CHAPTER 2

The Phylogeny of Amphibians: Current Knowledge and the Role of Cytogenetics

David M. Hillis
Department of Zoology
University of Texas
Austin, Texas

I. Introduction

Knowledge of evolutionary relationships is critical for interpreting all biological variation. Because species are not historically independent, all studies of comparative biology (including interspecific cytogenetic comparisons) must consider the phylogeny of the organisms studied (see Felsenstein, 1985b). The primary purpose of this chapter is to outline what is (and what is not) known about amphibian phylogeny and to assess how much confidence can be placed in our inferences about amphibian relationships. A secondary objective is to assess the contributions of cytogenetics to the field of amphibian systematics and to suggest areas in which future cytogenetic studies may contribute to our understanding of amphibian relationships.

Most of what is currently known about higher levels of amphibian phylogeny comes from study of morphology, while contributions from cytogenetics (e.g., Morescalchi, 1973) and molecular biology (e.g., Hillis and Davis, 1987; Larson and Wilson, 1989) have had a comparatively

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minor role to date. This is largely because of the great expanse of time that separates the living orders of amphibians, coupled with the fact that cytogenetic and molecular studies have tended to concentrate on highly variable characteristics and regions of the genome. In contrast, relationships within amphibian families and genera have been studied extensively with molecular (e.g., Wake *et al.*, 1978; Hillis and Davis, 1986) and, to a lesser extent, cytogenetic (e.g., Miyamoto, 1983; Green, 1986; Sessions and Kezer, 1987) techniques. It is of interest to study the relative reliability of cytogenetics compared with other methods of inferring phylogeny but *a priori* knowledge of phylogeny is almost never available. Two approaches can be used to assess confidence in phylogenetic inferences: the statistical approach (for a review, see Felsenstein, 1988) and studies of consensus and combination of different classes of data (see Hillis, 1987; Kluge, 1989). Both of these methods are used in this chapter to assess our current state of knowledge of higher-level amphibian phylogeny.

II. Methods

A. Data Sets

Seven data sets were analyzed in this study: Two concern the relationships of amphibians to other vertebrates and the relationships among amphibian orders, whereas the other five concern relationships among families within orders of amphibians. The recent study of Trueb and Cloutier (1991) reviewed the morphological evidence on phylogenetic relationships of amphibians to other vertebrates. This data set was used to evaluate ordinal and class relationships. A comparative molecular data set was also used to address relationships at this level. This data set was a combination of 28S ribosomal DNA (rDNA) sequences published by Ware *et al.* (1983; *Xenopus*), Hassouna *et al.* (1984; *Mus*), Hillis and Dixon (1989; *Latimeria, Notropis*), and Larson and Wilson (1989; *Typhlonectes, Rhyacotriton*). The rDNA sequences for *Typhlonectes* and *Rhyacotriton* were inferred from the published rRNA sequences. All characters were coded as unordered in these two data sets. Sequences from insects (Tautz *et al.*, 1988) were used as a taxonomic outgroup for the rDNA sequence data and the *Notropis* sequence was used as a functional outgroup based on the results of Hillis and Dixon (1989).

Relationships among families of salamanders have been examined by Duellman and Trueb (1986) based on morphology and limited cytogenetic data and by Larson and Wilson (1989) based on 28S rRNA sequences. The data matrix for morphology was that used by Duellman and Trueb (1986), with their characters I (condition of pterygoid), K (opercular apparatus), L (junction of periotic canal and cistern), M (flexures of periotic canal), N (basilaris complex of inner ear), R (number of larval gill slits), X (spinal nerves), and DD (karyotype) coded in ordered series. The remaining characters were unordered. All characters reported by Duellman and Trueb (1986) were polarized, so the outgroup consisted of a hypothetical plesiomorphic ancestor.

The sequences reported by Larson and Wilson (1989) were realigned and recoded. The region from 901 to 911 bp (numbering system for *Rattus* as reported by Hadjiolov *et al.,* 1984) could not be aligned with any degree of confidence among the species and was deleted from the analysis. The adjacent CGGG deletion at positions 2696–2699 in several species of salamanders was treated as a single character rather than four characters because it is highly unlikely that they are independent deletions. Otherwise, the alignment used was identical to that reported by Larson and Wilson (1989). All substitutions and insertions/deletions were given equal weight. Caecilian sequences (*Typhlonectes*) were used as an outgroup.

The data set for caecilian relationships was based on morphological data reported by Duellman and Trueb (1986), which was, in turn, based largely on data presented by Nussbaum (1979). This data set was modified to take the corrections of Nussbaum and Wilkinson (1989) into account. Duellman and Trueb's characters B (mouth opening), C (eye–tentacle relationship), D (annulation), and M (pterygoid) were multistate ordered characters. The remaining characters consist of only two states. Trees were rooted with a hypothetical plesiomorphic ancestor.

To infer frog relationships, I used the morphological data set reported by Cannatella (1985), which consists of 181 characters (some with multiple ordered states). To perform exhaustive searches and bootstrap analyses, only representative species of each family studied by Cannatella (1985) were used. These species were *Ascaphus truei* (Ascaphidae), *Leiopelma hochstetteri* (Leiopelmatidae), *Alytes obstetricans* (Discoglossidae), *Bombina orientalis* (Bombinatoridae), *Rhinophrynus dorsalis* (Rhinophrynidae), *Xenopus laevis* (Pipidae), *Megophrys montana* (Megophryidae), *Spea multiplicata* (Pelobatidae), *Pelodytes punctatus* (Pelodytidae), and *Limnodynastes peronii* (Neobatrachia). Morphological data on the remaining families of frogs were summarized by Duellman and Trueb (1986).

B. Phylogenetic Inference

For six of the data sets, all possible tree topologies were examined using the exhaustive search procedure of the Phylogenetic Analysis Using Parsimony (PAUP) software package, version 3.0 (Swofford, 1990). The data on neobatrachian relationships (Duellman and Trueb, 1986) were analyzed using the Branch and Bound option of PAUP. In addition, bootstrap analyses (Felsenstein, 1985a) were performed to determine the relative confidence in each node of the most parsimonious solutions. Bootstrap analysis involves random sampling with replacement of characters from the original data matrix to create many new data matrices (each the size of the original), each of which is then analyzed. A majority-consensus tree is then produced from the total number of new analyses. Well-supported nodes are much more likely to be represented in a large number of the bootstrap trees than are poorly supported nodes. One can use the method to construct confidence intervals for nodes on a phylogenetic tree, although it is perhaps better to view the method as providing an objective means for evaluating the *relative* confidence in particular nodes. Bootstrap analyses were performed with 1000 iterations using the branch and bound search algorithm of Hendy and Penny (1982). All uninformative characters were ignored. In cases where two data sets exist for the same taxa (e.g., morphological and molecular), data sets were combined as recommended by Kluge (1989).

