

SYSTEMATICS AT THE TURN OF A CENTURY

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1. INTRODUCTION

What is the status of plethodontid systematics as we end a century and begin a new one? This symposium brings together contributions from leading figures in this field, giving the reader an outstanding sample of research in this discipline. These papers also herald the triumphs of systematics in general. The systematics of plethodontids are also special in a couple of ways. Compared with other vertebrate groups, the sample sizes routinely used to study plethodontids are huge. Because of the fine scale of geographic differentiation, scores and sometimes hundreds of populations are sampled. Often a standard sample of 25 individuals per population is used for traits that vary appreciably within populations (e.g., allozymes). As a consequence of these standards, and the indefatigable efforts of leading investigators, we know more about species formation in plethodontids than in any other vertebrate group. Thus, the story of plethodontid systematics is the story of an especially successful variety of systematics.

In this paper, I review the history of plethodontid systematics, survey some of the prominent controversies in systematics, highlight some of the most interesting empirical findings, and suggest directions for future work. My aim is to provide a context for studying the important systematic contributions in this volume, and to do this in a brief and provocative way.

2. A BRIEF HISTORY

The reaction of European systematists to discoveries of North American plethodontids in the early 19th century was to sweep them into European genera. *Desmognathus*, *Eurycea*, *Gyrinophilus*, *Hemidactylum*, and *Plethodon* initially were all described as species of *Salamandra*. *Batrachoseps attenuatus* was described as a species

The Biology of Plethodontid Salamanders, edited by Bruce *et al.*
Kluwer Academic / Plenum Publishers, New York, 2000.

of *Salamandrina* (Frost, 1985). Later in the 19th century, new genera were erected for the North American species, but at first a few generic names (e.g., *Spelerpes*) served as dumping grounds. By the time of Dunn's (1926) masterful monograph, the distinctive character of plethodontids was apparent. At this time, about the same number of genera was recognized as today, although two extraordinary genera (*Haideotriton* and *Phaeognathus*) still had not been discovered (Carr, 1939; Highton, 1961). Despite its remarkable scope, Dunn's (1926) synthesis had little to say about relationships among genera. The major advance in this regard came 40 years later with the publication of Wake's doctoral dissertation. Basing his masterful analysis on osteology and other morphological traits, Wake (1966) recognized two subfamilies (desmognathines and plethodontines), the latter with three tribes. In Dunn's time, all of the neotropical species were packed into a single genus (*Oedipus*). During the second half of the 20th century, largely under Wake's guidance, many new species and genera of plethodontids were recognized in the neotropics. The big news in plethodontid systematics was that the tribe Bolitoglossini was the most speciose clade in the family and included numerous genera, an astounding discovery considering the diversity of plethodontids in the Appalachian Mountains (Wake, 1970, 1987). The latest chapter in this story is reported in this volume in Wake and Jockusch's (2000, this volume) analysis of *Batrachoseps* in southern California.

Throughout the first half of the 20th century, new species were recognized purely on morphological grounds. New species were discovered as students and faculty poured out of the eastern colleges and universities during the summer months to mine the rich veins of diversity in the Appalachian and Ouachita mountains (Fig. 1). Dentition, relative limb length, vertebral numbers (costal groove counts), and coloration were the characters of choice to describe the new finds. The conceptual framework for organizing the new discoveries was the polytypic species concept (Mayr, 1942, 1963). In this view, most wide-ranging species were composed of geographic races (subspecies) that were connected by narrow bands of intergradation which served as indicators of gene flow. In the case of eastern plethodontids, zones of intergradation often coincided with the boundaries of physiographic provinces, such as the Coastal Plain, Piedmont, and Blue Ridge. Faced with confusing lists of described forms, the systematists of this period often succeeded in recognizing the geographic races of polytypic species. This consolidation of taxa into polytypic species was set in motion by Dunn (1926) and continued throughout the remainder of the century. As consolidation continued, the formal naming of subspecies came under attack in the middle of the century. Wilson and Brown (1953) argued that subspecies were arbitrary constructs because traits within species tended to show different spatial patterns (discordant geographic variation). Because of

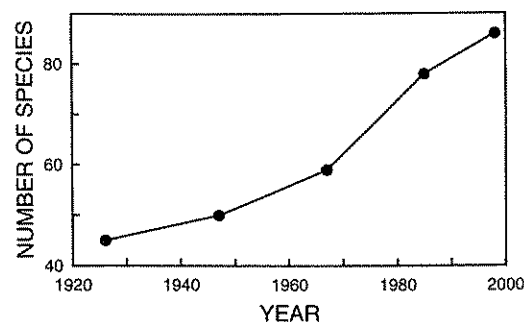


Figure 1. Total number of described species of plethodontid salamanders in the United States and Canada at five points in the 20th century (Dunn, 1926; Bishop, 1947; Brame, 1967; Frost, 1985; and Petranka, 1998).

