny to phylogeny in any given instance is a robust phylogenetic hypothesis.

Ontogenetic Reorganizations and the Evolution of Novelty—from Microevolution to Macroevolution

A significant source of novelty in salamander evolution is the diversification of ontogenetic trajectories by heterochrony (changes in relative timing of ontogenetic events), heterotopy (changes in the relative topographic occurrence of ontogenetic events), and heteroplasia (changes in the relative growths of organs or parts of organs in ontogeny) (Alberch & Blanco 1996). Add to this the persistence of developmental processes and one has a fertile field for evolutionary novelty and opportunity (Müller 2007). All of this is taking place within a framework of stability, resulting from constraints, canalization, and related phenomena that, in the end, are fundamentally shared inheritance. These are not new realizations; Darwin (1859) wrote about the opposing forces of natural selection and what he termed unity of type. The playing field is not open; it is constrained in many ways by history, development, function, physics, fabrication necessities, and the like. Although controversy has swirled around ideas of constraints, all that it means in the end is that it is easier for some things to evolve than for others (Alberch 1980), and this is not a result of absence of genetic variation.

Paedomorphosis in various forms is common in salamanders, but its alternative, peramorphosis (or shapes beyond, Alberch et al. 1979), also occurs. Both are associated with novelty and transitions from micro- to macroevolutionary realms. The arboreal salamander *Aneides lugubris* has juveniles that resemble adults of related taxa, but ontogenies are extended to achieve a novel state, characterized by hyperossification, enlargement of bones and teeth, and increased aggressiveness (Wake et al. 1983b). Evolutionary modifications of ontogenetic trajectories during phylogenesis are vividly evident in the hundred + million year old history of salamanders.

Reorganization of the tarsus of salamanders has led to homoplastic novelty—adaptation for scansorial locomotion and arboreal life in the unrelated plethodontids *Aneides* (North America) and *Chiropetrotriton* (Mexico); in each instance, their sister taxa have generalized tarsi. An identical, functionally significant, rearrangement of tarsal elements occurred by a developmental switch involving Schmalhausen’s element “m” (see above), so that the element shifts its connection from the fourth to the fifth distal tarsal, thereby increasing its size, changing its articulation pattern, and enabling the effective spreading of the entire foot and especially its outermost digit for enhanced clinging and climbing ability (Wake 1991). The variational potential inherent in the conserved developmental trajectory of salamander limbs was illuminated by study of a large sample of the salamandrid genus *Taricha* (Shubin et al. 1995). Schmalhausen’s “m,” typically unexpressed as an adult morphology in salamanders, was found in 5.4% of 902 limbs, and it was symmetrical in 1% of the individuals.

Highly projectile tongues have evolved repeatedly in salamanders, reaching morphological extremes of specialization (length, speed, power) (Deban et al. 1997, 2007) only when functional constraints are absent. Tongues of salamanders perform two main functions. They force air into the lungs during respiration and they extend from the mouth to the prey during prey capture. These two functions limit specialization in either direction. There is a third function in larvae; the parts that become the tongue of adults wave the gills and enable suction feeding. Lunglessness has evolved repeatedly in salamanders, and extreme tongue specialization has evolved in the

lungless hynobiids *Onychodactylus*, the lungless salamandrids *Chioglossa* and *Salamandrina*, and in all plethodontids. The extremes in plethodontids are achieved in *Hydromantes* and the tropical bolitoglossines, which not only are lungless but also lack larvae. The spelerpines have homoplastically evolved, long, fast tongues that use an alternative biomechanical pathway based on proportional differences in hyobranchial cartilages necessary for larval function in this clade (Wake & Deban 2000). Evidence that lung respiration and gill-waving act as constraints on specialization is the repeated evolution of tongue projection in independent clades lacking both.

