ORIGINAL ARTICLE

CAN PARALLEL DIVERSIFICATION OCCUR IN SYMPATRY? REPEATED PATTERNS OF BODY-SIZE EVOLUTION IN COEXISTING CLADES OF NORTH AMERICAN SALAMANDERS

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A classic paradigm in evolutionary biology is that geographically isolated clades inhabiting similar selective regimes will diversify to create similar sets of phenotypes in different locations (e.g., similar stickleback species in different lakes, similar *Anolis* ecomorphs on different islands). Such parallel radiations are not generally expected to occur in sympatry because the available niche space would be filled by whichever clade is diversified first. Here, we document a very different pattern, the parallel evolution of similar body-size morphs in three sympatric clades of plethodontid salamanders (*Desmognathus, Plethodon,* Spelerpinae) in eastern North America. Using a comprehensive, time-calibrated phylogeny of North American plethodontids from nuclear and mitochondrial DNA sequences, we show that these three clades have undergone replicated patterns of evolution in body size and that this parallel diversification occurred in broad-scale sympatry. At the local scale, we find that coexisting species from these clades are more similar in body size than expected under a null model in which species are randomly assembled into communities. These patterns are particularly surprising in that competition is known to be important in driving phenotypic diversification and limiting local coexistence of similar-sized species within these clades. Although parallel diversification of sympatric clades may seem counterintuitive, we discuss several ecological and evolutionary factors that may allow the phenomenon to occur.

KEY WORDS: Adaptive radiation, biogeography, community assembly, competition, diversification, salamanders.

One of the most striking patterns documented by evolutionary biologists is the parallel evolution of similar phenotypes in different geographic locations, or parallel diversification (Simpson 1953; Schluter 2000). For example, marsupial mammals in Australia exhibit striking convergence in feeding, locomotion, and morphology with placental mammals in other parts of the world (Futuyma 1998). Similarly, *Anolis* lizards have independently evolved the same ecomorphs on separate islands in the Greater Antilles (Losos et al. 1998), as have spiders on different islands of the Hawai-

ian archipelago (Gillespie 2004). In the Pacific Northwest, benthic and limnetic pairs of stickleback species (*Gasterosteus*) have evolved repeatedly in separate lakes (Schluter and McPhail 1992; Schluter 2000). Many other examples abound in the literature.

Given a strong relationship between phenotypic variation and resource use, an important corollary of the concept of parallel diversification is that sympatric lineages will preempt each other from evolving similar phenotypes in the same geographic location (e.g., Losos et al. 1998; Schluter 2000). This idea of
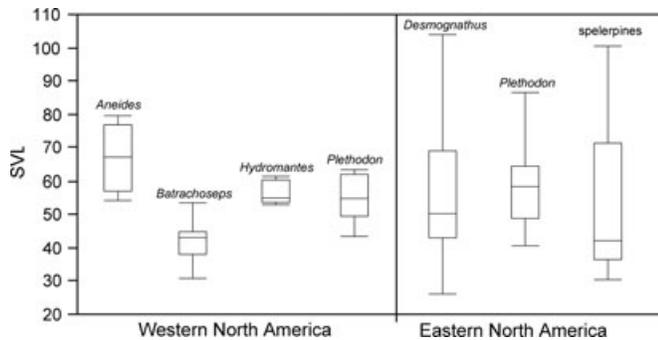
Figure 1. Box plots illustrating patterns of body-size variation among species in major clades of plethodontids from eastern and western North America. Boxes enclose the median of the average body size (snout-vent length in millimeters) among species and its 25th and 75th percentiles. Whiskers denote maximum and minimum mean body sizes among species within clades. Body-size data for individual species are provided in Appendix S3.

preemptive evolution has its roots in the ecological theory of adaptive radiation, which postulates that the colonization of geographic regions that lack resident competitors promotes phenotypic diversification, as lineages evolve phenotypes that allow them to use previously unoccupied portions of niche space or adaptive zones (Simpson 1953; Schluter 2000). However, as available niche space becomes "occupied" through phenotypic diversification (at least along the niche axis in question), competition is thought to constrain opportunities for other lineages to evolve similar phenotypes (e.g., Losos et al. 1998; Kozak et al. 2005; Wiens et al. 2006a). Thus, parallel patterns of phenotypic diversification are expected primarily in lineages that have radiated in geographically isolated locations (e.g., different islands, lakes, or continents).

In this article, we document a very different pattern: the parallel phenotypic diversification of clades that occur in sympatry. Specifically, we show that three sympatric clades of salamanders in eastern North America (ENA hereafter) have undergone similar patterns of morphological diversification, particularly in body size. ENA is dominated by three major radiations of plethodontids (genus *Plethodon* [55 species], supergenus *Desmognathus* [2 genera, 20 species], and subfamily Spelerpinae [5 genera, 34 species]; AmphibiaWeb 2007) (Fig. 1). These three clades are broadly sympatric and species from each clade occur together in most local communities in ENA that have been intensively studied (Fig. 2; see also Hairston 1949; Burton and Likens 1975; Petranka et al. 1993, 1994; Harper and Guynn 1999; Smith and Petranka 2000; Petranka and Murray 2001; Ford et al. 2002; Wilson and Dorcas 2003). Although there are some microhabitat differences between species within and between clades, species from these three clades occur microsympatrically in springs, seepages, streams, and adjacent forested habitats in ENA (e.g., Petranka and Smith 2005),

and some of the larger, predatory species are even known to feed on species in other clades (reviewed in Bruce 2007).

The pattern of parallel radiation in sympatric clades that we document here is particularly interesting in that there is already an extensive literature documenting the importance of interactions among species within these clades, and the importance of body size in these interactions. Specifically, competition among congeners is thought to drive patterns of coexistence, microhabitat use, and phenotypic evolution in both *Desmognathus* (e.g., Hairston 1986; Kozak et al. 2005) and *Plethodon* (e.g., Jaeger 1971; Hairston 1980a,b; Nishikawa 1985; Adams 2004, 2007). Most importantly, body size appears to be a key trait in determining coexistence of species within clades, in that species of similar body size within clades tend not to coexist, a pattern shown across all *Desmognathus* species (e.g., Kozak et al. 2005) and all *Plethodon* (Adams 2007) in ENA. This may occur because plethodontid salamanders in ENA are generalist predators (mostly on invertebrates; Petranka 1998) in which body size determines dietary resource use through the size of the prey that can be consumed (e.g., Burton 1976; Krzysik 1979; Adams and Rohlf 2000). Studies have also shown a reduction in prey consumption when congeneric species of similar size occur in sympatry (e.g., Adams and Rohlf 2000). Body size may also be important in terms of predation (e.g., some large plethodontids prey on smaller ones; reviewed in Bruce 2007) and other interactions between species (e.g., interference competition; Keen 1982; Southerland 1986a,b). Thus, body size seems important to resource use and ecological interactions between species, and is therefore very relevant to the idea of preemption discussed above (even if there is variation in other aspects of their ecology besides body size; Petranka 1998). Intriguingly, the diversification of plethodontids in ENA also contrasts strongly with that found in plethodontids that are restricted to western North America (WNA hereafter). In WNA, body-size differences are partitioned largely among the major clades of plethodontids (Fig. 1), and many of these clades are sympatric at regional and local scales (Petranka 1998).