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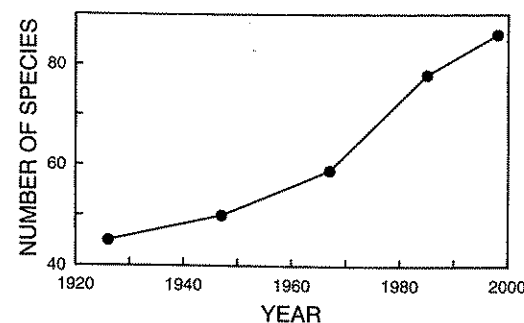


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this discordance, the borders of subspecies would vary depending upon which characters were used. Many workers stopped naming subspecies and instead used graphical and statistical methods to characterize geographic variation. Although out of favor with many systematists, subspecies live on in herpetological field guides (e.g., Conant and Collins, 1998; Stebbins, 1998).

Systematics was revolutionized by the use of molecular markers, starting in the mid 1960s. Beginning with protein electrophoresis, a succession of molecular techniques has been adapted to solving problems in systematics (Hillis et al., 1996). Perhaps the biggest conceptual fallout has been the realization that the phylogeny of populations within species (phylogeography) could be deduced (Avice, 1994). Prior to the use of molecular markers to estimate infraspecific phylogenies, the populations and geographic races that made up a species were viewed as purely geographic entities, disembodied from genealogy. Thus, major methodological advances in phylogenetic deduction, beginning in the late 1950s, coupled with molecular data, have completely revolutionized our concept of species. A second conceptual advance was the use of neutral molecular markers to provide a bridge between population genetics and systematics, giving systematics a solid rooting in Mendelism and theoretical population biology. The use of models in environmental biology increased dramatically in the 1960s and is still in the process of invading the discipline of systematics. Thirdly, molecular markers have revealed and highlighted evolutionary phenomena that were invisible—or only dimly visible—with only morphological data: cryptic species, parapatric distributions, and ancient lineages (Wake, 1981; Highton, 1985). Examples of all of these conceptual advances and phenomena can be found in this volume.

3. A SNAPSHOT OF CONTEMPORARY CONTROVERSIES AND PREDICAMENTS IN SYSTEMATICS

The systematics papers in this volume reflect current controversies and quandaries in systematic biology. Some controversies are right on the surface and acknowledged in the papers (e.g., disagreements over species concepts). Other differences are deeper and set the tone for debates, even though these differences are only barely acknowledged (e.g., differences in methodology for phylogeny reconstruction). Finally, all of the participants try to negotiate certain methodological quagmires. Some of these predicaments may seem quaint a few years from now, but for the moment they force systematists into compromising positions. Near the top of the list of tarbabies is the problem of combining molecular and morphological data in a single analysis of relationships.

3.1. Species Concepts

Systematics is waiting for a consensus to emerge on the meaning of species. Mayr (1963) set the stage for the current debate by defining a number of mutually exclusive concepts of species. One of those concepts—the biological species concept—gave center stage to reproductive isolation as a defining criterion and dominated thinking for most of the second half of the 20th century. The current debate is about the incorporation of criteria that could not be assessed in 1963 (monophyly, gene flow) or that can be assessed only poorly now (evolutionary fate) (de Queiroz and Donoghue, 1988; Frost and Kluge, 1994; Graybeal, 1995). An unfortunate aspect of today's debate is that

it is couched in terms of mutually exclusive concepts of species. At least seven concepts of species have been put forward in this spirit, often in an adversarial tone that offers little hope of reconciliation (Coyne, 1994). Much of the debate involves advocacy for one attribute of species as the sole arbiter of species status (e.g., monophyly). Alternatively, we could recognize that "species" is a multidimensional concept, one that involves dimensions of time, genealogy, reproductive isolation, and morphological divergence (de Queiroz, 1998). This recognition would mean abandoning the position that species can be infallibly diagnosed with just one methodology, such as phylogeny reconstruction. Flushed with the success of molecular markers and methodological improvements, systematists seem hesitant to admit that issues of reproductive isolation and gene flow need to be attacked with special methodologies.

