



AN INTEGRATED APPROACH TO EVOLUTIONARY STUDIES OF SALAMANDERS

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Abstract: This essay is an examination of the ways in which seemingly disparate studies focused on a single, rather small taxon, the Order Caudata, can provide a foundation for development of programs in evolutionary biology. Topics addressed include the use of individual characters as contrasted with complexly integrated systems of characters in phylogenetic analysis, the nature of integration and its impact on specializations and adaptation, and on the conceptualization and recognition of species. The possibility that salamanders and other amphibians might play a role as bio-indicators in environmental studies is raised.

INTRODUCTION

MY GOAL IS TO SHOW HOW STUDIES BROADLY focused on the organismal level can be used to develop a framework for an understanding of the evolutionary biology of a major taxon—in this case, the salamanders, Or-

der Caudata. Studying organisms from one perspective can inform and direct investigations that have other goals, and an integrative approach to organismal evolution can result. As evolutionary biologists, our ultimate goal is to understand how biological diversification occurs, and as herpetologists our primary focus is the whole organism, as exemplified by amphibians and reptiles. The examples I use are mainly from my own laboratory, because I can see most clearly in these cases how integration can be achieved. Although this paper is topical in nature, I attempt to provide connections between topics.

Any evolutionary study focused at the organismal level requires a framework of phylogenetics, with clearly stated hypotheses of relationships underlying all data analyses. At the same time, however, one must frankly acknowledge that the Caudata have been and remain a controversial group; we have yet to achieve a robust phylogenetic hypothesis for the group as a whole, or even for the genera within the larger families. Thus, I envision phylogenetics as an on-going process with reciprocal illumination occurring as we learn more of the nature of characters, character complexes, and novel data.

PHYLOGENETICS

There is growing acceptance that the living amphibians form a monophyletic group, the Lissamphibia, and that the Order Caudata

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also is a monophyletic group (Trueb and Cloutier 1991; Duellman and Trueb 1985). I will not deal with these issues further.

Progress has been made in understanding phylogenetic relationships at high taxonomic levels, but one of the most vexing problems facing a salamander biologist is the difficulties encountered in generating even a relatively robust phylogenetic hypothesis concerning the relationships of salamander families. The hypothesis of Duellman and Trueb (1985) serves as a convenient point of departure, for it displays the traditional arrangement of a basal position for the Sirenidae, a monophyletic group including Hynobiidae and Cryptobranchidae that is also relatively basal, and a deeply nested position for the Plethodontidae. Thus, the taxa with external fertilization (Hynobiidae and Cryptobranchidae, and presumably Sirenidae) are separated from a monophyletic group that practices internal fertilization.

New studies of morphological characters and of DNA sequences challenge this orthodoxy in several important respects. Cloutier (in prep.), using morphological traits, not only fails to find a monophyletic Hynobiidae + Cryptobranchidae, but also believes that the family Hynobiidae is paraphyletic. He does agree with earlier authors in recognizing the Plethodontidae as being deeply nested. In contrast, Larson (1991) has studied the nucleotide sequences of ribosomal RNA and concludes that the Hynobiidae + Cryptobranchidae is monophyletic but not basal, and that the Plethodontidae, Amphiumidae, and Rhyacotritonidae (a family recognized on morphological grounds by Good and Wake [in press]) are relatively basal. According to Larson, the remaining families form a monophyletic group and the Dicamptodontidae and Ambystomatidae are deeply nested sister taxa; this challenges the findings of Edwards (1976). Larson has re-evaluated the morphological

characters used by Duellman and Trueb (1985), and when these recoded characters were placed on the trees of Duellman and Trueb (based mainly on morphology) and of Larson (based on DNA sequences), the fit to the former was somewhat more parsimonious than to the latter; Larson argues that the difference is not statistically significant. Therefore, Larson's hypothesis is equally valid. Hillis (1991) has attempted to combine some of the morphological data and sequence data available at the time he wrote his paper, but as yet the result is not robust and has low internal consistency.

A major problem is the reconciliation of these different data sets. Larson (1991) has made an initial effort along these lines and his results demonstrate anew that any hypothesis of relationships of the salamander families requires extensive homoplasy for nucleotide, morphological, and other kinds of characters.

We need a fresh look at the nature of morphological and other characters and their interrelationship, and new characters. We must give attention to the ways in which characters are generated in development and the degree to which characters are developmentally and functionally linked. There is so much homoplasy in the morphological characters of salamanders that cladistic hypotheses that use states that cannot be directly observed in taxa are suspect. I especially distrust characters that, on the basis of a parsimony analysis alone, are considered to be lost and thus used as a synapomorphy low in a tree—yet reappear (attributed to evolutionary reversal) higher in the tree. In general, I mistrust characters whose state must be inferred rather than directly observed.