Here, we integrate approaches from phylogenetics and community ecology to test whether sympatric plethodontids clades in ENA have undergone parallel patterns of diversification. First, we reconstruct a comprehensive, time-calibrated phylogeny for North American plethodontids based on nuclear and mitochondrial DNA sequence data. Next, we use multivariate morphometric analyses to show that the major axis of morphological variation in these clades is body size, a variable shown to be of great ecological importance in plethodontid salamanders (e.g., Hairston 1986; Bruce 1996; Kozak et al. 2005; Adams 2007; Bruce 2007) and across organisms in general (e.g., McMahon 1973; Wilson 1975; Peters 1983; Calder 1984; Schmidt-Nielson 1984; LaBarbera 1986). We then map the timing and biogeographic context of body-size evolution on this tree, and show that
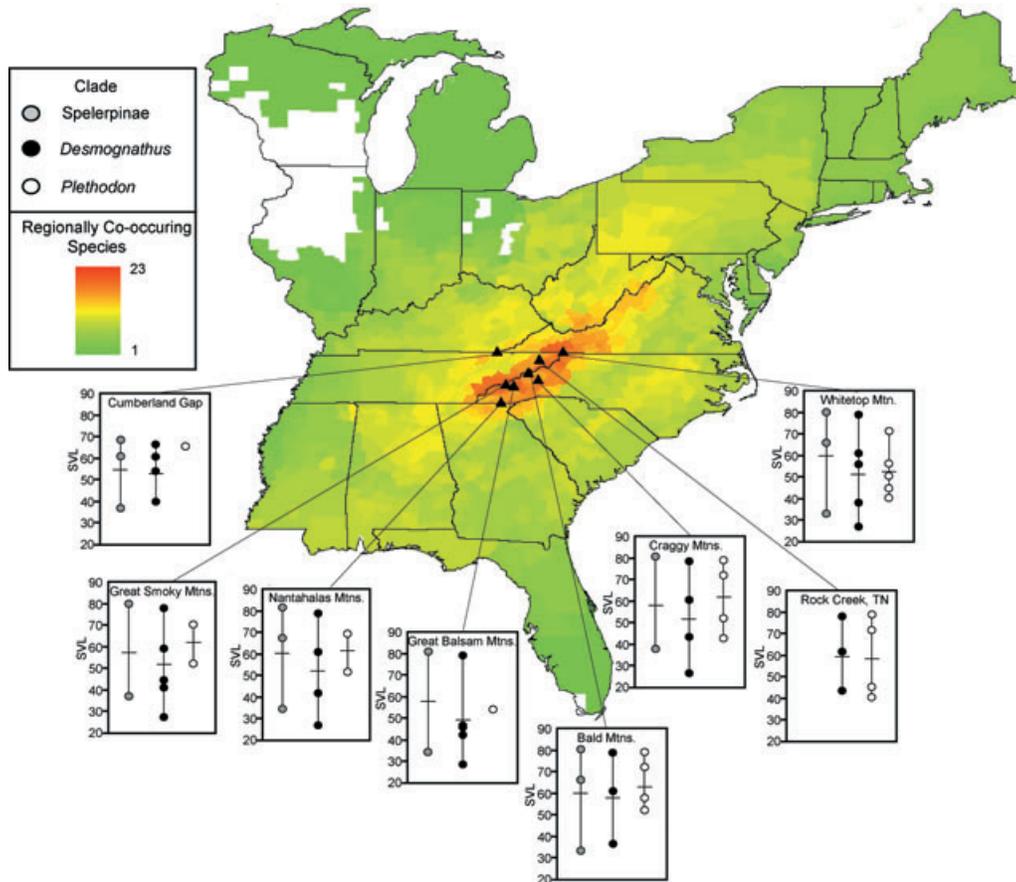
Figure 2. Map of the eastern United States showing the number of plethodontid species that are sympatric at the county level and the range in mean body size for coexisting species of spelerpines, *Desmognathus*, and *Plethodon* in eight local communities. Geographic variation in the number of regionally co-occurring species was mapped by overlaying species' geographic distribution maps (obtained from the National Amphibian Atlas for Amphibian Distributions, http://igsaceeswb00.er.usgs.gov:8080/mapserver/naa/) in Arc GIS 9.0. The mean body size for each species found in the community is shown with a circle. Filled, gray, and open circles correspond to spelerpines, *Desmognathus*, and *Plethodon*, respectively. Vertical bars connect the maximum and minimum values of mean body sizes among species in a given community. The horizontal bar corresponds to the average of the mean body sizes from all species from a clade that co-occur in the community. Note that only a single species of *Plethodon* occurs at the sites studied in the Great Balsam Mountains and Cumberland Gap. At the Rock Creek site, no species of spelerpines have been documented.

similar ranges of body-sizes evolved within each clade within ENA (although all three clades are actually more widely distributed). Using phylogeny-based simulations of morphological evolution (Garland et al. 1993) and models of random community assembly, we show that these clades significantly converged in their patterns of body-size evolution in ENA, and that locally coexisting species from different clades tend to be more similar in body size than expected by chance. We then discuss the possible causes of these counterintuitive patterns and their broader significance for evolutionary biology and ecology.

Materials and Methods
PHYLOGENETIC DATA AND ANALYSIS
Taxon sampling and DNA sequence data
Analyzing patterns of diversification in North American plethod-

ontid salamanders required an extensive species-level phylogeny. Unfortunately, previous phylogenetic analyses of Plethodontidae were not comprehensive (even for North American taxa) and are only partially overlapping in their sampling of genes and taxa. We therefore used a combination of new data and existing data from other studies (Jackman 1999; Jockusch et al. 2001; Mahoney 2001; Wiens et al. 2003; Bonett and Chippindale 2004; Chippindale et al. 2004; Mueller et al. 2004; Min et al. 2005; Kozak et al. 2005, 2006a,b; Wiens 2006b; Vieites et al. 2007) to generate a data matrix that had overlapping sets of taxa and genes for all major North American lineages of plethodontids. We included DNA sequence data from three mitochondrial (mtDNA) and three nuclear genes (nDNA). The mitochondrial genes included cytochrome *b* (cyt *b*; 649–1132 base pairs, bp), NADH dehydrogenase subunit 2 and the flanking tRNA[trp] gene

(ND2; 1107 bp), and NADH dehydrogenase subunit 4 (ND4; 686 bp). The nuclear genes included brain-derived neurotrophic factor (BDNF; 707 bp), proopiomelanocortin (POMC; 481 bp), and recombination activating gene 1 (RAG-1; 1467 bp). DNA amplification, sequencing, and alignment of genes followed Chippindale et al. (2004), Kozak et al. (2005), and Wiens et al. (2006b). The combined matrix, including 184 ingroup taxa and 5590 aligned characters, represents the most extensive analysis of plethodontid phylogeny to date.

Our analysis included 649 gene sequences, of which 195 were generated in this study. A complete list of the species, data sources, and GenBank accession numbers for each gene is included in Appendix S1. Because our data matrix included partially overlapping datasets from different studies, many taxa lacked data for one or more genes. On average, each taxon had data for 3.5 genes (range: 1–6 genes) and 58% of the 5590 aligned characters (range: 11.6–98.5%). A few of the taxa we included had data for only one or two genes (e.g., some species of *Batrachoseps* and Texas *Eurycea*, for which samples were not available to us). However, representatives of all plethodontid subfamilies and each of the North American genera were sampled for all six genes. Furthermore, most of the species within each of the major lineages in ENA had data for both a nuclear gene (RAG-1) and at least one mitochondrial gene. Although some readers may be troubled by the extensive missing data in some taxa, recent simulations and empirical studies indicate that when the overall number of characters is large (i.e., in the thousands), taxa with highly incomplete character sampling can be accurately placed in phylogenetic analyses (e.g., Wiens 2003; Driskell et al. 2004; Phillipe et al. 2004; Wiens et al. 2005), and their addition can even improve phylogenetic accuracy in some cases (Wiens 2005).