Distributions of all possible tree topologies are presented to indicate the relative structure in the data (see Hillis and Dixon, 1989; Hillis, 1991). Randomized data sets produce tree-length distributions that are nearly symmetrical. Data sets with significant nonrandom components, as would be expected under the constraints of phylogenetic history, produce tree-length distributions that are highly skewed (with a long left tail). Therefore, these distributions allow one to assess the degree of phylogenetic information contained in a data set and also allow one to assess how much better a most parsimonious solution is compared with all other possible solutions.

III. Monophyly and Relationships of Lissamphibians

There has been considerable debate about whether or not the living orders of amphibians (Lissamphibia) constitute a monophyletic group. The most commonly held position is that the three orders (Anura [frogs], Caudata [salamanders], and Gymnophiona [caecilians]) do make up a monophyletic group among living vertebrates (e.g., Parker, 1956; Szarski, 1962; Parsons and Williams, 1962, 1963; Remane, 1964; Thomson, 1964; Estes, 1965; Hecht, 1969; Colbert, 1969; Morescalchi, 1973; Lombard and Bolt, 1979; Gaffney, 1979; Gardiner, 1982, 1983; Rage and Janvier, 1982; Hennig, 1983; Rage, 1985; Bolt and Lombard, 1985; Duellman and Trueb, 1986; Trueb and Cloutier, 1991); however, a sizable minority opinion is that lissamphibians are para- or polyphyletic. Among the variations proposed are that salamanders and caecilians form a monophyletic group independent of frogs (e.g., Romer, 1945; Lehman, 1956, 1968; von Huene, 1956), that salamanders and frogs form a monophyletic group independent of caecilians (e.g, Haeckel, 1866; Noble, 1931; Eaton, 1959), or that all three orders have independent origins (e.g., Jarvik, 1980, 1986; Gregory, 1965; Løvtrup, 1985; Carroll and Holmes, 1980; Carroll, 1988).

The combined molecular and morphological data set strongly supports the monophyly of lissamphibians (Fig. 1). The bootstrap analysis suggests that this is a significant result, because the branch uniting lissamphibians was found in all 1000 iterations. The shortest tree that does not include a monophyletic Lissamphibia is 203 steps, which is 13 steps longer than the most parsimonious tree (Fig. 2). Therefore, the support for a monophyletic Lissamphibia seems compelling.

Even among supporters of the monophyly of lissamphibians, opinions differ on the relationships of amphibians to other vertebrate groups. Of particular concern are the relationships of lungfishes and coelacanths. Although most workers place amphibians as the sister group to amniotes to form the Tetrapoda, there is no consensus regarding the living sister group to tetrapods. Various authors place lungfishes, coelacanths, or actinop-

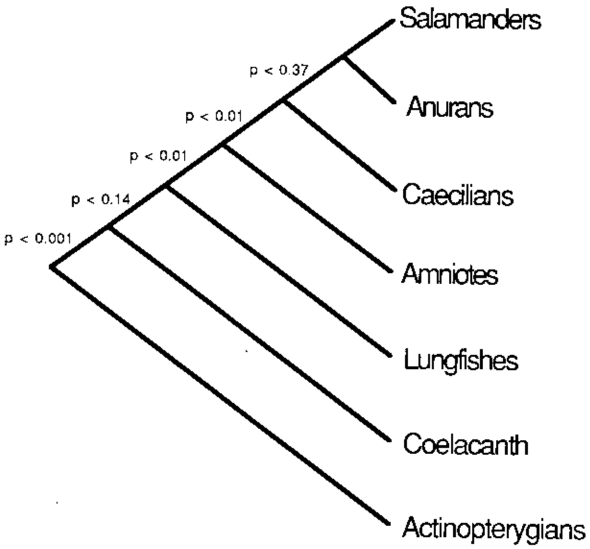


Figure 1. Most-parsimonious tree of vertebrate relationships, based on the combined morphological and molecular data set described in the text. The probabilities that the support for a given clade is not random are derived from bootstrap analysis with 1000 iterations (see text).