We lack critical information for various taxa. For example, it is amazing that we still do not know whether sirenids have external or internal fertilization. On the one hand, the absence

of cloacal glands suggests that no spermaphore is produced and that sperm storage does not occur. On the other, the fact that eggs are laid individually suggests that internal fertilization might occur.

My focus has been the Family Plethodontidae, which includes over 60% of the approximately 390 living species (Frost [1985] and subsequent descriptions). Homoplasy is extensive in the family (Wake 1966, and in press; Wake and Larson 1987) and has led to problems in developing a robust phylogenetic hypothesis. Since the publication of my comparative osteological study of the family (Wake 1966), however, there has been relative taxonomic stability. The monophyletic nature of the family is unquestioned, as is the monophyletic status of the two major sister taxa, the subfamilies Desmognathinae (3 genera and 14 species) and Plethodontinae (15 genera and about 235 species). The species of the Plethodontinae (still about 60% of living species!) were placed in three tribes: Plethodontini, Hemidactyliini (Dubois [1984] has argued that the correct name should be Mycetoglossini), and Bolitoglossini. Of these, the Bolitoglossini is an unquestioned monophyletic group and the Plethodontini is very likely a monophyletic group, but evidence for the monophyly of the third tribe is weaker.

Due to the extensive homoplasy and the relative dearth of characters that can be scored at the level of the tribes, I have argued that one might treat the feeding system (which involves many characters that could be separately coded) as an integrated and somewhat self-organized system that could be interpreted as one large, complex character. I have examined the implications of analysis of that system for understanding the phylogeny of the family (Roth and Wake 1985). Nevertheless, Lombard and I (1986) also attempted a cladistic analysis using all data then available to us. The two subfamilies and three tribes were

treated as four primary taxa. (Presch [1989] has questioned this procedure, which I defend on the grounds of the demonstrable homoplasy in the group; I reject Presch's reanalysis of our data, which involves recoding of data and use of groups of genera as primary taxa.) By accepting these four primary taxa, we are accepting that projectile tongues evolved independently at least three times in the family, and because we think that attached projectile tongues are functionally and phylogenetically independent of free projectile tongues, projectile tongues of some sort must have evolved at least five times, assuming that our four primary taxa are monophyletic. We examined the 15 dichotomous trees that could be generated for four groups, and found that two are more parsimonious than the alternatives. Our choice requires that the free projectile tongue evolved three times and the attached projectile tongue evolved three times in the family. It will be difficult with any rearrangement of taxa to reduce this more than by one; Lombard and Wake (1986) treated *Batrachoseps* and the supergenus *Bolitoglossa* as sister taxa, but if the sister taxon of *Bolitoglossa* is *Hydromantes* the number of evolutions of free tongues could be reduced by one.

At present we have too little information to choose among the various alternatives, but data have been building that support certain relationships. For example, details of the organization of the nervous system (such as the complete loss of the lateral line system, which is not a necessary condition associated with direct development [Wake et al. 1987]) support the concept of the monophyly of the Plethodontini + Bolitoglossini.

I would like to see the development of a comprehensive data matrix (including DNA sequence data) at the level of genera or species groups for the family, but to accomplish this will require sufficient insight into character ontogeny and evolution to deal, at least

in part, with the extensive homoplasy in the family. When such is available, it will doubtless continue to show substantial homoplasy, but by looking at different subsets of the matrix perhaps we will come to understand whether the integrated complex approach I have espoused is useful in phylogenetic analysis. I look forward to data sets based on nucleotide sequences, for they will be helpful in sorting out the morphological homoplasy. Eventually we will have to arrive at an understanding of relationships based on all available data, but how that will be accomplished is as yet unclear.

HIERARCHICAL APPROACHES

There is increasing interest in hierarchical approaches in evolutionary biology (for example, Eldredge 1985; Vrba 1989). Much discussion about hierarchies has focused on the question of whether or not selection occurs at the level of species, but my interest is in the interactions taking place between levels in the molecular-organismal-populational hierarchy. I can best illustrate this by focusing on the phenomenon of miniaturization in salamanders, for a great deal of useful information has accumulated in recent years concerning miniaturization and its effects (for recent reviews see Hanken, in press; Roth et al. 1990; Wake, in press).