Throughout the article, we follow the species-level and generic-level taxonomy of AmphibiaWeb (2007). In some analyses, we use two terminals for *Plethodon yonahlossee*, one of which corresponds to *P. longicrus*, which is not traditionally recognized but may represent a distinct species (Wiens et al. 2006b). We follow the subfamilial classification of Chippindale et al. (2004), rather than that of Vieites et al. (2007) because there is no phylogenetic justification for the changes suggested by the latter authors.

Phylogenetic analysis
We used maximum likelihood as implemented in RAxML 7.0.0 (Stamatakis 2006, 2008) to estimate the phylogeny. Previously published phylogenetic studies with broad taxon sampling across Plethodontidae concluded that GTR $+$ I $+$ Γ is the best-fitting model for four of the six genes (RAG-1, cyt-*b*, ND2, and ND4), GTR $+$ Γ is the best model for the other two (BDNF, POMC), and each gene should be partitioned by codon position (Kozak et al. 2005, 2006a; Wiens et al. 2006b; Vieites et al. 2007). We therefore conducted all phylogenetic analyses in RAxML using the GTRGAMMA nucleotide substitution model (with separate partitions for each gene and codon), which is the general-time reversible model with an among-site rate heterogeneity parameter, Γ. Although RAxML can incorporate a parameter for the proportion of invariant sites, we chose not to explicitly include it because the incorporation of Γ in the GTRGAMMA model in RAxML 7.0.0 mathematically accounts for this source of rate heterogeneity among sites by using 25 rate categories (and following the recommendations of Stamatakis [2008]).

Because it is theoretically possible for the mitochondrial gene tree and each of the nuclear gene trees to have partially conflicting evolutionary histories, we first analyzed the mtDNA and nDNA data separately to test for clades with strongly supported incongruence between these datasets (e.g., Wiens 1998). To examine support for individual nodes in each dataset, we used RAxML to conduct a bootstrap analysis (with 100 pseudoreplicates) of each data matrix. We found that the nDNA and mtDNA datasets produced generally congruent phylogenetic estimates (see Appendix S2), with most areas of discordance being restricted to branches with low support in one or both datasets (i.e., bootstrap proportions $<$ 70%; Felsenstein 2004). Exceptions involve the different placements of *Plethodon cinereus, P. serratus*, *P. hubrichti,* and *P. nettingi* within the *P. cinereus* group, of *Eurycea lucifuga* within the genus *Eurycea*, and the nonmonophyly of *Gyrinophilus* in the nDNA tree. The latter conflict likely results from retention of ancestral polymorphism in the nDNA tree given that *Gyrinophilus* is resolved as a clade upon the addition of more-rapidly evolving mtDNA genes in the combined dataset. The conflicting phylogenetic relationships within the *P. cinereus* group and *Eurycea* involve species that have very similar body sizes, and therefore should have little influence on subsequent comparative phylogenetic analyses. Given the overall congruence, the mtDNA and nDNA gene regions were combined to maximize the total number of characters for phylogenetic analysis and branch-length estimation.

The final estimate of plethodontid relationships was based on a maximum-likelihood analysis of the combined data, and support for clades was evaluated with nonparametric bootstrapping. To prevent our analyses of the combined dataset from becoming trapped on local optima, we conducted 40 separate maximum-likelihood searches. The tree with the best likelihood was then selected as the optimal tree. To evaluate the support for each node, we used the "fastbootstrap" algorithm in RAxML 7.0.0 with 200 bootstrap replicates.

Divergence time estimates
To estimate the ages of the major clades of North American plethodontids, we used penalized-likelihood (PL; Sanderson 2002) as implemented in r8s (Sanderson 2003) to transform the likelihood tree inferred from the combined data matrix into a

time-calibrated phylogeny. The program r8s requires that the age of at least one clade be specified, rather than merely using a minimum age constraint. We conducted three separate analyses, each fixing the crown-group root age of Plethodontidae to one of three plausible dates (48, 61, 69 million years old [Myr]; Wiens 2007). These dates were derived from a previous PL analysis of divergence times that included one or more representatives of most plethodontid genera and was based on slow-evolving RAG-1 sequences and 11 fossil-calibration points across all salamander families (Wiens 2007). In addition, we used information on known plethodontid fossils to constrain the minimum ages of the following crown-group clades (following Wiens 2007): (1) the most recent common ancestor of *Plethodon* is at least 19 Myr old; (2) the most recent common ancestor of *Aneides* is at least 19 Myr old; (3) subfamily Bolitoglossinae is at least 5 Myr old. Each analysis used the truncated-Newton algorithm and five replicate optimizations. The optimal smoothing parameter for each analysis was selected based on a cross-validated assessment with values ranging from 10^0 to 10^4 in exponential increments of 0.50.

We acknowledge that other studies have estimated somewhat older dates for the crown-group age of plethodontids (e.g., ∼96 Myr in Vieites et al. 2007) than Wiens (2007). However, for the present study, our analyses rely on relative branch lengths, not the absolute ages. Consequently, we found that assuming different root ages for Plethodontidae did not qualitatively impact our phylogeny-based analyses of biogeography and morphological diversification. For brevity, we therefore present the results of analyses that employed a root age of 61 Myr for Plethodontidae.

BIOGEOGRAPHIC RECONSTRUCTIONS

We used ancestral area reconstruction (Ronquist 1994) to estimate the biogeographic history of the major clades of North American plethodontids. We coded the geographic region inhabited by each species as an unordered multistate character using the following geographic areas as character states (following Duellman and Sweet 1999): ENA (east of the Mississippi River), Central Highlands (the Ouachita and Ozark mountains of Arkansas, Missouri, Oklahoma, and Kansas), Edwards Plateau of Central Texas, and WNA (the longitude of the eastern border of New Mexico and westwards). These regions correspond to major areas of plethodontid salamander endemism in North America; each region has multiple endemic species and there are relatively few species that occur in more than one region (Petranka 1998; Duellman and Sweet 1999). The few species with distributions that encompassed more than one geographic region were coded as having a separate polymorphic character state (with different states for different combinations of regions). We then used maximum likelihood (as implemented in MESQUITE, version 1.12; Maddison and Maddison 2004) to reconstruct shifts between major biogeographic regions on the phylogeny. Although dispersal-vicariance analysis

(DIVA; Ronquist 1997) has some advantages for biogeographic analysis relative to ancestral area analysis, it is also disadvantageous in that it does not incorporate branch-length information, as does our likelihood-based analysis. Ideally, we would have employed recently developed maximum-likelihood methods that also incorporate models of lineage dispersal and local extinction for estimating ancestral geographic ranges (e.g., Ree et al. 2005). However, software for implementing this approach was not available during the present study. Finally, we point out that our primary goal in analyzing the biogeography was to determine in which regions major shifts in body size occurred, rather than to infer details of dispersal and vicariance.