Consequently, it seems likely that a consensus about species will not emerge until well into the next century.

3.2. How Many Species Should We Name?

The naming of species and other taxa (taxonomy) is only one aspect of systematics. Although no one wants to be called a taxonomist, the naming of species is far from trivial. Changes in nomenclature are often the most visible consequences of a systematic study, with implications for everything from field guides to public policy.

The participants in this volume have strikingly different positions on the issue of naming species. Viewpoints are most clear in a pair of papers on *Ensatina* published just before the symposium for this volume was convened (Highton, 1998; Wake and Schneider, 1998). Both camps agree on some key points. *Ensatina* is a complex of forms that vary in morphological distinctiveness, molecular divergence, and how they interact in contact zones. Despite this agreement, Highton (1998) would recognize at least 11 forms (species and semispecies) while Wake and Schneider (1998) argue that—at least for the time being—only a single species should be recognized. What is the basis for such different opinions? The difference arises from three issues in translating the phylogenetic analyses into taxonomy: the importance of monophyly as a criterion for species (important to Wake and Schneider, less so to Highton), whether species need to be diagnosed with discrete characters (essential to Wake and Schneider, not to Highton), and the interpretation of broad zones of contact (viewed as indicating gene flow by Wake and Schneider, viewed as hybrid zones by Highton). These same differences in interpretation characterize papers in this volume.

3.3. Phylogeny Reconstruction

Although debates still rage within systematics, the field is moving out of a period of especially contentious dispute over methodologies for reconstructing phylogeny. The last 20 years have been characterized by labeling of camps with different methodologies, unbridled advocacy, and polarized opinion. At the present time, three major categories of methods coexist: parsimony methods (which seek trees with the fewest characters changes), maximum likelihood methods (which seek trees with the highest likelihood, given the observed data and an explicit model of character evolution), and distance methods (which minimize the discrepancy between matrices that measure the distance between taxa and the tree) (Felsenstein, 1988a). The distinctions between these categories are beginning to erode. First, parsimony and maximum likelihood methods

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have a number of important features in common. They are criterion-based methods that seek the extreme value (maximum or minimum) of an optimality criterion, sometimes known as an objective function (Swofford et al., 1996). Second, there is growing (if not universal) acceptance that statistical concepts provide a framework for comparing and evaluating the different methods. Thus, the parsimony and maximum likelihood criteria are equivalent if evolutionary rates are sufficiently slow (Felsenstein, 1973, 1988a). Such a statistical justification for parsimony is more powerful and integrating than an appeal to Occam's razor (Frost, 2000, this volume). Third, as a practical matter, software packages such as PAUP* allow workers to move between the three categories and exploit their separate advantages. One can use a distance method such as neighbor-joining to find a reasonably good tree to start a search for a tree that maximizes an objective function under parsimony or maximum likelihood. Such hybrid analyses are increasingly common.

3.4. Combining Data from Multiple Data Sets

Leaders in the field of systematics have repeatedly made the point that different kinds of data all provide important information about phylogenetic relationships (Avice, 1994; Moritz and Hillis, 1996). Much is to be gained from combining the insights that molecular and morphological data sets offer (e.g., Jackman et al., 1997). Unfortunately, we still lack a strong conceptual basis for merging molecular and morphological data into one phylogenetic analysis. How much weight should be given to a single morphological trait or to an allozyme locus compared with a nucleotide substitution so that they can be combined in one analysis? The default solution of giving all characters equal weight simply sidesteps the conceptual issue of justifying the weighting system. In the absence of a conceptual solution, systematists often use other, ad hoc solutions to the problem of combining disparate kinds of data. One solution is to base the phylogeny reconstruction only on molecular data and use that phylogeny for discussing morphological data. Another solution is to analyze the morphological, allozyme, and sequence data separately, compare the resulting trees and perhaps combine them (Huelsenbeck et al., 1996; Wiens, 1998). A predicament arises, however, if the trees have different branching patterns. In that case, one could compute the consensus of the different patterns, but that consensus is often bland and uninteresting; in extreme cases the consensus shows little or no resolution of relationships. Thus, modern systematics continues to wrestle with the problem of how to combine disparate data.