Miniaturization is found in several families of salamanders, but it is particularly evident in the Plethodontidae, in which it has evolved repeatedly. I consider any species that becomes sexually mature at body sizes of about 30 mm to be miniaturized, and by this criterion there are numerous miniaturized plethodontids (some examples are: *Desmognathus aeneus*, *D. wrighti*, *Hemidactylium scutatum*, *Eurycea quadridigitata*, *Plethodon websteri*, *Batrachoseps attenuatus*, *Bolitoglossa rufescens*, *Chiropterotriton dimidiata*, *Dendro-*

triton bromeliacia, *Nototriton richardi*, and all species of *Thorius*).

Ecologists generally accept that body size is the single most important variable in an individual from the perspective of population or community ecology. Thus, one expects that the evolutionary dynamics operative at the population and community levels of organization must have an important role in determining size at first reproduction, maximum body size, and the minimum size at which independence can occur. Furthermore, many investigators have shown that the size of a female is strongly related to the numbers of eggs produced, with each species having a relatively narrow and predictable relationship between egg size and egg number (Houck 1977; Kaplan and Salthe 1979).

Genome size in salamanders is huge (Sessions and Kezer 1991; Sessions and Larsen 1987; Morescalchi 1990; Olmo 1983) in relation to other amphibians and especially in relation to other vertebrates. In fact, the largest genome size in salamanders is exceeded among vertebrates only by the lungfishes (Morescalchi 1990). An empirical relationship exists between genome size (amount of DNA per haploid nucleus) and cell size. Some researchers believe that genome size increases in a ratchet-like manner, tending to increase unless countered by other factors (the "selfish DNA" hypothesis). While there is some evidence that genome sizes can both increase and decrease within lineages of salamanders (Sessions and Larson 1987), there might be something of a ratchet effect, since all of the bolitoglossines have relatively large genome sizes, and in different genera (for example, *Hydromantes* and *Bolitoglossa*) the genomes have become enormous.

In some miniaturized species, such as *Desmognathus aeneus*, no special problem arises with miniaturization, for these small

salamanders also have small cells. As genome sizes increase, however, problems arise. This is perhaps most evident in members of the Mexican genus *Thorius* (Figure 1), studied extensively by Hanken (1982, 1983a, 1984) and Roth et al. (1990). In such cases hierarchical interactions have profound impact on organismal-level phenomena, such as structure of the skull and of the limbs, and especially of the central nervous system.

Most species of *Thorius* inhabit small spaces under bark or surface cover, and their eyes protrude only slightly beyond the limits of the head. Since the cells are large, however (because the genome is large), the eye must be large in relation to the head in order to be image-recording. *Thorius* has the most highly specialized projectile tongues (Lombard and Wake 1977), and they are dependent on relatively high visual acuity for feeding, as are all plethodontids (Roth 1987). The eyes impinge strongly on the space for the brain, however, which lies between the eyes in the platytrabic (that is, broad and flat, lying between two cranial trabeculae) brain case. The impinging eyes lead to deformation of the forebrain and to posterior displacement (illustrated by Roth et al. 1990). There are only about 25,000 photo-

receptors in the eye (contrasted with about 450,000 in a frog such as *Rana*), but there are 30,000 retinal ganglion cells; thus, the whole retina is a functional fovea (Roth, Rottluff et al. 1988). The main integration center in amphibian brains, the optic tectum (which contains the cells that are directly related to visual function), has only about 35,000 cells (Roth et al. 1990), as compared to about 500,000 cells in that of *Rana*.

Although the *Thorius* brains are extraordinarily tiny, visual acuity and distance perception remain at effective levels. A number of compensatory changes in the brain have been identified by Roth et al. (1990). The visual centers are relatively enlarged at the expense of other areas, such as the forebrain. Cell-packing approaches perfect packing density—apparently at the expense of glial cells, which are extraordinarily reduced in number. Glial cells are generally thought to play critical roles in brain function, and in other vertebrates they are about as abundant as neurons. This trade-off does not occur even in humans, with our greatly expanded brains and trillions of neurons. The close packing and large size of the neurons in these tiny animals has many implications for such phenomena as cell migra-