We acknowledge that some of these regions could be subdivided further. For example, ENA could be subdivided into additional subregions (e.g., Duellman and Sweet 1999). However, we found such divisions to be very arbitrary, and many species occurred in multiple subregions. For example, even though the Appalachian Mountains contain endemic species, many species are widespread in both the lowlands and highlands (Petranka 1998; Duellman and Sweet 1999).

MORPHOLOGICAL DATA AND ANALYSES

To quantify morphological variation in plethodontids, we first analyzed data for seven standard morphometric variables of the head, body, limbs, and tail from adult specimens of 73 species representing the subfamily Spelerpinae, supergenus *Desmognathus*, and the genera *Plethodon*, *Aneides*, *Batrachoseps*, *Ensatina*, and *Hydromantes* (variables, measurements, and data taken from Wiens and Hoverman [2008]). Specimens were considered to be adult based on the presence of secondary sexual characteristics, having body sizes similar to the largest individuals sampled within a given species, and based on published descriptions of the range of adult body sizes within a species (e.g., Petranka 1998). A principal-component analysis (not shown) revealed that divergence in overall size explained most of the morphological variation among species (i.e., all variables loaded positively and evenly on PC1, which accounted for 90%). Given these results, and a strong correlation between ln-SVL and PC1 among these 73 species ($r^2 = 0.90$), we used ln-SVL data as a standard proxy for body size in subsequent analyses. This allowed us to use SVL data from the literature for many additional species, given that data for all seven morphometric variables were available for a much smaller set of species. We obtained published SVL data from an additional five *Batrachoseps* species, 13 *Desmognathus*, five *Eurycea,* and 13 *Plethodon* (see Appendix S3 for literature sources). We also collected new SVL data for five presently undescribed species of *Eurycea* reported in Kozak et al. (2006b). In total, the dataset included body-size data for 109 species (see Appendix S3). The mean ln-transformed SVL for each species was then used in all subsequent analyses.

We note that although our analyses average SVL across the ranges of species, geographic variation in body sizes should have little impact on our conclusions about patterns of phenotypic diversification within and among clades. Despite geographic variation in body size, large and small species generally show little overlap in body size with each other (e.g., Tilley and Bernardo 1993; Carr 1996; Ryan and Bruce 2000). In addition, such variation in body size is also minor in comparison to the total range of body sizes that evolved within each clade (upon which our analyses of body-size overlap among clades are based; see below).

BODY-SIZE EVOLUTION

To visualize the evolutionary history of body-size diversification, we reconstructed the evolution of body size on the chronogram using the linear generalized least-squares method of Martins and Hansen (1997) as implemented in COMPARE version 4.6 (Martins 2004). We acknowledge that reconstructed values for continuous traits may not be completely accurate (but see simulation results of Martins 1999). However, they serve as a heuristic for examining the extent to which clades have evolved similar or different body-size distributions. For illustrative purposes, we also categorized species into four body-size classes based on their mean SVL: small = 26–42 mm; medium = 43–60 mm; large = 61–89 mm; very large ≥ 90 mm. Delimitation of these four body-size classes is arbitrary and is presented simply to summarize the extent to which different clades have evolved species with similar or different body sizes. Neither the reconstructions nor the body-size categories were used in the statistical tests of parallel diversification.

The hypothesis of parallel diversification leads us to predict the independent evolution of a large range of similar body-size morphs in different clades, and that clades will exhibit greater overlap in their body-size distributions than expected from a model of stochastic diversification in body size. To assess the extent to which clades overlap in their body-size distributions, we used relative disparity analyses (Harmon et al. 2003) to quantify how body-size variation is partitioned within and among clades in ENA. To accomplish this, we first separately calculated the mean-squared pairwise distance in SVL between all plethodontid species in ENA (total disparity) and within each of the three major clades of plethodontids (clade disparity) in ENA. Next, we calculated the relative disparity of each clade by dividing its clade disparity by the total disparity calculated for ENA. Finally, we averaged the relative disparity values across clades to obtain a mean relative disparity for the major clades compared to the total disparity of ENA. A mean relative disparity near 0 indicates that body-size differences among all species are partitioned largely among clades (and thus clades found within the same region show little overlap in their body-size distributions). In contrast, a mean relative disparity near 1.0 indicates that species in different clades overlap extensively in body-size and that the codistributed clades have independently evolved similar body-size distributions (Harmon et al. 2003).

To quantify whether the mean relative disparity of clades differed from that expected under a model of stochastic evolution in body size, we simulated 1000 body-size datasets along the ENA chronogram, first assuming a model of Brownian motion. For each simulation, we constrained the mean and the variance of the simulations to be equal to those observed at the tips of the tree (Garland et al. 1993). For each of the 1000 simulated datasets, we calculated the mean relative disparity as described above. This procedure generated a null distribution of mean relative disparity values expected under a model of stochastic diversification in body size. We considered the average relative disparity to be significant if it fell within the upper or lower 2.5% of the simulated values.

Brownian motion is a widely used null model in comparative evolutionary studies (e.g., Garland et al. 1993; Harmon et al. 2003; Butler and King 2004; Kozak et al. 2005). However, to test the robustness of our results to alternate evolutionary models, we also simulated null distributions for the mean relative disparity values expected under speciational (punctuated change) and stabilizing selection (Ornstein–Uhlenbeck) models. The Brownian motion model assumes that the trait variance among taxa increases at a constant rate that is proportional to their divergence time. Under the speciational model of diversification, the trait variance among taxa is independent of divergence time and instead is assumed to increase in proportion to the number of nodes (i.e., speciation events) separating taxa (Pagel 1997). In the stabilizing selection model, trait values are "pulled" toward a selective optimum. The rate and strength at which traits are pulled toward the selective optimum are modeled with the parameter alpha (Hansen 1997; Butler and King 2004). We estimated the maximum-likelihood value of alpha in GEIGER (Harmon et al. 2008), which we then used to model the relationship between divergence time and trait variance in all subsequent simulations.

BODY-SIZE VARIATION AND LOCAL COMMUNITY STRUCTURE

To determine whether patterns of body-size overlap among locally sympatric species parallel those observed at a regional scale, we tested whether locally co-occurring species from different clades are more or less similar in mean body size than expected by chance. We obtained data on local species composition from eight intensively studied sites across the southern Appalachians (Fig 2; Table 1). Data on species that co-occur on the same study plots were gathered during field collections by KHK (this study) and obtained from the literature (Organ 1961; Smith and Petranka 2000; Petranka and Murray 2001; Beachy and Bruce 2003; Petranka and

Table 1. Mean nearest-neighbor distances (MNND) in mean body sizes among *Desmognathus*, *Plethodon*, and spelerpine species that coexist in eight local communities in eastern North America (Appendix S4; Fig. 2). The observed MNND is the average nearest-neighbor distance in body size between each species and the species in the other clades that coexist in the same local community (i.e., *Desmognathus* vs. *Plethodon*, *Desmognathus* vs. spelerpines, *Plethodon* vs. spelerpines). The *P*-value is the proportion of simulated communities that had an MNND smaller than that observed for the real community. See Materials and Methods for a detailed description of the calculations and simulations.