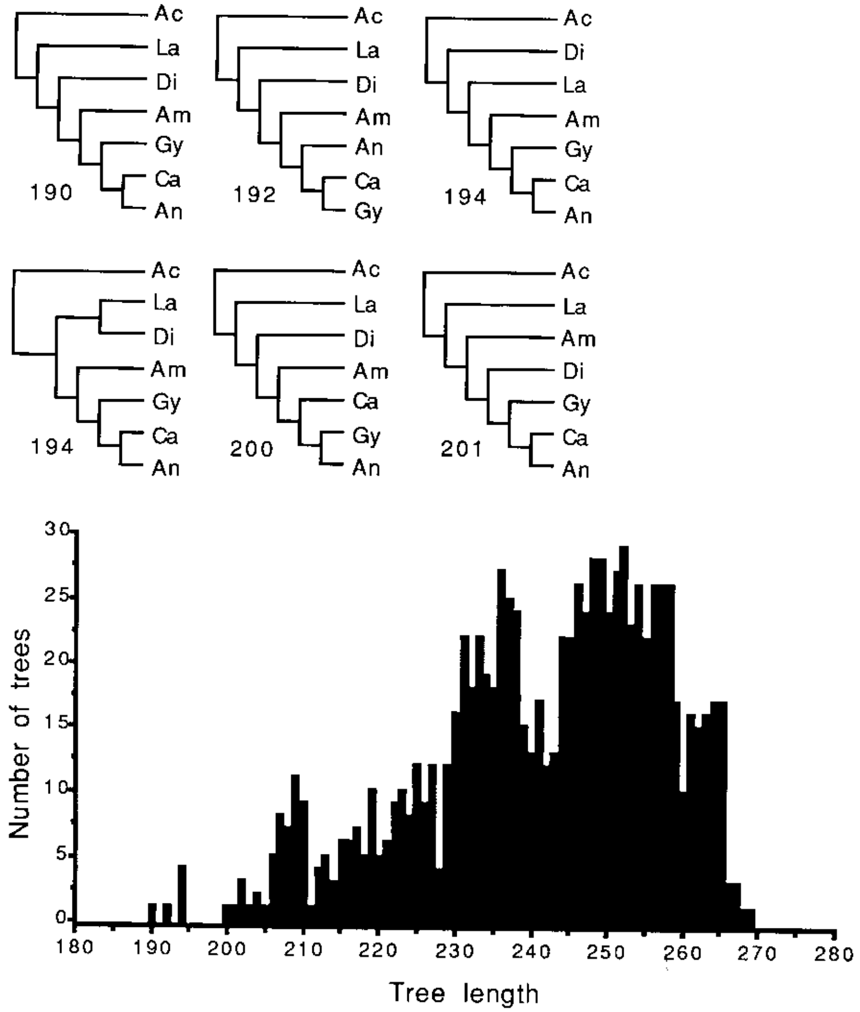


Figure 2. Lengths of alternative hypotheses of vertebrate relationships (above), and distribution of tree lengths for all possible topologies of the relevant taxa (below). Left-skewed distributions are indicative of strong phylogenetic signal; note that relatively few alternative trees are near the length of the most parsimonious tree. Ac, Actinopterygii; Am, Amniota; An, Anura; Ca, Caudata; Di, Dipnoi; Gy, Gymnophiona; La, *Latimeria*.

terygians (ray-finned fishes) as the sister group (see reviews by Rosen *et al.*, 1981; Schultze, 1981; Duellman and Trueb, 1986; Goodman *et al.*, 1987; Trueb and Cloutier, 1991). The relationships of coelacanths (represented by the single living species *Latimeria chalumnae*) are particularly controversial and almost every conceivable relationship among the vertebrates has been suggested for this group (see Forey, 1988; Hillis and Dixon, 1989).

The combined molecular and morphological analysis (Fig. 1) resolves the relationships among the osteichthyian vertebrates fairly strongly. This analysis suggests that amniotes are the sister group to amphibians, that lungfishes are the sister group to tetrapods, and that coelacanths are the basal sarcopterygians. All of these groupings are significant at $p < 0.01$, with the exception of the position of lungfishes ($p < 0.14$). This is due in part to the lack of rDNA sequence data for lungfishes, combined with the relatively large number of missing morphological characters for this group. All of the solutions near the most parsimonious solution involve alternative positions of lungfish (as well as changes within amphibians [see below]). Thus, although the most support falls in favor of the relationships shown in Fig. 1, other hypotheses of lungfish relationships (e.g., sister group to coelacanths, or outside of coelacanths plus tetrapods) are not a great number of steps from the shortest solution (Fig. 2). However, another commonly suggested relationship for lungfish, namely as sister group to amphibians, requires 11 additional steps (Fig. 2). Hypotheses of relationships of coelacanths outside of Osteichthyes (see Goodman *et al.*, 1987) can be rejected on the basis of the rDNA sequence data (Hillis and Dixon, 1989; Hillis *et al.*, 1991).

IV. Relationships among the Orders of Lissamphibians

Of the three possible relationships among the three orders of living amphibians, two have received support from morphological studies. Trueb and Cloutier (1991) reviewed the morphological data that bear on this point and concluded that characters of soft anatomy group salamanders and caecilians together whereas osteological features group salamanders and frogs together. They further noted that the total morphological data set supported the view from osteology. The rDNA sequences, on the other hand, support the view that salamanders and caecilians are more closely related than either are to anurans (Larson and Wilson, 1989).

In the combined data set, it is probably not surprising that the two shortest trees (Fig. 2; 190 and 192 steps) are the two alternative hypotheses that are suggested by morphology and molecules. The morphological solution—that salamanders and anurans are sister groups—is the more parsimonious solution but the alternative tree cannot be rejected (Figs. 1 and 2). The final possibility—the tree uniting caecilians and anurans apart from salamanders—has never been seriously entertained and, in fact, requires 10 additional steps (Fig. 2). The closeness of two of the three solutions suggests that there has been considerable convergence in one of the two sets of characters. Additional data clearly are needed to resolve this problem and three sources of information seem especially worthy of investigation. First, the morphological data can be examined in greater detail to look for potential cases of convergences as well as additional informative characters. Second, sequences of additional conserved genes can be obtained. Convergences across unrelated gene sequences related to nonhistorical factors seem unlikely. Third, molecular cytogenetic data on conserved linkage groups may prove useful in resolving this controversy.

V. Relationships within the Orders of Lissamphibians

A. Caecilians

The caecilians are the least studied order of amphibians. They are also the least speciose group. However, the systematic relationships of the families of caecilians are better resolved than for either of the two better-known orders (Figs. 3 and 4). Inferences of phylogeny of caecilians are currently based primarily on morphological characters, although limited cytogenetic data (see Nussbaum, Chapter 3, this volume) are consistent with morphology. The least stable part of the phylogeny concerns the placement of the Uraeotyphlidae. Alternative positions for this family require only two additional steps on the tree (Fig. 4).

Alternative familial classifications for caecilians have been suggested

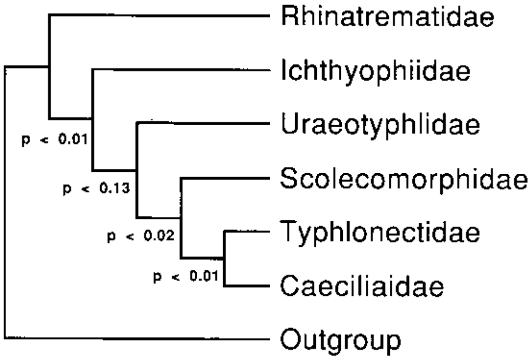


Figure 3. Most parsimonious tree of caecilian families, showing bootstrap probabilities as in Fig. 1.