Adaptive Radiations Can Evolve Slowly

The tropical plethodontids (bolitoglossines) are members of a single deeply nested clade. There are currently 243 species (or about 42% of all salamanders), with more being described every year. This clade experienced an adaptive radiation unusual in that it occurred within a framework of constraints on the direction of evolution (Wake 1987, Wake & Larson 1987). Bolitoglossines lack larvae; life-history modification is not a component of this adaptive radiation. Furthermore, all species have a highly specialized feeding mechanism that involves a long, fast, protrusible tongue (see above), including specialized neuroanatomy and vision (Lombard & Wake 1986, Roth & Wake 2001). The tongue varies little throughout the clade; a few species have enlarged jaws, but these may relate more to social behavior and aggression than to feeding, as in other plethodontids (Staub 1993). Accordingly, feeding, often a component of adaptive radiations, is not a factor in this adaptive radiation. The brains of these salamanders have very few cells and a simplified structure that might limit structural and functional diversification (Roth & Wake 2001). In contrast, diverse locomotor adaptations have evolved in the context of the invasion of diverse and sometimes novel habitats and microhabitats (Wake 1987, Wake & Lynch 1976), including caves; epiphytes (moss and liverwort mats and clusters, bromeliads); leaf axils; soil; and even, in one instance, homoplastic return to an aquatic habitat in a cold mountain stream (Wake & Campbell 2001). Locomotor specializations include increases in numbers of vertebrae and reduction of limbs in burrowing species of *Oedipina*; increases in the length of individual vertebrae and reduction of limbs in burrowing species of *Lineatriton*; expansion of the manus and pes, and dramatic increase in interdigital webbing of cave-dwelling members of *Chiropterotriton*; paedomorphic reduction of limbs leading to an undifferentiated pad in leaf-axil dwelling members of *Bolitoglossa*; re-evolved digits in high-elevation members of *Bolitoglossa*; interdigital webbing coupled with development of hooks on terminal phalanges of arboreal *Bolitoglossa*; whole body miniaturization and limb reduction of crevice-dwelling species of *Thorius*; and whole body miniaturization coupled with specialized digits and prehensile tails of bromeliad-dwelling members of *Chiropterotriton*, *Cryptotriton*, *Nototriton*, and *Dendrotriton* (Jaekel & Wake 2007, Wake 1987, Wake & Lynch 1976). The bolitoglossine adaptive radiation fits all parts of Schluter's (2000) definition except rapid speciation; there may have been many bouts of rapid speciation, but the continuing phenomenon has been in progress for a long time, possibly 50 Myr or more (Vieites et al. 2007, Wiens et al. 2007).

Homoplasy Is even More Common than I, or perhaps Anyone, Has ever Imagined

As is evident from nearly all parts of this review, homoplasy, the independent evolutionary origin of characters so similar as to appear to be homologues, is a common phenomenon in salamanders, and cladistic analyses based on morphological traits are likely to give incorrect results (e.g., Wiens et al. 2005). Consider the evolution of highly projectile tongues in plethodontids. Lombard & Wake (1986) argued that such tongues had evolved three times, with three additional evolutionary events

giving rise to semiattached protrusible tongues. Subsequent analyses of molecular data (Mueller et al. 2004) bolsters this conclusion, suggesting that homoplasy is even more extensive and unanticipated than earlier work (using a morphologically-based phylogenetic hypothesis) indicated. It is difficult to find a single trait in salamanders that can conclusively be demonstrated as a unique synapomorphy. We should not be surprised that homoplasy is ubiquitous; Hall (2007) has argued persuasively that although structures might be lost, their genetic-developmental backgrounds may be retained so that environmental or selective pressures may trigger the reappearance of similar features in organisms that do not share a recent common ancestor. Similarly, novelties in one clade may appear independently in other clades (e.g., projectile tongues) because of shared genetics, developmental and organismal backgrounds that enable selection to call out the trait in question (Wake 1991).

SOME FUTURE RESEARCH DIRECTIONS

Knowledge gained from evolutionary studies of salamanders has also highlighted research opportunities. Examples below are detected patterns for which we lack understanding of processes.