Local community	Observed MNND	*P*-value
1. Nantahala Mountains, North Carolina	0.027	0.011
2. Great Smoky Mountains, North Carolina	0.038	0.031
3. Great Balsam Mountains, North Carolina	0.042	0.040
4. Craggy Mountains, North Carolina	0.019	0.005
5. Rock Creek, Tennessee	0.007	0.004
6. Bald Mountains, North Carolina	0.006	0.001
7. Whitetop Mountain, Virginia	0.021	0.005
8. Cumberland Gap, Kentucky	0.003	0.001

Smith 2005; J. W. Petranka, pers. comm.). Each of the study plots encompassed an area where all species could be encountered by a single investigator in a short walking distance (generally < 1200 m^2). Plots often contained a mixture of stream and forest microhabitats, but were found in the same general habitat type (e.g., mesic, mid-elevation forest). For the seven sites assayed by KHK or by Petranka, each site was searched by two to five investigators on at least two separate occasions between 1994 and 1997. The species composition observed for each site matches expectations based on range maps and elevational distributions (e.g., Petranka 1998), suggesting that all species likely to be present were indeed encountered. The precise location and species composition of each community is given in Appendix S4, and the localities are mapped (and mean species body sizes are summarized) in Figure 2.

We focus on communities in the southern Appalachians for three reasons. First, reconstructions of elevational distributions of species on the phylogeny suggest that all three clades originated at higher elevations in southeastern North America (i.e., the southern Appalachians; K. H. Kozak and J. J. Wiens, unpubl. data). Second, our analyses of body-size evolution suggest that the major differences in body size within each of the three clades in ENA evolved relatively early within each clade, in association with the most basal splits, and that the taxa with extreme body sizes in each clade still occur in the southern Appalachians. Third, communities in the surrounding lowlands and to the north are generally similar, but contain fewer species and a subset of the body sizes that have evolved in each clade. For example, range maps

(Petranka 1998) and our own observations suggest that most communities in ENA minimally contain a large *Plethodon* (*glutinosus* group), a small *Plethodon* (*cinereus* group), one or two large spelerpines (*Gyrinophilus* and/or *Pseudotriton*), a small spelerpine (*Eurycea bislineata* complex), and a small *Desmognathus* (e.g., *D. ochrophaeus* complex).

We used phylogenetic simulations to test whether locally coexisting species from different clades (i.e., *Desmognathus, Plethodon*, Spelerpinae) were more (or less) similar in body size than expected by chance alone. For every species in a given community, we found its nearest neighbor (i.e., the species from which it shows the smallest squared difference in body size) in each of the different clades that coexists with it. For example, for a spelerpine that is found in a community with one *Plethodon* species and one *Desmognathus* species, we recorded the nearest-neighbor distance (NND) in body size between that spelerpine species and the sympatric *Plethodon* species, and between that spelerpine species and the sympatric *Desmognathus* species. We calculated the NND for every species found in the same local community and then calculated the mean nearest-neighbor distance (MNND) for each community. To determine whether the MNND for a community was smaller or greater than expected by chance, we conducted 1000 simulations of body-size evolution along the PL chronogram for ENA. For each of the 1000 simulated datasets, we pruned out all taxa except those found in the community of interest. We then calculated the MNND for each of the simulated communities as described above. If fewer than 5% of the simulated communities had MNND values smaller or larger than the actual MNND, we considered the results to show statistically significant deviations from community assembly that was random with respect to species body sizes.

We employ this approach because we are interested in how species from different clades are assembled into local communities and to account for the nonindependence of species trait values due to shared evolutionary history. Thus, we could not apply recently developed metrics of trait variation in communities (e.g., mean pairwise trait distance among taxa [MPD] and mean distance to nearest-neighbor trait distance [MNTD] implemented by Webb et al. [2008]) because they are calculated across all co-occurring species and do not explicitly incorporate the phylogenetic relationships of species.

We note that because we used average body sizes calculated across species' geographic ranges, we cannot detect whether some size-adjustment occurs at each locality (i.e., the body size of a given species in a given community might shift due to the presence or absence of species of similar body size in the same local community). Nonetheless, our approach still allows us to address whether locally coexisting species with different mean body sizes are more similar to each other than expected by chance, given the range of body sizes that have evolved in each clade and

across ENA. Furthermore, even if there is some size adjustment in local communities, this presumably occurs within a restricted range of known adult body sizes (i.e., small species are small in every community).

Results

PHYLOGENY

Maximum-likelihood analysis of the combined data divides Plethodontidae into two major clades (designated A and B in Fig. 3). The first clade (A) contains the subfamilies Hemidactylinae (*Hemidactylium*), Bolitoglossinae, and Spelerpinae. The second clade (B) corresponds to subfamily Plethodontinae (including *Plethodon, Karsenia, Hydromantes, Ensatina, Aneides, Phaeognathus*, and *Desmognathus*). Our analysis supports the continued recognition of a subfamily Plethodontinae that includes *Hydromantes* (formerly considered a bolitoglossine) and the former subfamily Desmognathinae (*Desmognathus, Phaeognathus*). In contrast to the most recent molecular phylogenetic study of higher-level plethodontid relationships (Vieites et al. 2007), our analyses do not place *Hemidactylium* as the sister taxon to Bolitoglossinae (*Batrachoseps* + supergenus *Bolitoglossa*). Instead, we found strong support (bootstrap = 96%) for placing *Hemidactylium* as the sister taxon to a well-supported clade containing subfamilies Spelerpinae and Bolitoglossine (bootstrap = 81%). The phylogenetic position of *Hemidactylium* was poorly supported in all previous analyses of Plethodontidae (Chippindale et al. 2004; Mueller et al. 2004; Vieites et al. 2007; Wiens 2007).

Within Spelerpinae, we found strong support for a clade that groups *Gyrinophilus*, *Stereochilus*, and *Pseudotriton*; this clade in turn is the sister taxon to *Eurycea* (Fig. 3). *Pseudotriton* is the sister taxon to *Stereochilus* + *Gyrinophilus*. Within *Eurycea*, four major clades are resolved. The first contains all the endemic species from the Ozark Highlands, and is the sister taxon to the remaining species, which occur across ENA and on the Edwards Plateau of Texas. The second clade groups the *E. quadridigitata* sample from Mississippi with the 10 species from the Edwards Plateau. The third clade groups *E. longicauda*, *E. lucifuga,* and *E. guttolineata*. Finally, the fourth clade consists of the *E. bislineata* species complex (*E. aquatica, E. bislineata, E. cirrigera*, *E. junaluska*, and *E. wilderae*).

Within Plethodontinae (Fig. 3), the genus *Plethodon* is a strongly supported clade that is the sister group to a well-corroborated clade containing *Karsenia, Hydromantes, Ensatina, Aneides*, and supergenus *Desmognathus* (*Desmognathus, Phaeognathus*). However, the relationships among genera within this latter clade are generally poorly supported. Our analysis confirms recent studies of supergenus *Desmognathus* that showed that: (1) *Phaeognathus hubrichti* is the sister taxon of *Desmognathus*, (2) *D. wrighti* is the sister taxon to all other *Desmognathus*, and (3) all biphasic species exclusive of the *D. folkertsimarmoratus-quadramaculatus* clade form a monophyletic group, within which most lineages are separated by short, poorly supported branches suggesting a period of rapid lineage splitting (Titus and Larson 1996; Rissler and Taylor 2003; Kozak et al. 2005). In contrast to previous phylogenetic studies, the combined dataset resolved a strongly supported clade containing *D. ochrophaeus*, *D. orestes*, *D. welteri*, and northern populations of *D. fuscus*. This clade was only poorly supported by mtDNA data alone (Kozak et al. 2005).