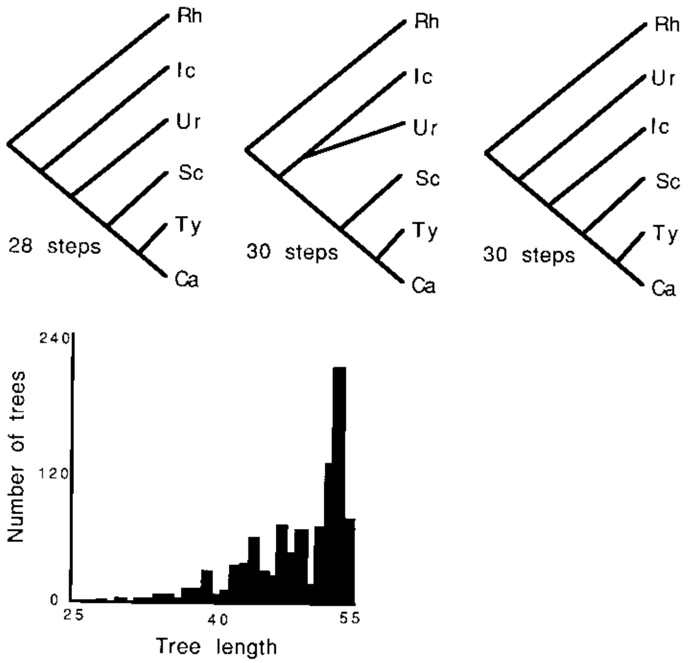


Figure 4. Alternative trees of caecilian relationships (above), and distribution of tree lengths for all possible topologies (below). Ca, Caeciliaidae; Ic, Ichthyophiidae; Rh, Rhinatrematidae; Sc, Scolecomorphidae; Ty, Typhlonectidae; Ur, Uraeotyphlidae.

by Laurent (1984, 1986) and Lescure *et al.* (1986). These authors recognized additional families of caecilians by breaking the probably paraphyletic Caeciliaidae (see Wake, 1977; Nussbaum, 1979; Nussbaum and Wilkinson, 1989) into additional families. Lescure *et al.* (1986) also divided the Typhlonectidae into two families. However, Laurent (1984, 1986) did not present data to support his revision and the data of Lescure *et al.* (1986) do not support their classification (Nussbaum and Wilkinson, 1989). Each of the new families recognized by Lescure *et al.* (1986) is either not monophyletic or else leaves another family para- or polyphyletic according to their data (Nussbaum and Wilkinson, 1989). Therefore, until convincing data are presented that bear on the phylogeny of the Caeciliaidae, the only way to make all families of caecilians monophyletic would be to place typhlonectids within the Caeciliaidae.

B. Salamanders

Phylogenetic relationships of salamanders have been studied fairly extensively from both a morphological standpoint (reviewed by Duellman and Trueb, 1986) and a molecular standpoint (e.g., Larson and Wilson, 1989). However, these analyses are remarkable for their lack of concordance (see Figs. 5–10). This author obtained somewhat different results than those obtained by the original authors by reanalyzing the morphological and molecular data sets (Figs. 5 and 8) but the revised results are no closer in agreement than the original solutions. Neither morphological nor molecular resolutions are particularly robust (Figs. 7 and 10) and the strongest points of each study contradict the strongest points of the other study. The most robust portion of the morphological tree is at the base, where the Cryptobranchidae and Hynobiidae are found to be the sister groups to the remaining families of salamanders (Fig. 7). The lungless salamanders (Plethodontidae) are placed with salamandrids and ambystomatids. However, in the molecular tree, the positions of virtually all the families are changed and the plethodontids are placed at the base of the tree (Fig. 10). The morphological tree is a very poor solution to the molecular data set, and vice versa. Because there is no *a priori* reason to accept one tree over the other, it is of interest to combine the data set to see which tree provides the best global solution.

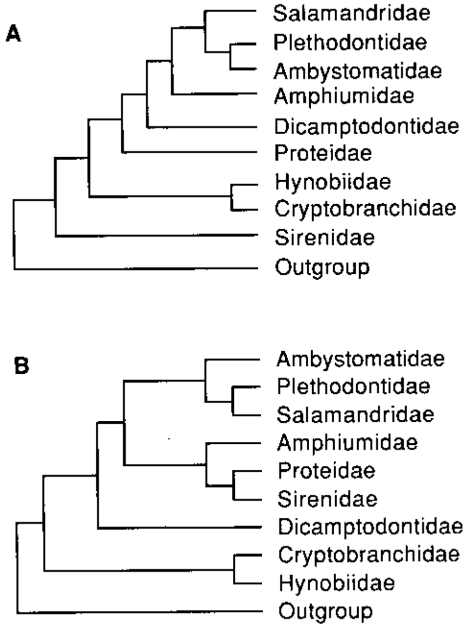


Figure 5. A) Tree of relationships of salamander families shown by Duellman and Trueb (1986), based on analysis of morphology. B) Shortest solution to the data set of Duellman and Trueb (1986). This tree is seven steps shorter than the topology shown in A.

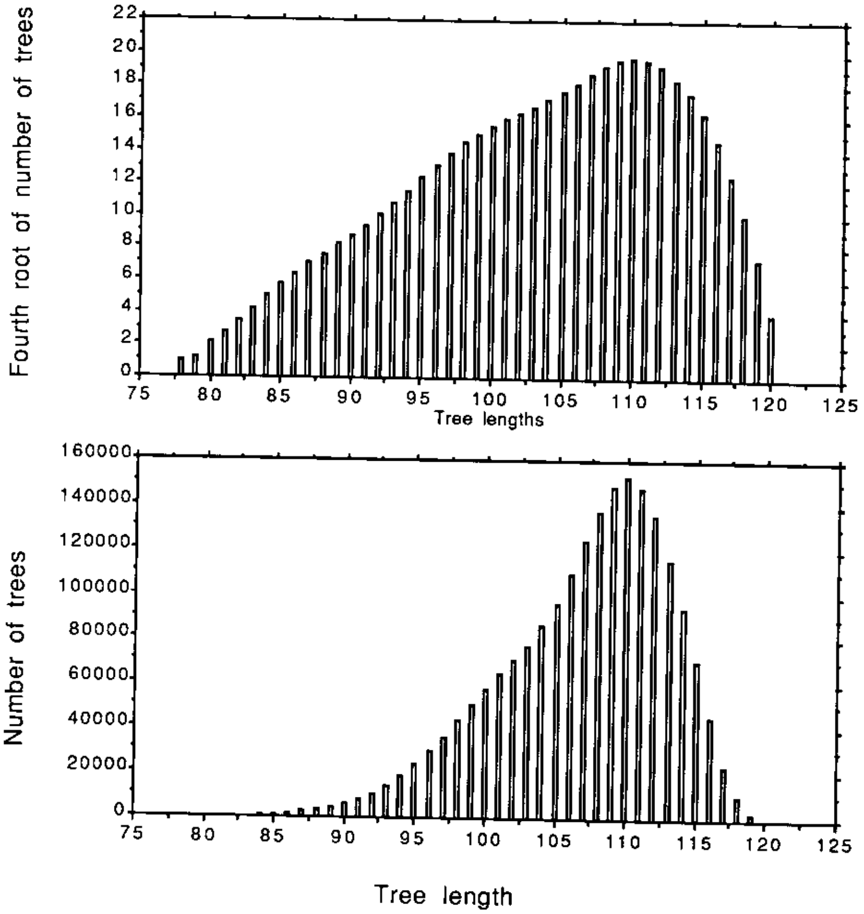


Figure 6. Distribution of tree lengths of all possible topologies of salamander families based on the data of Duellman and Trueb (1986). The Y-axis in the upper graph is the fourth root of the number of trees, which is shown so that the categories with relatively few trees can be seen.