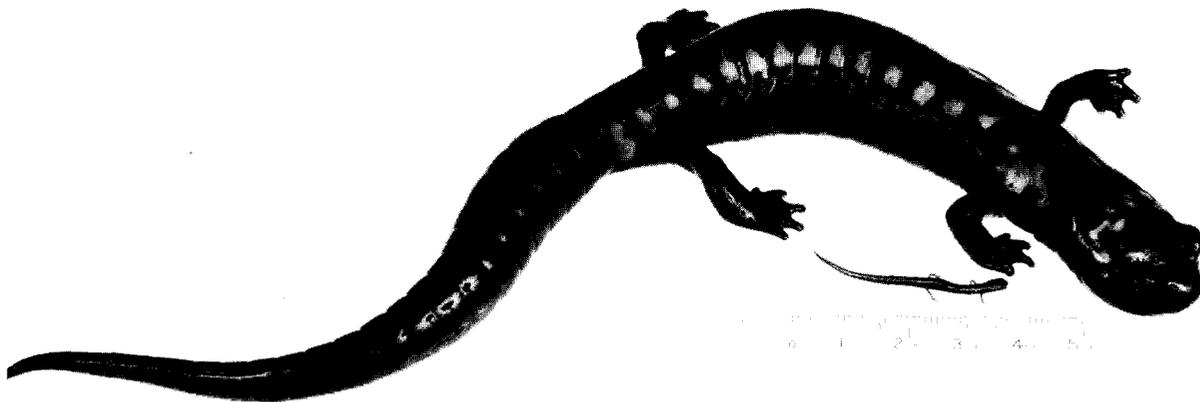


FIGURE 1. *Thorius pennatulus* (from near Orizaba, Mexico) and *Pseudoeurycea bellii* (from near Putla de Guerrero, Oaxaca, Mexico), showing the smallest and largest species of tropical salamanders (family Plethodontidae, tribe Bolitoglossini). Scale is in centimeters.

tion (which seems to be strongly impeded) and may well contribute to the general impact of paedomorphosis in bolitoglossines.

Exactly what these tradeoffs and compromises mean to these species functionally is unclear, but one might expect the effects to be most strongly felt at hatching. It turns out that the hatchlings of *Bolitoglossa*, which we have studied in the laboratory at Berkeley, are very altricial, and they do not move much for some days. Occasionally we find, however, a one-month-old animal climbing to the top of its container (a normal behavior for larger animals). For some weeks they do not eat, and perhaps they are incapable of using their eyes at first. Other general effects might be predicted. For example, the forebrain of bolitoglossine salamanders is relatively small, and Sever et al. (1990) have shown that these animals also have poorly developed cloacal glands. I predict that tropical bolitoglossines, in particular, will turn out to be less oriented to olfactory cues in daily and courtship behavior than are North American species such as *Plethodon cinereus* and *Desmognathus ochrophaeus*, which use olfactory cues for many functions (David and Jaeger 1981; Horne and Jaeger 1988; Houck and Reagan 1990; Jaeger 1990; Mathis 1990). I would also expect them to show relatively little territoriality as a consequence. Further studies are needed to test these predictions.

I have used miniaturization to show how evolution at one hierarchical level can have an effect on another, in both upward and downward directions. The organism is in essence "squeezed" by the combination of downward effects from the population level imposing miniaturization and upward effects from increased genome size imposing large cells. Something has to give, and does. It presents us with an excellent opportunity to explore the role of certain morphological features in behavior and ecology.

ONTOGENY AND PHYLOGENY

The existence of the axolotl (*Ambystoma mexicanum*) and the widely known larval state of such curious organisms as *Necturus* and *Proteus* force salamander biologists to accept the fact that paedomorphosis has played an important role in salamander evolution. What has been recognized recently is that we have focused too much attention on the phenomenon of larval reproduction, and this has diverted us from appreciating how profound the influence of paedomorphosis has been in direct-developing salamanders that lack a larval stage. These direct-developing organisms display ontogenetic repatterning, an extensive reordering of morphogenetic events in time and space that can lead to novelty (Wake and Roth 1989). Ontogenetic repatterning has been of profound influence in amniote evolution and I believe that it is important in direct-developing amphibians as well.

There is a tendency to use the terms "neoteny" and "paedomorphosis" for ecologically labile conditions. I prefer to restrict the terms to phylogenetic contexts, but this may be a losing battle. The present state of affairs is a muddle, with some biologists even calling late-metamorphosing larvae that reproduce in a single reproductive season paedomorphic. I recommend using the terms "larval reproduction," or "perennibranchiate," for larvae that reproduce in normally metamorphosing species, and "delayed metamorphosis" for those individuals that do not metamorphose at the expected time. I will discuss here the phylogenetic phenomena and will restrict paedomorphosis to this level (compare with Reilly, in press). To illustrate my point, I will focus on the bolitoglossine plethodontid salamanders, for they have been studied most extensively in regard to ontogenetic phenomena (for example, Alberch and Alberch 1981; Hanken 1982, 1983a, 1984; Wake 1966, 1989).