BIOGEOGRAPHY

Our biogeographic results (Fig. 4) show that there are major clades of plethodontids that are restricted to ENA and to WNA, and that these clades appear to have been diversifying within each of those regions for at least the last 20 Myr (although *Hydromantes* appears to have a relatively shallow evolutionary history in WNA). We also find that plethodontid lineages from ENA have been an important source of colonizing lineages for the Central Highlands and Edwards Plateau. Specifically, the Edwards Plateau was colonized twice by lineages from ENA (once by spelerpines and once by *Plethodon*), whereas the Central Highlands have been independently colonized from ENA six times (once each by spelerpines and *Desmognathus*, and four times by *Plethodon*). However, the deeper biogeographic history of plethodontids is somewhat ambiguous (Fig. 4), and beyond the scope of this study. Phylogenetic reconstructions of biogeographic history using parsimony (not shown) showed qualitatively similar results to those obtained using maximum likelihood.

BODY-SIZE EVOLUTION

Reconstruction of body-size evolution and biogeography on the chronogram shows that the three major clades of plethodontids in ENA (*Desmognathus, Plethodon*, Spelerpinae) have each independently evolved similar body-size morphs (Fig. 4). All four body-size morphs have evolved within *Desmognathus*. *Plethodon* and spelerpines have also evolved small, large, and very-large body-size morphs in parallel. All three clades appear to have evolved these replicated patterns of body-size evolution within ENA, and over a similar period of time (30–40 Myr ago; Fig. 4). Intriguingly, in WNA the body-size differences tend to be partitioned among the major clades (Fig. 1), despite the generally similar ages in comparison to the three clades in ENA (Fig. 4).

We used relative disparity analyses to test whether the major clades of plethodontids in ENA showed greater or lesser overlap in their body-size distributions than expected by chance alone. In general, we found that each clade partitions a large amount of the body-size variation found across all species in ENA (mean relative disparity = 1.05; relative disparity of spelerpines = 1.31; relative disparity of *Desmognathus* = 1.43; relative disparity of eastern
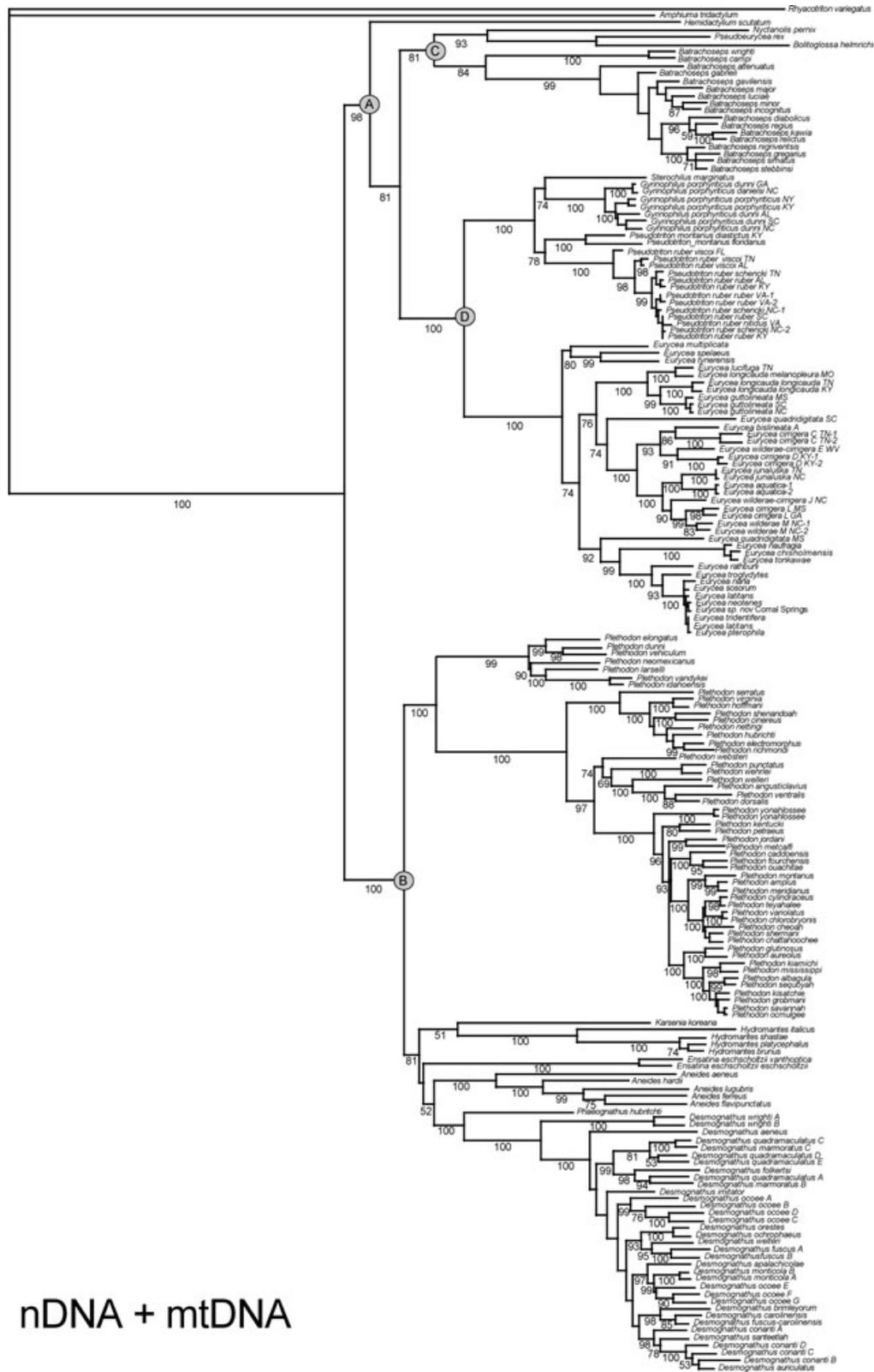
Figure 3. Maximum-likelihood phylogeny of plethodontid salamanders obtained from combined analysis of three nuclear genes and three mitochondrial genes. The tree has a ln-likelihood of −101662.414. The proportion of times each node was resolved among 100 bootstrap replicates is shown under each branch. Bootstrap proportions < 50% are not shown. Support values from some near-terminal branches within Spelerpinae have been omitted for illustrative purposes. Major clades of plethodontids are listed as follows: (A) Hemidactylinae + Bolitoglossinae + Spelerpinae; (B) Plethodontinae; (C) Bolitoglossinae; (D) Spelerpinae.
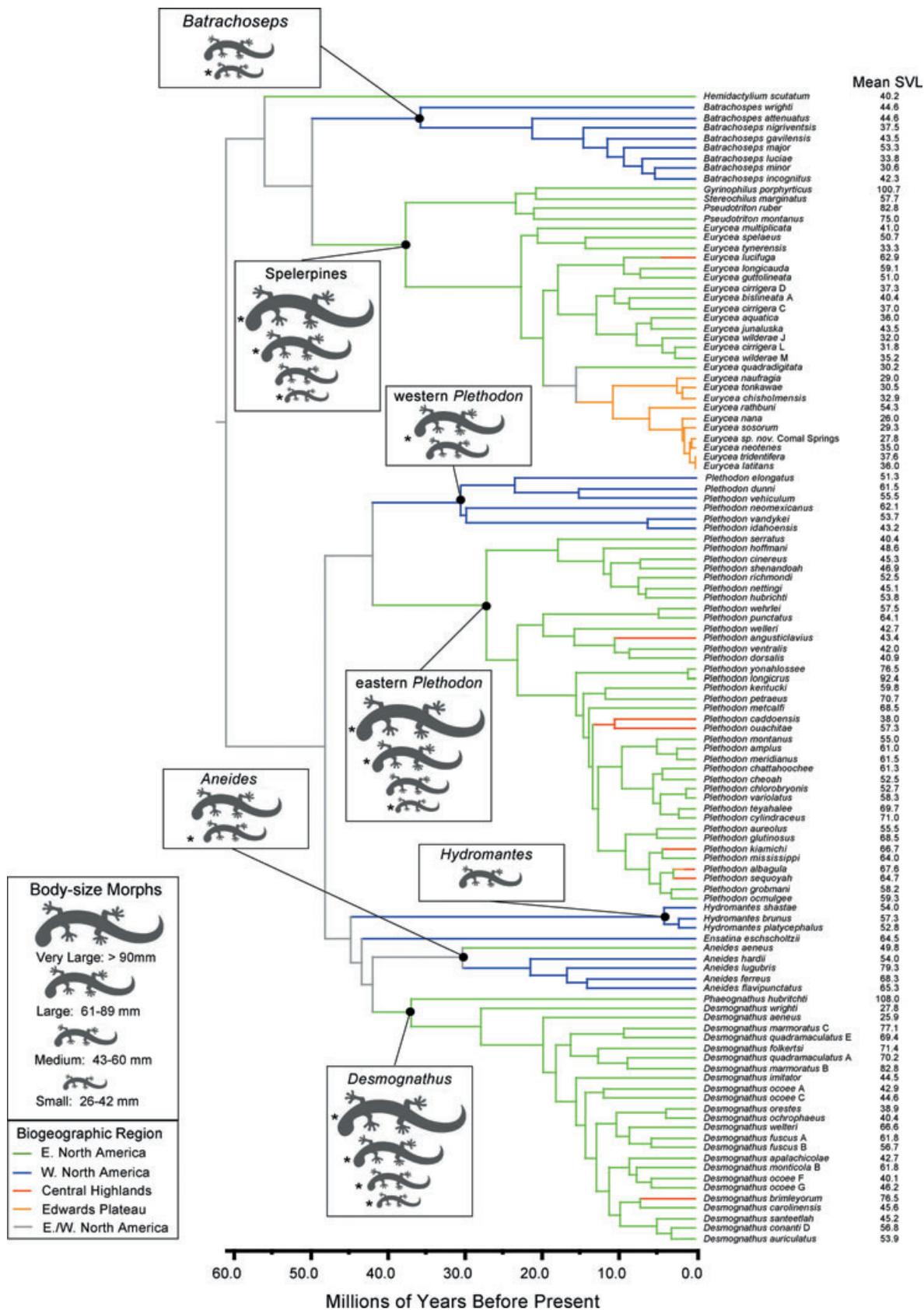
Figure 4. Summary of divergence-time estimates, biogeography, and body-size evolution for major clades of North American plethodontids. The chronogram was generated using penalized-likelihood analysis, with branch lengths from the maximum-likelihood phylogram