The combined data set produces somewhat of a surprise (Figs. 11 and 12). There is a single most-parsimonious solution that is two steps better than any other tree (Fig. 12). This solution is like the morphological tree in that cryptobranchids are the basal family (hynobiids were not included in this analysis because molecular data are lacking for the group). The relationships of the remaining families are different than for either the molecular or the morphological data sets alone, although none of the findings are very robust (Fig. 11). Given the lack of agreement between the two data

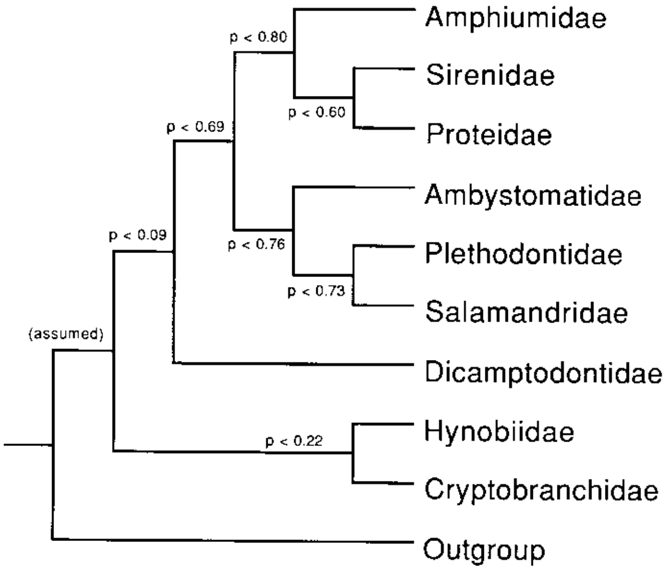


Figure 7. Results of a bootstrap analysis (1000 iterations) of the data of Duellman and Trueb (1986).

sets, combined with the lack of any significant groupings for either data set (Figs. 7 and 10), it is safe to say only that we still know very little about salamander relationships. Contributions from all sources (molecular, morphological, and cytogenetic) are needed to address this problem.

C. Frogs

The vast majority of living amphibians are anurans. Frogs and toads are found worldwide and occupy a great array of different habitats. However, despite the large number of species and the diversity of habitats, anurans are morphologically quite conservative. This fact has made morphological explorations of anuran phylogeny extremely difficult. The morphological variation of the basal families (the paraphyletic Archaeobatrachia) has been examined in detail by Cannatella (1985). The remaining families (which compose the Neobatrachia) have been examined in less detail, but the morphological variation was summarized by Duellman and Trueb (1986). My results of reanalysis of these two data matrices differ somewhat from the findings of the original authors and are shown in Figs. 13–15.

Except for the relationships of the basal families, very little is resolved in anuran phylogeny (Figs. 13 and 15). The relationships among the neoba-

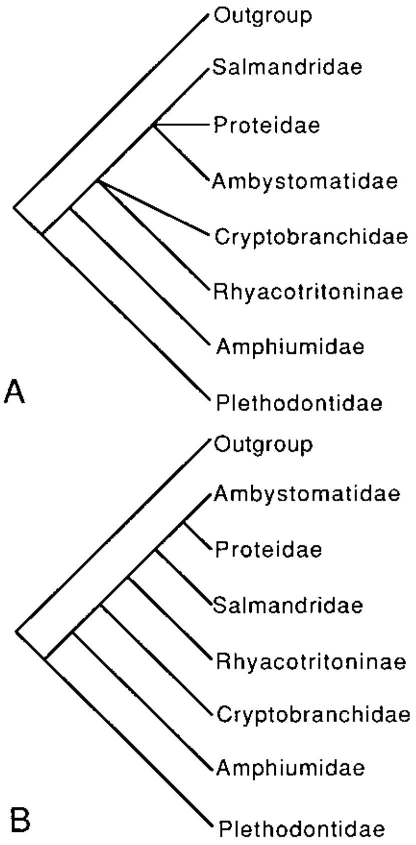


Figure 8. A) Strict consensus of the four equally parsimonious trees obtained from analysis of the modified (see text) data matrix of Larson and Wilson (1989). B) Tree presented by Larson and Wilson (1989); this is one of the four shortest trees in the reanalysis.

trachians have been, and remain, particularly troublesome. The paucity of morphological data that bear on this problem indicates that another source of data is needed. Even the resolved clades in Fig. 15 are supported by only a single morphological character, and none of these nodes is significant in a bootstrap analysis. Part of the problem may be that most of the families of neobatrachians arose over a short period of time so that resolution may be difficult based on any data set. However, the possibility also remains that frogs simply have a morphology that is highly constrained and that data from molecular biology may be able to resolve this radiation. At present, however, frog phylogeny remains one of the major puzzles of tetrapod evolution.