4. PLETHODONTID PATTERNS

Modern systematics has revolutionized our understanding of plethodontid species and how they evolve. Often, the most exciting results are tucked away in systematic monographs, and they do not get the attention that they deserve from the rest of the scientific community. I was impressed with the emerging patterns that are discussed below because of their implications for behaviorists, ecologists, and evolutionary biologists. Additional reviews of evolutionary patterns in the family Plethodontidae are given by Hairston (1987), Highton (1985), Tilley (1997), Wake (1981, 1992, 1993), and Wake and Larson (1987).

4.1. Mosaic Distribution of Clades and Species: Sympatry between Species with Relatively Deep Divergence

A common—although not universal—pattern in the geography of plethodontid species has been revealed by recent systematic work. Closely related species usually replace one another in space, with abutting geographic ranges. The seven species of the *Plethodon glutinosus* complex are a good example of this pattern (Highton and Peabody, 2000, this volume). Because geographic races within species often show this same pattern, there is no change in geographic distribution that denotes speciation. Sympatry evolves much later, often after deep divergence (Chippindale, 2000; Highton and Peabody, 2000; Wake and Jockusch, 2000, all this volume). Apparently it is only during this long period of differentiation in allopatry that resource utilization diverges and behavioral mediation of competition evolves (Jaeger, 1971; Nishikawa, 1985).

4.2. Different Kinds of Species

Species differ in a variety of attributes, including age. At one end of the age continuum are young species, with short branches on molecular phylogenies, little geographic variation in morphology, no sexual isolation (even between widely separated populations), and sometimes with appreciable geographic ranges. *Desmognathus monticola* appears to be such a newcomer (Tilley, 1997; Verrell and Mabry, 2000, this volume). Thus, ecological interactions with *D. monticola* may be actively evolving in many areas of sympatry. At the other end of the continuum are ancient species with deep molecular branches, with at least detectable geographic variation in morphology, sexual isolation between populations a few scores of kilometers apart, and extensive geographic ranges. *Ensatina eschsholtzii* and *Desmognathus ocoee* may both be such species (Jackman and Wake, 1994; Moritz et al., 1992; Tilley and Mahoney, 1996; Wake, 1997). These species have weathered invasions of competitors and benefitted from the extinction of rivals on a geological time scale.

4.3. Signatures of Pleistocene Dispersal

Some species bear the signature of recent northward expansion on the heels of glacial retreat at the end of the Pleistocene (Highton and Webster, 1976; Larson et al., 1984; Tilley and Mahoney, 1996). The northern ranges of these species are characterized by a loss of genetic variation at neutral loci, both within and among populations. In contrast, southern populations have high levels of heterozygosity and are deeply differentiated. Such species are intriguing subjects for ecological studies. Have bottlenecks been so severe and recent during northward expansion that genetic variation for life history traits and other adaptations has been compromised? Are northern populations of these species prone to local extinction because of an inability to respond to selection?

4.4. Ancient, Peripheral Isolates

Some species show arrays of ancient, peripheral isolates. Sometimes these arrays take the form of a necklace strung in an arc to the south of the main distribution (Highton, 1997; Highton and Peabody, 2000, this volume; Highton and Webster, 1976). The implication here is that populations were left behind to flourish in small pockets

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of favorable habitat during ancient contractions of the species' range. The older view was that peripheral isolates differentiated because gene flow was cut off (Mayr, 1942). The emphasis was on interruption of gene flow rather than on the time that might have elapsed since isolation was established. Gene flow among plethodontid populations is low even among populations in the main body of the range (Slatkin, 1985). The surprising thing about peripheral isolates is their ancient status rather than their interrupted gene flow.

4.5. Unusual Departures from Morphological Stasis

Most plethodontid species groups are remarkably uniform in morphology. *Batrachoseps*, *Desmognathus*, *Plethodon*, and the *Eurycea* of the Balcones escarpment are bewildering radiations for the beginning herpetologist, exasperating because the characters that distinguish species are so subtle. Major morphological innovations some-time spring from the middle of such speciose, morphologically uniform clades. *Typhlomolge* is a good example of this phenomenon (Chippindale, 2000, this volume). This pattern represents the invasion of a new adaptive zone (deep caves in the case of *Typhlomolge*), in which a population responds to a whole suite of new selective pressures and fundamentally alters its life style and appearance (Simpson, 1944). Tilley (2000, this volume) may have captured the process at an early stage in his study of rockface populations of *Desmognathus imitator*. Thus, plethodontids offer outstanding opportunities to study shifts in adaptive peaks.