Recently, it was discovered that the bolitoglossines differ from other salamanders in many aspects of central nervous system morphology (Roth, Nishikawa et al. 1988; Wake et al. 1988), and we believe that much of this derives from paedomorphosis (Roth et al., in press). For example, in the bolitoglossines—which differ from other plethodontids in having higher to much-higher genome sizes and thus different cellular characteristics (larger cells with longer cell cycles)—there is a striking difference in the organization of the motor nuclei that control feeding. In *Plethodon* and other outgroups (virtually all other tetrapods), these motor nuclei have a row of relatively undifferentiated cells in a medial column and a row of differentiated cells in a lateral column. In bolitoglossines there is a different organization; cells arise medially from the ependymal layer, as in other vertebrates, but they fail to migrate fully and they never fully differentiate (Wake et al. 1988; Nishikawa et al. 1991). As a result, there is only a single, rather ragged column of undifferentiated cells. These cells are retarded, that is, they are paedomorphic in relation to the homologous cells in less-derived taxa. This phenomenon is evident in other parts of the brain as well, which in salamanders in general and bolitoglossines in particular has a very simple, embryonic appearance, as noted by many authors (Roth et al., in press). We believe that the paedomorphic cells in the motor nuclei of bolitoglossines are a manifestation of a general ontogenetic repatterning in these species, and that there are some important functional implications. For example, among salamanders, it is only the bolitoglossines that fail to lunge forward during feeding (Larsen et al. 1989); we believe that this is connected to ontogenetic repatterning (Wake et al. 1988; Nishikawa et al. 1991).

Of course, paedomorphosis is evident at several levels of organization, from the cellular to the organ to the organismal. Historically, com-

parative osteology has been used to demonstrate paedomorphic effects. The absence of late-developing bones, such as the prefrontal and septomaxillary in many tropical salamanders, is probably the result of this phenomenon (Wake 1966; Wake and Elias 1983). Such paedomorphic evolution leads to profound difficulties (because of extensive homoplasy) in using such characters in phylogenetic reconstruction (Wake 1989, and in press).

Homoplasy is very common in salamanders; paedomorphosis is only one of the reasons. Some years ago (Wake 1970), I pointed out that structural opisthocoely (a bony, cartilage-covered cap on the anterior end of a vertebral centrum, fitting into a bony, cartilage-covered depression on the posterior end of a more anterior vertebra) arises from two very different developmental patterns in salamanders. One is the result of an extension of the normal pattern of development of ancestral taxa, while the other results from a novel pattern of development in highly derived, miniaturized taxa. Regardless of the developmental pathway, there is only a limited structural response, so we find a true convergence (Wake, in press).

A final example illustrates the complexity of the evolutionary pathways leading to the diversification of structure in the premaxillary bone of plethodontids. To oversimplify, the bone is either paired or fused, but the ways in which this happens varies (Wake 1966, 1989; Wake and Larson 1987). Larval plethodontids all have a single bone, although the ancestral condition must have been paired. The ancestral state might result from metamorphosis or from continued development in direct-developing taxa. Conversely, the larval state might be retained (by either paedomorphosis or peramorphosis). There has been extensive convergence, and possibly reversal, and this single apparently simple character reveals the complexities that arise from knowledge of ontogeny.

RECOGNITION OF SPECIES

Studies of protein variation are revealing previously undetected diversity in the genetics of species and genera of salamanders. Salamanders live in a chemical world, and mate-recognition signals are largely not visible to us. Thus, our impression is that they are as monotonous genetically as some of them appear to be morphologically. This is very misleading. I will present just a few examples and use them to raise questions concerning the criteria we should use to recognize species-level taxa in salamanders.

In 1987, David Good, Gloria Wurst, and I presented data that showed that the Olympic salamander, *Rhyacotriton*, is really far more complicated than it appears. This group of small, stream- and spring-dwelling salamanders occurs from Washington State to northern California, west of the Cascade Mountains. It was discovered late (Gaige 1917) and attracted little attention for many years, because it was thought to be some kind of ambystomatid (Dunn 1920). Stebbins and Lowe (1951) examined geographic variation in morphology and coloration, and concluded that two subspecies should be recognized, one in the north and along the coast and one in the south and inland to the Cascade Mountains, but the degree of differentiation was thought to be relatively slight. The zone of intergradation between these two was mapped as being about as extensive as the range of either.

We undertook our study of protein variation because I had detected some geographic variation in osteology (Wake 1981a) and wanted to reject the possibility that there were different species involved. I had had extensive experience with the species and thought that it was clearly a single species. We were shocked to discover that there was extensive subdivision within the genus, and we only refrained from breaking the group into different

species because we had inadequate geographic sampling (we did examine 29 populations, which also says something about the need for far more extensive and in-depth sampling than has been characteristic of previous work). Subsequently, additional sampling was done (Good and Wake, in press). We now conclude that there are four distinct species and that the most recent divergence is several million years old. There are some color differences, and some subtle osteological differences, but there are no detectable differences in morphometry, and there are only small ecological differences (some species mature in 4¹/₂ to 5 years, but others in 5¹/₂ to 6 years).