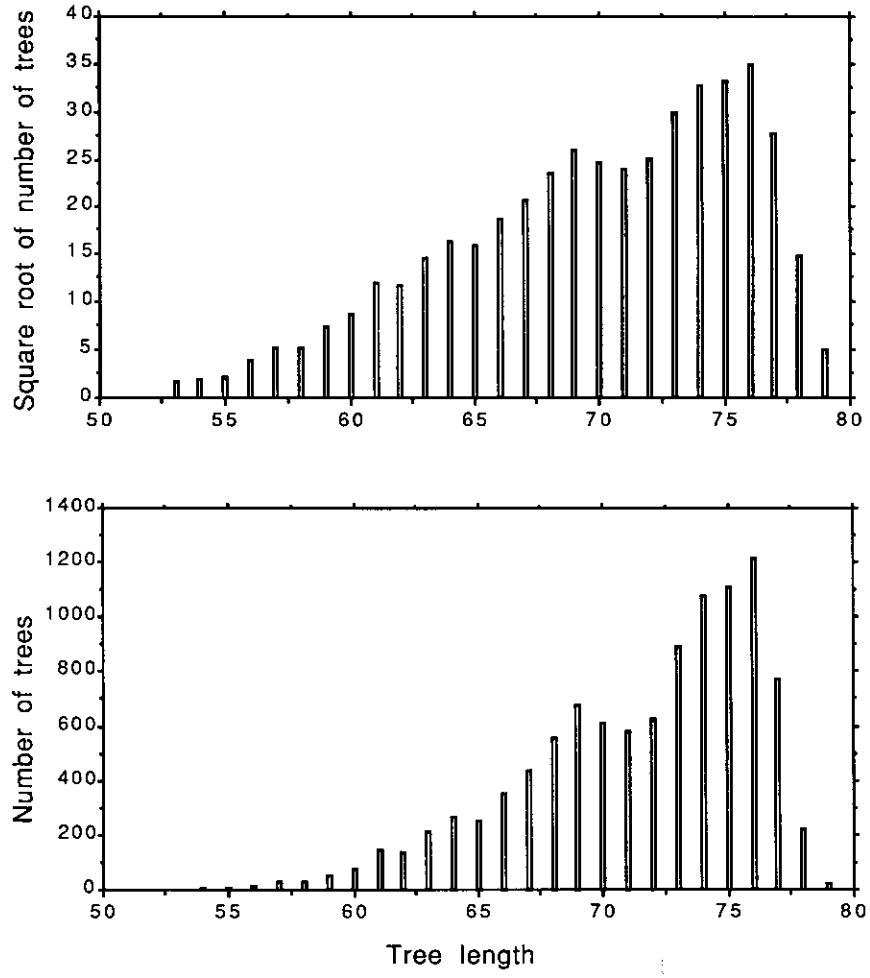


Figure 9. Distribution of tree lengths of all possible topologies of salamander families based on the data of Larson and Wilson (1989). The Y-axis in the upper graph is the square root of the number of trees, which is shown so that categories with relatively few trees can be seen.

VI. Past and Future Roles of Cytogenetics in Amphibian Phylogeny

As should be clear from the foregoing analyses and discussion, there is much about amphibian phylogeny that is unknown or poorly resolved. The monophyly of amphibians has been firmly established, as have their rela-

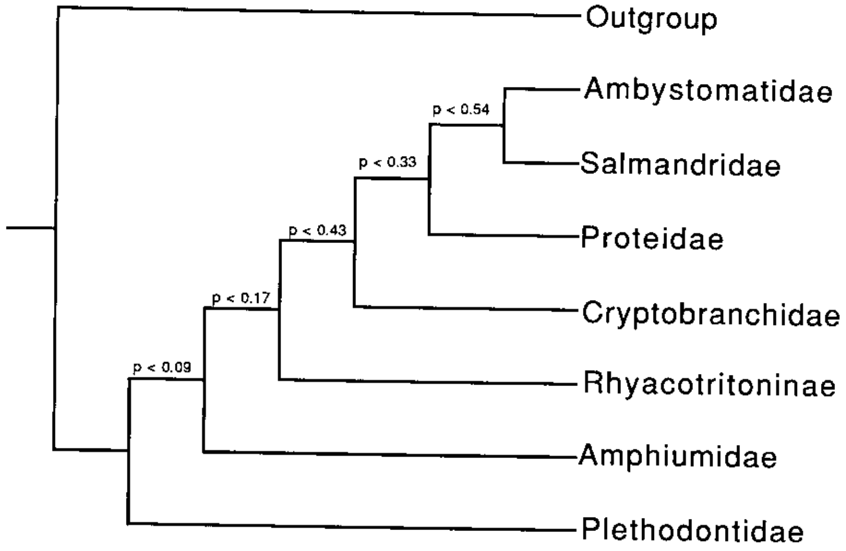


Figure 10. Results of bootstrap analysis (1000 iterations) of the rDNA data presented by Larson and Wilson (1989).

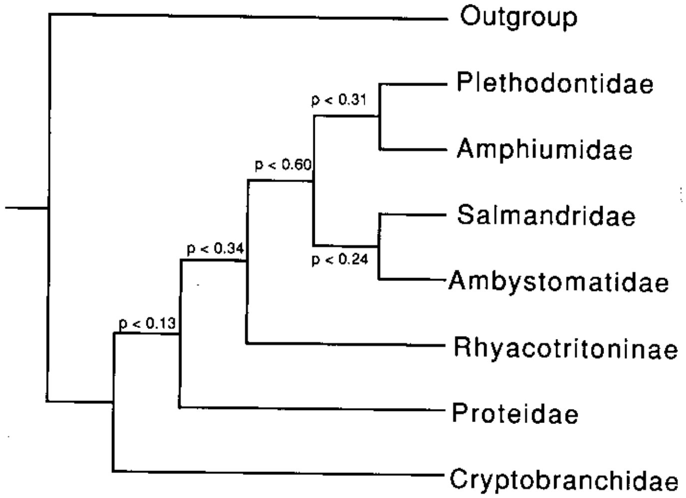


Figure 11. Results of bootstrap analysis (1000 iterations) of the combined molecular–morphological–cytogenetic data set.