5. THE NEXT CENTURY

What directions will systematics take in the century that lies ahead? One profitable direction would be to integrate experimental methods and modeling into systematics and to broaden the data base to include more kinds of characters. In particular, we seem poised to make progress on at least five major fronts. Trends towards all of these directions are apparent in the current literature.

5.1. The Experimental Study of Reproductive Isolation and Its Incorporation into Systematics

One complaint with the biological species concept is that its emphasis on reproductive isolation makes it nonoperational. This complaint does not apply in the case of plethodontids. Experimental protocols have been established for the study of one major component of reproductive isolation—sexual isolation—and extensive surveys have been conducted in two genera, *Desmognathus* and *Plethodon* (Arnold et al., 1993). We do not have to make assumptions about reproductive isolation: we can measure it! Despite the availability of procedures, the measurement of sexual isolation has so far had relatively little impact on plethodontid systematics. When data on sexual isolation are available, they have been taken into account (Tilley and Mahoney, 1996; Highton and Peabody, 2000, this volume), but generally they are used to support conclusions reached by other means. Perhaps the best hope for more meaningful incorporation in the future is to focus experimental studies on those problematic issues and cases that can not be reconciled by other means, e.g., the status of allopatric populations.

5.2. Larger Samples of the Nuclear and Mitochondrial Genomes

As technology advances and expertise improves, the bar is raised in systematics. As gene sequencing becomes easier and cheaper, more and more data are used to assess genetic differentiation. From this perspective, it seems likely that many vexing issues in today's results will be resolved when larger samples are brought to bear on the problems. In an illuminating study of sampling issues, Cummings et al. (1995) found that samples of a single mitochondrial gene rarely succeed in retrieving the whole-genome tree. A better strategy was to take smaller samples from throughout the genome. Furthermore, convergence on the whole-genome tree typically did not occur until several thousand base pairs had been sampled.

5.3. Plethodontid Genomics

The molecular systematics of plethodontids is in a relatively primitive state because there is no plethodontid genome project. In model organisms (e.g., *Drosophila*, *Mus*, *Caenorhabditis*, humans), for which genome-wide sequencing is well advanced, primers for nuclear genes can easily be designed and used in systematic work. In the absence of a genomic survey, primers for nuclear genes must be developed on a case-by-case basis. Consequently, plethodontid systematics is stalled in the mitochondrial genome, at least for the moment. Microsatellite loci are one viable shortcut and several labs are trying to design and employ primers for these highly variable, nuclear genes. But this is palliative. In the next century, someone will weigh the advantages and take the plunge into plethodontid genomics. One outstanding issue is the huge size of the plethodontid genome (Hanken and Wake, 1993; Roth et al., 1997). Is the genome's huge size a major factor in plethodontid evolution or is it an epiphenomenon that lacks a causal role?

5.4. Morphological and Molecular Data Together

Systematics urgently needs a solution to the problem of combining morphological and molecular data sets in a single phylogenetic analysis. Until that problem is solved, morphological traits will increasingly be an addendum to molecular analyses of phylogeny. To combine data in a single analysis, we need solutions to two problems: independence and weighting. Our current methodologies for phylogeny reconstruction ignore the problem of correlation among characters, treating characters as if they evolved independently (Felsenstein, 1982, 1988b; but see Shaffer, 1986). The assumption of independence often may be reasonable for molecular traits, but it is patently false for morphological traits. The evolutionary trajectories of many morphological traits are coupled due to pleiotropy and linkage disequilibrium (Falconer and Mackay, 1996; Roff, 1997). We need assessments of how serious nonindependence can be to the accuracy of phylogeny reconstruction. If violation of the independence assumption can produce serious errors, then we need methodologies that allow for character correlation. Secondly, we need a solution to the problem of character weighting. One solution might be to use a common currency (e.g., nucleotide substitutions) for all character change. For morphological traits, we might need the following kind of equivalencies: "A change of one genetic standard deviation in a linear dimension is equivalent to 100 nucleotide substitutions." It is conceivable that studies of quantitative trait loci will produce enough results in the next century to give us the needed conversion table. If

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The molecular systematics of plethodontids is in a relatively primitive state because there is no plethodontid genome project. In model organisms (e.g., *Drosophila*, *Mus*, *Caenorhabditis*, humans), for which genome-wide sequencing is well advanced, primers for nuclear genes can easily be designed and used in systematic work. In the absence of a genomic survey, primers for nuclear genes must be developed on a case-by-case basis. Consequently, plethodontid systematics is stalled in the mitochondrial genome, at least for the moment. Microsatellite loci are one viable shortcut and several labs are trying to design and employ primers for these highly variable, nuclear genes. But this is palliative. In the next century, someone will weigh the advantages and take the plunge into plethodontid genomics. One outstanding issue is the huge size of the plethodontid genome (Hanken and Wake, 1993; Roth et al., 1997). Is the genome's huge size a major factor in plethodontid evolution or is it an epiphenomenon that lacks a causal role?