Stasis in Morphology and Ecology

Morphological evolution of salamanders has progressed slowly and, apart from the bizarre paedomorphic lineages, most salamanders have highly conserved, even static, morphology (Wake et al. 1983a). Causes for this conservation are unclear, but phylogenetic inference suggests that it has extended for 100 million years or more. Stasis extends to ecology as well (see below). Yet, there have been episodes of rapid morphological change within the framework of stasis. For example, molecular evidence led to the hypothesis that the attenuate (very slender with diminutive limbs) and elongated species of *Lineatriton* arose recently from within a deeply nested clade of morphologically generalized and conservative *Pseudoeurycea* (Parra-Olea & Wake 2001).

Niche Conservation and Nonadaptive Radiation

Congeneric species of salamanders are often very similar in ecology and morphology, even in speciose genera such as *Desmognathus*, *Batrachoseps*, *Plethodon*, and *Thorius*. Wiens (2004, phylogenetic niche conservation) and Wake (2006, nonadaptive radiation) argued that ecological and morphological stasis contribute to the formation of geographically isolated (allopatric) lineages. Upland species of *Plethodon* (e.g., *P. shenandoah*, *P. hubrichti*) in eastern North America are geographically isolated from each other, apparently because though once widespread they were forced upward into allopatry by interactions with a newer, more widespread species (*P. cinereus*) that is a superior competitor (Wiens 2004). In contrast, species of *Batrachoseps* in California were initially isolated when geological fragmentation and movement of newly formed landmasses distributed associated populations geographically (Wake 2006). Doubtless combinations of these two phenomena are associated with the buildup of salamander diversity.

Tropical Diversity Patterns

Bolitoglossines are highly successful in tropical America, using numbers of species, extent of distribution, and degree of ecological and morphological divergence as criteria. The species occur from northern Mexico to central Bolivia and from sea level to over 5000 m in elevation, and include miniatures and giants, fossorial, terrestrial, and arboreal species (Wake 1987). Bolitoglossines

represent the pinnacle of modern success for salamanders. Tropical salamanders had a North Temperate origin and they are phylogenetically deeply nested (Vieites et al. 2007); at some point late in the evolutionary history of plethodontids they became associated with tropical environments and diversified. A mid-elevational bias in species richness is directly related to the inferred age of occupancy (Wiens et al. 2007). Increased tropical diversity is not a mid-domain effect, however; diversity is highest in the narrowest part of the Americas (Central America), and falls off dramatically in South America (Wake 2005). On elevational gradients, highest species abundance is at or near the tops of mountains, except where they exceed about 3000 m (Wake et al. 1992).

There are two main categories of hypotheses as to why there are latitudinal gradients of diversity, with the peak in the tropics (Mittelbach et al. 2007): (a) time and area, and (b) rate of diversification. The first is associated with the idea that temperate taxa are younger—demonstrably false in this case. The second is associated with the idea that diversification rates are higher in the tropics, or that extinction rates are lower. Tropical salamanders are concentrated in mid-elevational habitats, and if phylogenetic niche conservatism is operative, perhaps salamanders became associated with tropical environments in the north at some point during Tertiary when temperatures were much warmer, and they moved south as temperatures generally cooled, following the path of least resistance from both climatic and ecophysiological perspectives (Wiens 2007). Perhaps from a global perspective there has not been an increased diversification rate (Wiens 2007) because the tropical salamander clade is about as old as several northern plethodontid clades; if one uses a stem approach, the diversification rate is higher, but if one considers crown groups only it is not. Wiens speculates that perhaps extinction rates have been lower. However, if one considers local diversification in the tropics one is struck by the high degrees of local endemism—the occurrence of close relatives at different elevations on a given elevational transect—and by evidence supporting in situ parapatric and allopatric species formation along local elevational gradients (e.g., García-París et al. 2000, Parra-Olea et al. 2001).

Whatever accounts for the success of tropical bolitoglossines, it is a fact that the deeply nested supergenus *Bolitoglossa* is the most speciose salamander clade. Its evident evolutionary success runs counter to the dogma that the tropics are crowded and hostile to invaders.