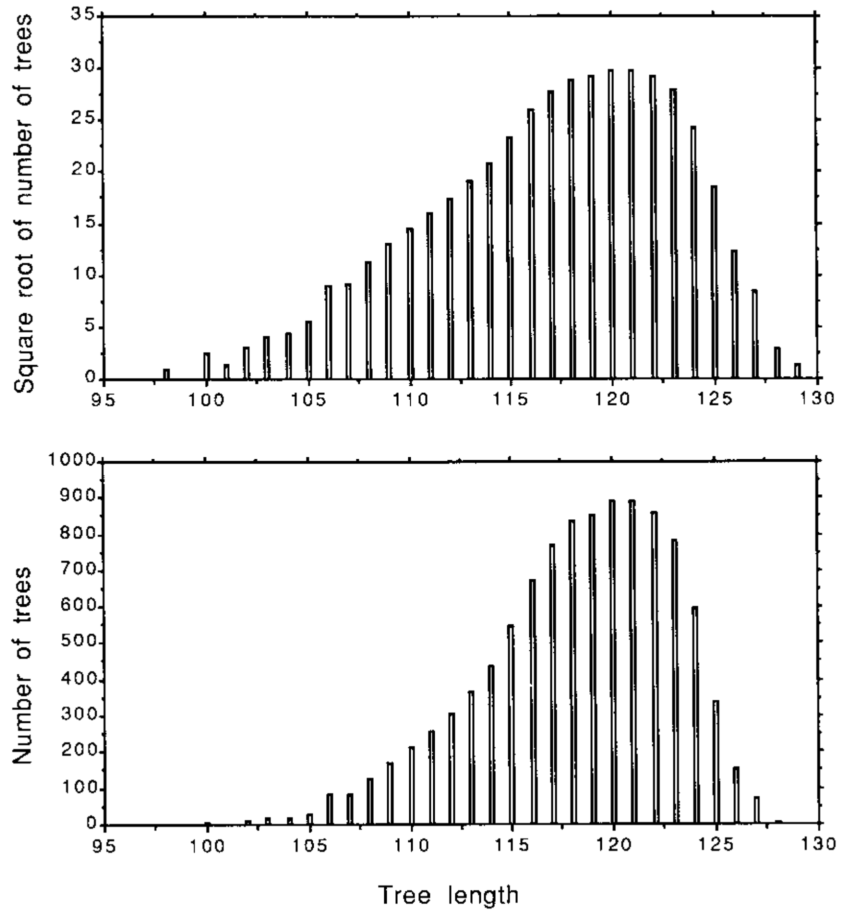


Figure 12. Distribution of tree lengths of all possible topologies of salamander families based on the combined molecular–morphological–cytogenetic data set. The Y-axis in the upper graph is the square root of the number of trees, which is shown so that categories with relatively few trees can be seen.

tionships to other vertebrate groups. The phylogeny of caecilian families also appears to be highly resolved, a surprising finding given the relative paucity of biological information on this poorly studied group. However, the relationships among the three orders of lissamphibians is still open to question, as are the relationships among the families of salamanders. Morphological and molecular data provide conflicting answers to these two systematic problems. The basal anuran radiation is fairly well established, but the relationships among the vast majority of anuran families is completely unresolved.

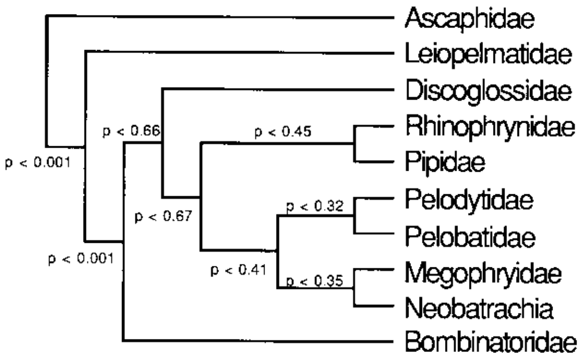
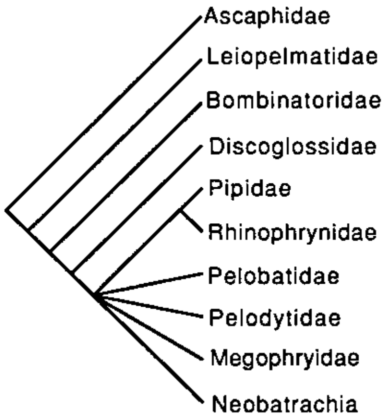
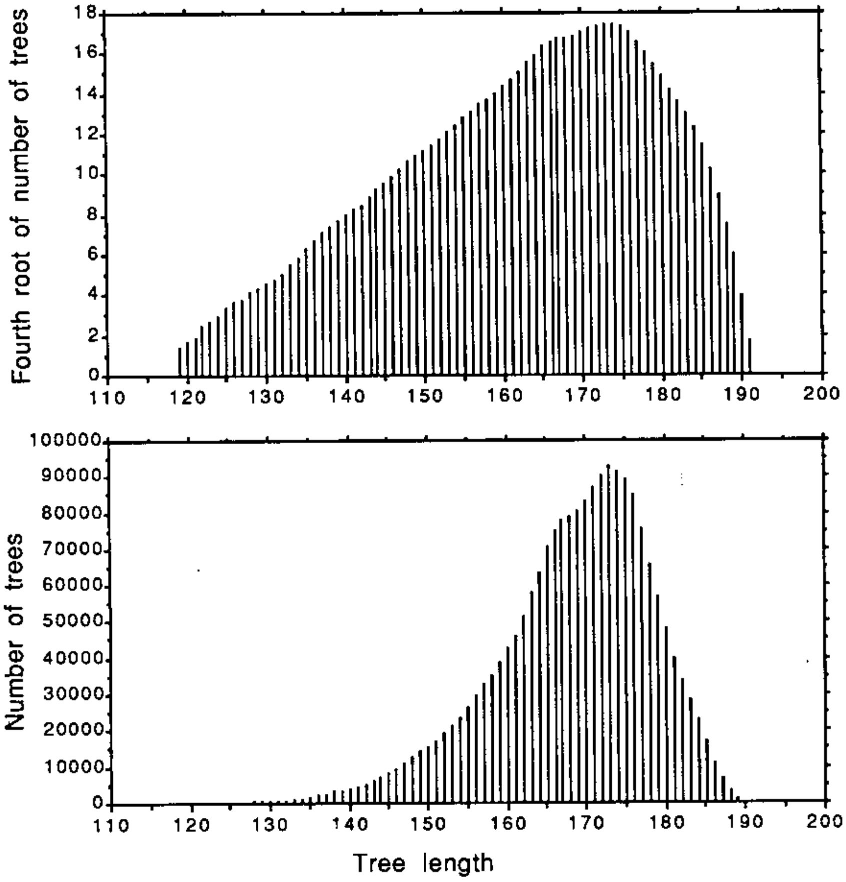


Figure 13. Results of bootstrap analysis (1000 iterations) of the morphological data set of Cannatella (1985) on archaeobatrachians.