The more general message from this study relates to criteria for recognition of species. My position is rather close to that of Frost and Hillis (1990). In the case of *Rhyacotriton*, we used the criterion of genetic cohesion, together with a criterion of genetic and geographic distinctiveness. When we plotted Nei genetic distance against geographic distance, we found a straight line going through the origin for all comparisons within species, but the regression line rises along the Y-axis between species. For us this was a very useful factor in designating species limits and borders. It suggests that the world of salamanders is very large indeed, and that isolation by distance is a phenomenon that might generally apply.

Highton's (1989) taxonomic revision of the *Plethodon glutinosus* complex has proven to be controversial, but if the same criteria we used with *Rhyacotriton* are applied—rather than his apparently (see below) arbitrary level of Nei genetic distance—results very similar to his are obtained (Good, in prep.). When geographic distances are plotted against genetic distances, the regression lines are nearly flat and intercept the Y-axis well above the origin, or the comparisons form polygons positioned relatively high on the Y-axis. This indicates that the taxa identified by Highton are dis-

crete units by this criterion as well as by the criterion he reported (discrete clusters in a UPGMA phenogram; see also Highton [1990]).

What is not emphasized by Highton is that he and his group, in extensive field and laboratory work, have focused on the borders between taxa. For example, Duncan and Highton (1979) examined in detail the secondary contact between *Plethodon ouachitae* and *P. fourchensis*, and showed that there is a very sharp gene frequency cline between the two, occurring in a restricted geographic zone. One could take the position that these kinds of interactions illustrate that reproductive isolation has not yet evolved, and that these are semi-species. Even if that is the case, however, I support Highton in recognizing such groups as species-taxa. While I would emphasize pattern over magnitude of genetic differentiation (and hence largely agree with Frost and Hillis [1990]), I also believe that the rule of thumb level of genetic distance ($D = 0.15$) that Highton has adopted is informed by his extensive and in-depth knowledge of interactions on the borders of the ranges of genetically cohesive groups. Furthermore, his apparently arbitrary approach using level of differentiation is not arbitrary when coupled with geographic analysis that elucidates the relation to history of the taxa. Certainly there is no disagreement with the recognition of cryptic, sympatric species; the controversy arises solely in respect to the recognition of allopatric and parapatric forms (see also Larson 1984).

Another study that relates directly to the controversy over recognition of species-taxa is based on an experimental approach to reproductive isolation (Tilley et al. 1990). *Desmognathus ochrophaeus* is a small salamander that is widespread in northeastern North America, but becomes fragmented geographically in the southern portions of its range. In this study, salamanders were selected from 11 populations for allozymic and

ethological studies. Based on laboratory courtship experiments, an ethological isolation index was devised. A multiple-correlation analysis showed interactions between genetic, geographic, and ethological isolation; when genetic distance is held constant there is a significant relationship between geographic and ethological distance. When geographic distance is held constant, however, the relation between genetic distance and level of ethological isolation is not significant. Tilley and his colleagues concluded that ethological isolation and genetic divergence reflect the gradual divergence of allopatric populations.

Somewhat surprising, then, is their finding that the relationship of geographic to genetic distance is not significant when ethological isolation is held constant. A clue is provided by the published plot of genetic against geographic distance. There are two dramatic outliers, in which genetic distance is much higher than expected. These spots both involve the northernmost population (Mount Rogers, Virginia). One reported value of genetic distance is shockingly high: $D = 0.643$ for the comparison of Mount Rogers with Mount Mitchell, North Carolina, a geographic separation of only about 100 km. Furthermore, the smallest genetic distance of Mount Rogers to any of the other populations is 0.384, a relatively high level of differentiation in such a small geographic region (compare with the results reported by Highton 1989). The authors noted that genetic distances for comparisons with the Mount Rogers population show no tendency to increase with geographic distance. From data presented, one might conclude that the northernmost population is most appropriately placed in a separate species-taxon, but the situation is more complex than presented (Tilley, pers. comm.). Perhaps it would have been useful to tag this outlying population throughout the study and indicate that it is a "candidate-species" (that is, it might be assignable to *D. ochrophaeus* [*sensu stricto*],

while the others might be best considered *D. carolinensis*). For example, it would be useful to see how the Mount Rogers population ranks as an out-group, for then one could appropriately examine the break-up of what appears to be a separate historical entity.