Diversity is Fractal

The numbers of salamander species have changed dramatically since 1926 when Dunn recognized 71 species of plethodontids; today there are 394 (approximate; discoveries and taxonomic revisions are on-going—see AmphibiaWeb 2009). New discoveries, especially in the New World tropics, account for many (tropical species have increased from 30 to 243). Even in the United States and Canada numbers have increased from 39 to 142, largely the direct result of the application of increasingly sophisticated molecular methods. Striking increases involve North American genera thought to be well known in 1926: *Batrachoseps* (from 6 to 20), *Desmognathus* (from 7 to 20), *Eurycea* (from 7 to 26), and especially *Plethodon* (from 11 to 55). A breakthrough was the use of allozymes by Highton (1989), who examined widespread species and discovered them to consist of genetically and geographically cohesive subdivisions, which he recognized as species. Almost all of them are parapatric or allopatric in relation to close relatives. Many taxa display complex patterns of hybridization or genetic interaction with adjoining species (Highton & Peabody 2000; Weisrock & Larson 2006). An alternative classification might recognize several polytypic ring species complexes, analogous to *Ensatina*, but Highton (2000) has stoutly defended his taxonomy of *Plethodon*, which is widely but not universally (Petranka 1998) accepted. Application of DNA sequencing methods uncovers even more diversification; the closer one looks the more diversity one finds. Following detailed studies of *Batrachoseps*, which resulted in taxonomic revisions recognizing 20

species (Jockusch & Wake 2002), *B. attenuatus* was examined closely for phylogeographic structure using mitochondrial and nuclear DNA sequences (Martínez-Solano et al. 2007). Extreme population subdivision was found; 183 specimens examined from a densely sampled area centered around San Francisco Bay uncovered 138 haplotypes. Five geographic segments were recognized with intergroup divergencies averaging from 5 to 9.5%. No taxonomic changes were proposed, but some systematists would recognize several species. Gene diversity within each ranged from 0.96 to 0.99, and genetic distances within two of the five units exceeded 5%. Apparently, almost literally, no end to the diversity of salamanders, notably the terrestrial species that lack larvae, has been found. The world is a very big place for these salamanders, which have differentiated on an extremely local scale.

The number of species of salamanders recognized has increased dramatically recently. The number jumped from 355 in 1985 to 506 in 2002; at present there are 582 species recognized and many new species are described annually (AmphibiaWeb 2009). Much of this increase in Europe and North America can be attributed to taxonomic revision motivated by molecular data. However, amazing discoveries have been made, notably the first Asian plethodontid, *Karsenia koreana* (Min et al. 2005), which qualifies as one of the most surprising biogeographical discoveries since 1900. Many new species are plethodontids, particularly from the American tropics where there have been great increases in field research, but notable discoveries from well-studied regions include *Salamandra lanzai* from a small area in the Cottian Alps of France and Italy, *Batrachoseps gabrieli* from very near Los Angeles, California, and several species *Lyciasalamandra* from the Mediterranean coast of southern Turkey (AmphibiaWeb 2009). There is no indication that the rate of discovery is slowing. Paradoxically, much new discovery is related to the attention that salamanders and other amphibians are getting because of amphibian declines (see below), which has attracted more researchers and stimulated field activity. Diversity of the small, terrestrial, direct-developing species does indeed seem to be fractal.

WHAT IS IN STORE FOR SALAMANDERS?

Salamanders, as we know them, have been around for more than 150 million years and perhaps far longer. Now they are facing unprecedented challenges.

Salamanders and Climate Change

Climate change will profoundly affect salamanders. They are ectotherms that are temperature and humidity limited, and many of them have surprisingly small distributional limits. Furthermore, many species are restricted to specific habitats and microhabitats (e.g., bromeliads) and narrow elevational zones. This is particularly true of tropical species, but there are also species restricted to high elevation in the Appalachian Mountains, the Alps, and the Sierra Nevada of California. Patterns of elevational distribution of tropical plethodontids have been studied intensively, starting with the pioneering work of Schmidt (1936) on a community of salamanders associated with Volcán Tajumulco in western Guatemala. This was followed by our extended study of this region (Wake & Lynch 1976) and our recent retrospective analysis of changes over a roughly 30-year period (Rovito et al. 2009). We found 15 species distributed from near sea level to 3900 m, with the most diverse communities (7 at one spot) found in upper elevational (2400–2800 m) cloud forests. Between 1978 and 2005, three species disappeared and two others experienced upward shifts in their distributions of between 300 and 500 m. These results have frightening implications. Most tropical salamanders have narrow distributions, many are restricted to specific habitat types, and many occur near the tops of mountains and have nowhere to go. Data exist for 10 elevational