5.4. Morphological and Molecular Data Together

Systematics urgently needs a solution to the problem of combining morphological and molecular data sets in a single phylogenetic analysis. Until that problem is solved, morphological traits will increasingly be an addendum to molecular analyses of phylogeny. To combine data in a single analysis, we need solutions to two problems: independence and weighting. Our current methodologies for phylogeny reconstruction ignore the problem of correlation among characters, treating characters as if they evolved independently (Felsenstein, 1982, 1988b; but see Shaffer, 1986). The assumption of independence often may be reasonable for molecular traits, but it is patently false for morphological traits. The evolutionary trajectories of many morphological traits are coupled due to pleiotropy and linkage disequilibrium (Falconer and Mackay, 1996; Roff, 1997). We need assessments of how serious nonindependence can be to the accuracy of phylogeny reconstruction. If violation of the independence assumption can produce serious errors, then we need methodologies that allow for character correlation. Secondly, we need a solution to the problem of character weighting. One solution might be to use a common currency (e.g., nucleotide substitutions) for all character change. For morphological traits, we might need the following kind of equivalencies: "A change of one genetic standard deviation in a linear dimension is equivalent to 100 nucleotide substitutions." It is conceivable that studies of quantitative trait loci will produce enough results in the next century to give us the needed conversion table. If

we go down this path, it is clear that we will need increased interaction between systematics and quantitative genetics.

5.5. Why Is Plethodontid Morphology So Conservative?

We are still struggling to understand the remarkable morphological conservatism of many plethodontid genera. The type specimen of this phenomenon is *Hydromantes*, which has kept its distinctive Bauplan even though the genus is millions of years old and its species are distributed on two continents (Jackman et al., 1997; Wake, 1993). Two broad categories of explanation have been offered for this kind of stasis. The first category is internal constraints arising from development and genetics. The problem with this class of explanation is that genetic variation has generally been found within populations for most kinds of traits when it has been searched for (Charlesworth et al., 1982). It is unlikely that *Hydromantes* has kept its Bauplan because it lacks genetic variation to change (e.g., due to developmental constraint). The second category is external constraints. Here, we imagine that *Hydromantes* has kept its ecology constant over geological time (perhaps by habitat selection), and the stabilizing selection that arises from that constancy has kept its Bauplan intact. The range of microhabitats where *Hydromantes* occurs in Sardinia, Italy, and California are remarkably similar. Nevertheless, we lack direct evidence that the far flung populations of *Hydromantes* experience similar patterns of stabilizing selection. A challenge for the next century will be to test this and other predictions of the stabilizing selection model. In a similar vein, Wake et al. (1983) discuss the possibility that morphological stasis in plethodontids is a consequence of an ability to behaviorally compensate for environmental change.

6. SUMMARY

Plethodontid systematics progressed through several stages and revelations during the 20th century. In the first stage, new discoveries in North America were placed in pre-existing European genera or in "waste basket" genera. With the revelation that a vast radiation had occurred in North America, new genera were created and many new species were described using morphological traits. In the last couple of decades, the biggest revelations have been that the largest radiation of plethodontids is in Middle America, and that molecular methods can reveal cryptic species and ancient lineages.

At the end of the 20th century, plethodontid systematics is pervaded by controversies and predicaments that involve: species concepts, the naming of species, phylogeny reconstruction, and methodology for combining disparate kinds of data.

The plethodontid radiation is characterized by a number of important evolutionary patterns. These patterns include: the mosaic distribution of closely related forms, great variety in kinds of species, signatures of Pleistocene dispersal, peripheral isolates of great age, and radical departures from morphological stasis.

A number of trends in plethodontid systematics seem likely to continue into the 21st century. These easily extrapolated trends include: more experimental studies of reproductive isolation and their incorporation into systematics, larger samples of mitochondrial and nuclear genomes, a complete characterization of a plethodontid genome, the development of well-justified techniques for combining molecular and

morphological data, and the development of testable models that will solve the puzzle of morphological stasis in plethodontids.

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