To return briefly to the *Rhyacotriton* example, in that study the critical factor in determining species status is at what point one moves from a pattern of gene flow within a group, or among recently separated groups, to a hierarchical pattern that indicates phylogenetic independence. I once argued that allopatric populations should not be given species status on grounds of allozyme differentiation alone "in the absence of significant morphological differentiation" (Wake 1981b). Findings since that time, and in particular, my experience with *Rhyacotriton*, have led me to modify that view. In the case of *Rhyacotriton*, we see an instance in which only trivial morphological differentiation has occurred and in which morphometric analysis shows that populations are more similar to genetically distinct nearby populations than to geographically more-remote but genetically less-distinct populations. This and other studies lead me to conclude that it is the pattern of genetic differentiation and the degree to which genetically cohesive units can be recognized that are the critical elements in recognition of cryptic and sibling species. In general, I agree with many recent authors in arguing that it is more useful to finely divide taxa so as not to obscure information. We lose more information by "lumping" than by "splitting," and this is especially important when we are concerned with maintaining biological diversity and studying its implications for evolutionary and biogeographic analyses.

It is not simple, even with extensive data bases, to make decisions concerning the taxonomy of differentiated and fragmented groups. My own work on the celebrated "ring-

species," *Ensatina eschscholtzii*, has shown that there are two morphologically and genetically differentiated species living in sympatry in southern California (Wake and Yanev 1986; Wake et al. 1986, 1989), but the geographic scale of the studies conducted was too coarse to permit detection of species borders. Frost and Hillis (1990) argued that the two groups in southern California should be recognized taxonomically as separate species, and that other species in the complex would be recognized in the future (see also Collins 1991). What they did not know was that large, unpublished data sets fill in many of the geographic gaps in the previous studies. What we are seeking, before disrupting the current long-stable taxonomy, is some evidence of monophyly for the separate segments of the ring and of a hierarchical structure within the complex. To date, it appears that genetic distance builds largely as a function of isolation and distance throughout the ring, and that the geographic gaps in distribution correspond more or less to what one would predict for the measured genetic distances, based on regions of continuous distribution in the ring. Furthermore, data from nucleotide sequences of mitochondrial DNA shows that the classical interpretation of Stebbins (1949), gradual divergence in allopatry, cannot be rejected (Moritz et al., in press). I agree with Frost and Hillis that we should refer to the components of this group as the *E. eschscholtzii* complex until the taxonomy is settled.

SPECIES DIVERSITY

Recognition of species relates directly to species diversity. Species of salamanders continue to be discovered at a high rate. Some are subdivisions of taxa formerly considered to be single species (see examples above), but others represent new discoveries. In particular, in the plethodontid tribe Bolitoglossini, centered in Middle America, there is very high diversity

as measured in relative number of species and in terms of numbers of co-occurring species and the degree of ecological segregation.

According to my most recent calculation, plethodontids constitute about 65% of the species of salamanders. Other large groups include the families Hynobiidae (as currently recognized), with a large number of species in far eastern Asia into Central Asia and as far as the Ural Mountains; Ambystomatidae, which are exclusively North American in distribution; and the Salamandridae. The last is the only family that approaches a cosmopolitan distribution, in the sense that it occurs in all major centers of distribution of salamanders except the New World tropics. The Plethodontidae is represented by a few species in the central Mediterranean region of Europe, but is otherwise exclusively New World in distribution. The plethodontid subfamily Desmognathinae and a number of genera of the subfamily Plethodontinae (including groups with larval development and with direct development) occur in eastern North America, long considered to be the area of origin and center of diversification of the family (Dunn 1926). Only one supergenus of the plethodontine tribe Bolitoglossini is found in the New World tropics, but this group is of great interest because of its impressive diversity (Figure 1). It now includes about two-thirds of the plethodontids and over 45% of all species of salamanders.

Despite the relatively great numbers of tropical salamanders, these salamanders are fairly obscure, even to the scientific community. A recent example of the failure to appreciate this diversity is the account of salamanders (Halliday and Verrell 1986) in "The Encyclopedia of Reptiles and Amphibians," which almost completely ignores the tropical species and gives no indication that nearly half of the salamanders in the world are tropical!

For a number of years my colleagues and I have attempted to work out the patterns of relationships of the tropical salamanders, and we have also attempted to discern the main features of the local and regional distribution of taxa (Wake and Lynch 1976; Wake and Elias 1983; Elias 1984; Larson 1983; Wake 1987). We have focused our attention on studies of local elevational transects, for since the time of Schmidt (1936), it has been recognized that elevational zonation of the tropical species is much more marked than is that of extratropical species. In nearly every instance in which we have conducted detailed local studies along elevational transects, we have uncovered novelties, which suggests that we still have a great deal to learn—even at the level of species recognition—as far as tropical salamanders are concerned.