transects, ranging from central Veracruz, Mexico, to the vicinity of the Costa Rica-Panama border (García-París et al. 2000; Wake 1987, 2005; Wake et al. 1992). Species extend to the tops of the highest mountains in many areas, and a number of these species restricted to the highest peaks are at risk of being “pushed” off the top into extinction.

Salamanders have faced dramatic climate change in the past and yet have prospered. Past episodes of global warming may have been factors in the invasion of the Old World by plethodontids, and the reinvasion of western North America from Asia by *Hydromantes* (Vieites et al. 2007). As these organisms were forced northward, new territory became available to them and invasion routes shortened progressively toward the North Pole. What differs about the current global warming is that it is happening very rapidly, so rapidly that salamanders have little time to adapt, and furthermore, there has been so much habitat modification and destruction that migration routes are blocked in many if not most instances.

Salamanders are tough, and there is pretty strong evidence that they are capable of sustaining themselves in the face of environmental extremes. The southern Appalachian mountains are famous for their extraordinary salamander diversity (Duellman & Sweet 1999). Many of these species have small geographic ranges, and within these ranges they display substantial phylogeographic structure, attesting to their age and low dispersal tendencies, and also suggesting that they have not experienced much recent change in distribution. During glacial stages of the Pleistocene, conditions in the Appalachians were much different than today, yet salamanders appear to have persisted. In California, species of *Batrachoseps* are restricted to remote desert canyons that might imply they are Pleistocene relicts, yet they, too, show phylogeographic structure implying that they are old and that their distributions were largely unaffected by major periods of warming or cooling in the past (Yanev & Wake 1981).

The Crisis of Declining Amphibians

The profound decline of amphibians has been well documented (Stuart et al. 2004, Wake & Vredenburg 2008). The most compelling evidence relates primarily to frogs, but salamanders, too, are declining and even disappearing. Habitat modification explains most salamander decline in North Temperate regions. Profound losses of salamanders in Central American cloud forests likely are the combined result of habitat destruction and climate change (Rovito et al. 2009). A virulent fungal disease is responsible for the most dramatic losses of frogs and, because the fungus is also known to infect salamanders, it may play a role in losses of some species.

Salamanders have survived since late Paleozoic times, if not earlier (Marjanovic & Laurin 2008, Roelants et al. 2007). They are survivors, many of them capable of persisting through major periods of climate change. They are facing unprecedented challenges, nearly all traceable to direct and indirect effects of *Homo sapiens*. We have learned much from them and we have much yet to learn, and I am confident that at least some members of the major clades will outlast the species that poses the greatest threats to their existence.

CONCLUSIONS

Taxon-centered research programs have the advantage of enabling integrated, multidimensional analyses in evolutionary biology. In this review, I have tried to illustrate many of the research themes that have emerged from approaching a taxon, the Order Caudata, from different perspectives: phylogeny and systematics, functional morphology and biomechanics, developmental morphology, and evolutionary ecology. Each of these areas gains from knowledge generated using alternative perspectives from other areas. Sometimes the pursuit of explanation in a strictly



Darwinian, adaptationist framework works well, but in other instances more structuralist approaches are necessary to understand such phenomena as constraints on the direction of evolution, limitations on the extent of change, and opportunities emanating from dynamic changes in ontogenetic trajectories such as heterochrony (Wake 1991, Wake & Larson 1987). Multidimensional, hierarchical approaches to problems in evolution within taxa will be increasingly necessary as the knowledge base continues to grow. Not only morphology and ecology, but also disparate areas including physiology, development, and behavior benefit from such perspectives (Autumn et al. 2002). The challenge for the future, in an era of steadily increasing specialization and technical and analytical sophistication, is to see the whole in relation to all the parts.

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