It also should be clear from the foregoing that most of what we know about higher amphibian relationships is from study of morphology and, to a lesser extent, molecular biology. The attention of cytogeneticists has been occupied more by variation among and within species than at higher levels. These data have been used effectively for inferring relationships within some genera (e.g., Miyamoto, 1983; Green, 1986). Most broad-scale systematic studies of amphibian genera to date have concerned basic karyotypes (e.g, Blommers-Schlösser, 1976, 1978; Bogart, 1967, 1970, 1972, 1973, 1974, 1981; Bogart and Nelson, 1976; Bogart and Tandy, 1981; Cole, 1974; Morescalchi, 1975, 1977; Morescalchi and Ingram, 1974; Wake and Case, 1975), which often provide sufficient information to define major species groups. Although techniques for high-resolution banding of amphibian chromosomes have been developed (e.g., Schmid, 1978a,b, 1980; Schmid *et al.*, 1979, 1983; Sessions, 1990), relatively few studies have taken advantage of these techniques explicitly for the purpose of reconstructing phylogenies of amphibian groups. Studies in other vertebrate groups, such as mammals (e.g., Yunis and Prakash, 1982; Baker *et al.*, 1983), have demonstrated the usefulness of banding for phylogenetic studies, although problems with convergence have been noted (e.g., Baker and Barnett, 1981; Robbins and Baker, 1981). Green (1986) found C-banded karyotypes of frogs of the *Rana boylii* group to be useful for inferring the phylogeny of that group but noted that homologies among C-bands were sometimes difficult to establish. As noted by Green (1986) and discussed by Sessions (1990), molecular methods can be used to establish firmly homologies of various chromosomal regions but these methods rarely have been used to date in studies of vertebrate phylogeny. Some applications of molecular techniques to systematic cytogenetics of amphibians are described in the other contributions to this volume.


Ascaphidae
Leiopelmatidae
Bombinatoridae
Discoglossidae
Pipidae
Rhinophrynidae
Pelobatidae
Pelodytidae
Megophryidae
Neobatrachia


Fourth root of number of trees
18
16
14
12
10
8
6
4
2
0
110
120
130
140
150
160
170
180
190
200
Number of trees
100000
90000
80000
70000
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Tree length

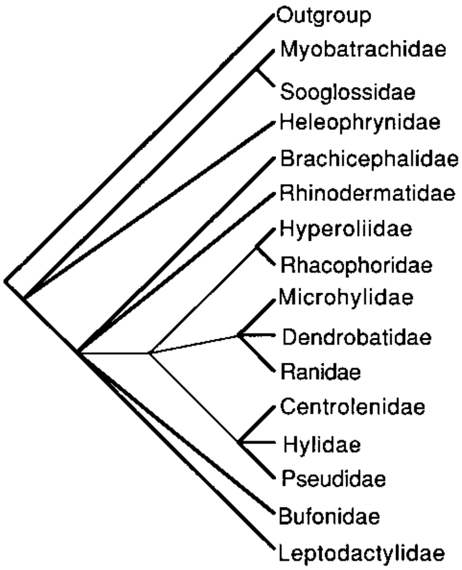

Figure 15. Majority consensus tree of neobatrachians based on the morphological data presented by Duellman and Trueb (1986). The heavy lines indicate clades represented in all the shortest trees; the light lines indicate clades represented in four of the six shortest trees.

Until recently, comparatively little attention has been focused on cytogenetic characters that could shed light on higher-level problems of phylogeny. As noted by Morescalchi (1973) and Green (1986), determining homologies between characters derived from traditional karyotypes is very difficult and the problem becomes increasingly severe as one moves to progressively distantly related species. This is beginning to change with the influx of molecular methodology into cytogenetics (see Sessions, 1990). Techniques such as *in situ* hybridization can be used to test hypotheses of homology generated from work with standard karyotypes.

In addition to characters such as C-, G-, and Q-bands, nucleolus organizer regions, centromere position, and other traditional cytogenetic data, two cytogenetic character sets that are likely to contribute grist to the mill of amphibian phylogenetic research are conserved linkage groups and mechanisms of sex determination. Some linkage groups are conserved throughout the vertebrates, and others show levels of variability that are

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Figure 14. Distribution of tree lengths of all possible topologies of archaeobatrachian families based on the morphological data of Cannatella (1985). The tree is the consensus tree of the four shortest solutions. The Y-axis of the upper bar graph is the fourth root of the number of trees, which is shown so that categories with relatively few trees can be seen.

probably useful for studying relationships among families of amphibians (see O'Brien, 1987). Recent work on sex determination in amphibians has revealed a wealth of information that should be applicable to studies of amphibian phylogeny (Schmidt *et al.*, Chapter 16, this volume; Hillis and Green, 1990). In fact, the boundary between molecular genetics and cytogenetics has closed to the point that it is difficult to distinguish between the two fields. As molecular approaches to cytogenetics continue to develop (see Sessions, 1990, and contributions to this volume), cytogeneticists will have ample opportunity to contribute to knowledge of higher levels of amphibian phylogeny.

Knowledge of phylogenetic relationships also can be informative about macroevolutionary cytological patterns. For instance, Hillis and Green (1990) used knowledge of amphibian phylogeny to examine the evolution of sex-determining mechanisms in salamanders and frogs. By mapping the changes in sex chromosome systems on amphibian phylogeny, these authors could detect an apparent bias in the evolution of male heterogamety from female heterogamety, rather than vice versa. In a similar manner, knowledge of amphibian phylogeny could be used to test other propositions of cytological evolution. Examples of testable hypotheses of chromosomal evolution include Morescalchi's (1973) suggestion of karyotypes evolving from asymmetrical to symmetrical, or the commonly held belief that the loss of microchromosomes is a derived feature. However, many comparative studies of chromosome evolution are currently restricted by the relatively poor knowledge of amphibian relationships at the familial level. Other cytogenetic features, such as changes in band positions, or Robertsonian fissions/fusions in some groups, may be best studied within genera or families for which external (i.e., noncytogenetic) information on phylogeny exists. Such studies are critical for understanding the processes of amphibian karyotype evolution.

VII. Summary

Data from morphological and molecular studies strongly support the monophyly of the living amphibians and place the Lissamphibia as the sister group to the amniotes. In addition, morphological, molecular, and cytogenetic studies among genera and species of amphibians have shed considerable light on relationships at this level. However, the relationships among the orders and families of amphibians remain largely unresolved. Knowledge of relationships at the familial level is critical to an understanding of the macroevolutionary trends in chromosome evolution. Recent developments in molecular approaches to cytogenetics provide new opportunities for contributions to higher-level amphibian systematics, as well as for understanding cytological evolutionary mechanisms.

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