Generalities are beginning to emerge. Whereas in the temperate parts of the globe salamander diversity is related to habitat diversity, life-history diversity, and larval adaptations, in the tropics the picture is very different. First, all of the species are strictly terrestrial, and second, all of the species utilize the same feeding mechanisms based on a highly projectile tongue. Relatively few species are microsympatric—I believe the greatest number is six, in Guatemala, but this number might be greater in the Tapanti forest reserve in Costa Rica (Wake and Lynch 1976; Wake 1987; Wake et al., in press). The main factors separating species are altitude and microhabitat. There has been extensive homoplasy based on microhabitat specialization, and species in different genera have independently evolved adaptations for climbing and for fossorial life. Arboreality is especially common. In the lowlands, where competition with other vertebrates is expected to be most intense, one rarely encounters a salamander on the soil surface. Rather, the species are almost universally arboreal or fossorial.

At the present time I know of approximately 25 well-differentiated species of tropical salamanders that await description, and there are a number of additional cryptic species that have been detected by electrophoretic analysis (for example, Hanken 1983*b*). Some of the recently described species have been among the most spectacular salamanders yet discovered. Destruction of tropical habitats, however, is proceeding at such a rapid rate that we are at risk of losing many species before they are even discovered. A good example is the newly described genus and species of scansorial salamander from Chiapas, Mexico—*Ixalotriton niger* (Figure 2)—which is capable of leaping and bounding along vertical rock faces and on tree trunks (Wake and Johnson 1989). We fear that it may already be extinct. Within the past ten years two other genera of equally interesting tropical salamanders have been discovered, *Nyctanolis* and *Bradytriton*.

A PLEA FOR CONSERVATION

The world's biota faces a crisis of unprecedented proportions. Habitat destruction has accelerated to an alarming rate. Up to now, conservationists have appropriately stressed the megafauna, such as Kemp's ridley turtle, for

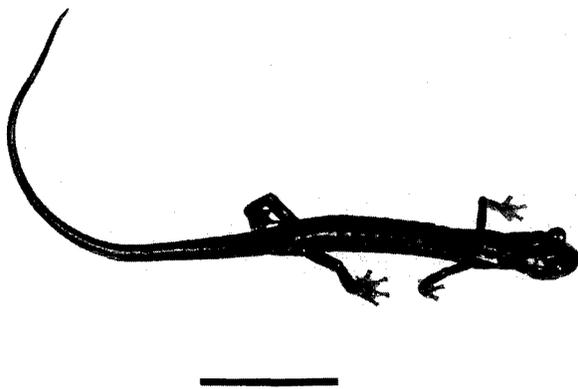


FIGURE 2. *Ixalotriton niger* (Wake and Johnson 1989), a remarkable, newly discovered salamander from Chiapas, Mexico, that was placed in a new genus and species. This taxon may already be extinct. Scale is 25 mm.

the public can more easily relate to such symbols. But the megafauna has no more intrinsic worth than these remarkable tropical salamanders. We who are informed have a special obligation to work in the political and social arena at local, regional, national, and international levels to check the destructive forces that are permanently altering the evolutionary potential not only of salamanders, and other amphibians and reptiles, but also of *Homo sapiens*.

At the First World Congress of Herpetology, many of us first became aware of the fact that many amphibians are in danger of becoming extinct and that amphibian populations are in decline in many parts of the world. I had personally been aware of the decline of frog populations in California and of the decline of populations of frogs and salamanders in Costa Rica, but the congress focused attention on what was apparently a general phenomenon. I returned to the United States to work with colleagues on the Board on Biology of the National Research Council to convene a workshop to examine the issue. The meeting resulted in an understanding that there was indeed a problem, and elicited a great deal of attention in the news media. A number of recommendations resulted (Blaustein and Wake 1991), and there has been substantial progress (Wake 1991). The most noteworthy development has been the establishment of a Task Force on Declining Amphibian Populations by the Species Survival Commission of the International Union for Conservation of Nature and Natural Resources (IUCN). Perhaps a more lasting outcome has been a growing awareness that amphibians in general, because of a whole complex of physiological, morphological, ecological, behavioral, and life historical factors, may have extraordinary potential as indicators of environmental degradation and even of global climate change. We may yet come to see how apparently esoteric studies of simple salamanders have global impact.

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