Plethodon = 0.45), with *Desmognathus* and spelerpines showing the most body-size variation and overlap (Fig. 1). Phylogenetic simulations suggest that observing a mean relative disparity value of 1.05 across three clades is highly unlikely under Brownian motion ($P = 0.029$), punctuated ($P = 0.001$), and stabilizing selection models of evolution ($P = 0.026$). Thus, the major clades of plethodontids have evolved body-size distributions that overlap more extensively than expected under a wide range of diversification models. Together, these results strongly support a model of parallel diversification.

BODY-SIZE VARIATION AND LOCAL COMMUNITY STRUCTURE

Patterns of body-size overlap among locally coexisting *Desmognathus*, *Plethodon*, and spelerpine species mirror those observed at the clade level. In most communities, the different clades also show similarly broad ranges in mean body size (Fig. 2). Furthermore, phylogeny-based simulations show that the MNND (for body size) for each local community is smaller than expected (Table 1). Thus, locally coexisting assemblages of *Desmognathus*, *Plethodon*, and spelerpines tend to be comprised of species that are more similar than expected based on a null model in which communities are randomly assembled with respect to body size.

Discussion

A classic paradigm in evolutionary biology is that given similar selective regimes, geographically isolated lineages will diversify to create similar sets of phenotypes in different locations. Examples include the repeated evolution of benthic and limnetic species of sticklebacks in different lakes in northwestern North America (Schluter and McPhail 1992), of *Anolis* and treefrog ecomorphs on different islands in the Greater Antilles (Losos et al. 1998; Moen and Wiens 2009), and of snake-like ecomorphs of squamates in different continental regions (Wiens et al. 2006a). It is assumed that these parallel radiations do not happen in sympatry because once a given clade evolves a range of phenotypes that allows them to utilize a set of resources along a given niche axis, the other clade would be prevented from evolving a similar range

of phenotypes (even if the clades are not ecologically identical on every possible niche axis).

Our results from plethodontid salamanders in ENA provide an intriguing counterexample to this paradigm of preemptive evolution. We find that there has been repeated evolution of similar body-size morphs in three clades (*Desmognathus*, eastern *Plethodon*, and spelerpines) that are broadly sympatric at both regional and local scales. Our phylogenetic simulations of body-size evolution suggest that these clades are more similar than expected under a wide range of models of trait evolution. We also find that local assemblages of *Desmognathus*, eastern *Plethodon*, and spelerpines tend to be comprised of species that are more similar in body size than expected by chance. Furthermore, this pattern of diversification and community assembly in ENA contrasts with that found in WNA, where clades of plethodontids exhibit the expected pattern of largely nonoverlapping body-size distributions (Fig. 1), despite the similar amount of time available for within-clade diversification (Fig. 4). Together, these findings suggest that natural selection has driven the major clades of plethodontids in ENA into overlapping portions of body-size space.

The pattern of replicated diversification that we document here is particularly interesting given the strong relationships among body size, resource use, and ecological interactions between species within each of the major plethodontid clades in ENA. Plethodontid salamanders are generalist predators (mostly on invertebrates; Petranka 1998) in which body size strongly influences resource use through the size of the prey that can be consumed (e.g., Burton 1976; Krzysik 1979; Lynch 1985; Petranka 1998; Adams and Rohlf 2000). Previous ecological studies suggest that species of similar size within the same major clade compete for food and/or cover objects (Kryzsik 1979; Keen 1982; Hairston 1986; Southerland 1986a,b; Roudebush and Taylor 1987; Adams and Rohlf 2000). Furthermore, recent analyses of plethodontid community structure in ENA suggest that species of similar body size (within a given clade) tend not to occur in sympatry because of competitive interactions and such interactions drive divergence in body size. For example, an extensive analysis of *Plethodon* communities in ENA showed that *Plethodon* species of different size classes co-occur more frequently than expected

from the combined-data analysis (Fig. 2). Results were qualitatively unaffected by assuming different root ages for plethodontids. For brevity, we therefore present the results of analyses that employed a root age of 61 Myr for Plethodontidae. Branch colors correspond to the generalized geographic ranges of species and clades reconstructed using maximum likelihood. Green, blue, red, or yellow branches indicate that a branch receives significant support for the corresponding biogeographic region using a likelihood-ratio test. Gray branches are ambiguous (but were inferred to have ancestral distribution of either ENA or WNA). Body-size morphs (based on snout-vent length) contained in each of the six major clades are shown. Asterisks denote body-size morphs that have evolved within each clade, based on reconstructions of body-size evolution on the chronogram (but note that these arbitrary morph categories were not used in any statistical analyses). Note that species that do not occur in North America (i.e., tropical bolitoglossines) have been excluded for illustrative purposes but are shown in Figure 2.

by chance, suggesting that congeners of similar size are unable to coexist because of competition (Adams 2007). Similarly, within *Desmognathus*, competition and intraguild predation seem to have driven body-size diversification, patterns of microhabitat use, and the assembly of communities comprised of different-sized species (Hairston 1986; Kozak et al. 2005). Although less is known about the relationship between body-size variation and ecological interactions among species of spelerpines, the available data suggest that body-size differences also mitigate competitive and predatory interactions (Beachy 1994, 1997; Bruce 2003).

We note that the high degree of local sympatry among the major clades of plethodontids (especially *Desmognathus* and spelerpines) does not preclude the possibility that these clades evolved overlapping ranges of body sizes in allopatry in different subregions within ENA and became sympatric only later. Under this scenario, the evolution of a large species in one subregion could not constrain the evolution of a large species in another clade in another subregion, even though these clades are sympatric now. Distinguishing between sympatric diversification and allopatric diversification followed by secondary sympatry is extremely difficult given that these clades began radiating at least 30–40 Myr ago. Nevertheless, there is some evidence that supports a hypothesis of sympatric diversification in the southern Appalachians. Many of the species with extreme body sizes in each clade are endemic to, or at least co-occur, in the southern Appalachians (e.g., *Desmognathus quadramaculatus*, and *D. wrighti*; *P. cinereus* and *P. yonahlossee*; *E. wilderae* and *Gyrinophilus porphyriticus* [Bruce 1991; Petranka 1998; Duellman and Sweet 1999]), and these extreme body sizes seemingly evolved early in the history of each clade (Fig. 4). All three clades have their highest species richness in the southern Appalachians (Duellman and Sweet 1999), suggesting long occupancy of this region. Ancestral reconstructions of elevational ranges also suggest that all three clades in ENA arose in montane regions (i.e., the Appalachians; K. H. Kozak and J. Wiens, unpubl. data). Finally, many codistributed species from each of these clades show deep phylogeographic structuring across the southern Appalachians (Crespi et al. 2003; Kozak et al. 2005, 2006b; Sites et al. 2004; Weisrock et al. 2005; Weisrock and Larson 2006), suggesting a long history of association among clades that have evolved similar ranges of body size. But even if these clades evolved their body-size distributions in allopatry and later became sympatric, the pattern of sympatry between clades is still counterintuitive because species within each clade would have to invade habitats in which ecologically and phenotypically similar species from a different clade already occurred (e.g., as in the southern Appalachians; Petranka 1998).

Given that ecological interactions seemingly promote body-size divergence and limit the coexistence of similar-sized species within clades, why are sympatric species of *Desmognathus*, eastern *Plethodon*, and spelerpines more similar in body size than expected by chance? A straightforward and intuitively appealing explanation for the parallel diversification of these sympatric clades is that divergence in other ecologially relevant traits (apart from body size) allowed the evolution and coexistence of sympatric species with similar body size from different clades. For example, most *Desmognathus* and spelerpine species rely on aquatic sites for reproduction and larval development and tend to occur adjacent to streams and springs (Petranka 1998). Therefore species of these clades might not interact as extensively with species of *Plethodon*, which lack an aquatic larval stage and are fully terrestrial (but see Grover and Wilbur 2002). If so, there may have been limited opportunity for *Desmognathus* or spelerpines to constrain the range of body sizes that could evolve in sympatric lineages of *Plethodon* (and for *Plethodon* to constrain the diversification of *Desmognathus* and spelerpines).

However, clade-specific differences in microhabitat use alone cannot explain how parallel diversification occurred in all three clades without any constraints from preemptive evolution. First, similar-sized species of *Desmognathus* and spelerpines exhibit extensive microsympatry in and around streams, springs, and headwater seepage areas across the southern Appalachian Mountains (Hairston 1949; Petranka et al. 1993; Smith and Petranka 2000; Ford et al. 2002; Petranka and Smith 2005), which raises the question of why one clade did not preempt the body-size diversification of the other. Second, in further contrast to the expectations of the hypothesis of preemptive evolution, it is these two clades that occur in the most similar microhabitats that actually show the strongest pattern of body-size overlap (see Results). Third, even the most aquatic *Desmognathus* (*D. quadramaculatus*) and spelerpines (*G. porphyriticus*) often use terrestrial microhabitats and consume terrestrial prey, including syntopic species of *Plethodon* (Bishop 1941; Hairston 1949; Bruce 1972; Burton 1976; Formanowicz and Brodie 1993; Beachy 1994; Hairston 1996; Beachy 1997; reviewed in Bruce 2007). Thus, despite some microhabitat differences, it is clear that all three clades are syntopic. Of course, divergence along other niche axes could still be important in facilitating parallel diversification in these sympatric clades, but there do not seem to be obvious ecological differences between these clades that would strongly support this hypothesis. For example, despite impressive differences in some aspects of feeding mechanics between these three clades (e.g., Lombard and Wake 1977), there do not appear to be clade-specific differences in diet, and diet instead seems to be determined primarily by body size (Petranka 1998).

Ecological theory (both young and old) does suggest a scenario whereby the pattern of parallel diversification in sympatry might actually be expected. According to the ecological theory of adaptive radiation (Schluter 2000), ecological interactions are expected to drive phenotypic divergence during the early stages of

diversification and community assembly, when species richness is low and there are many "open" niches to invade. But as species richness increases over time and species increasingly subdivide available ecological space, other theory predicts that the adaptive landscape will shift such that ecological interactions can actually promote the evolution and coexistence of species with similar ecologically relevant phenotypes. For example, MacArthur and Levins (1967) showed that when species are closely packed in ecological space, it may be easier for a species to invade a community if it has a phenotype that is very similar to that of a resident species, rather than being phenotypically intermediate between two resident species. Although this prediction may seem counterintuitive, an invader with a phenotype that is intermediate between two resident species may overlap in its resource requirements with both species, but not be as good a competitor as either, and may thus be more likely to be competitively excluded from the community. In contrast, if the invader is very phenotypically similar to an existing species in the community, this very similarity may prevent it from quickly being outcompeted by a resident species (because heterospecific competition will be similar in strength to intraspecific competition). Of course, over time, one species should ultimately exclude the other (MacArthur and Levins 1967). However, recently developed models (e.g., Scheffer and van Nes 2006) suggest that such competitive exclusion may take place over evolutionary timescales (i.e., thousands of generations). Most importantly, these models predict the assembly of communities that contain separate "clumps" of ecologically and phenotypically similar species (Scheffer and van Nes 2006), similar to what we find in plethodontids in ENA. We do not claim that this scenario necessarily explains what occurred in plethodontids in ENA, but we emphasize that there is theory that can potentially explain these seemingly counterintuive patterns, and without invoking ecological differences in other traits besides body size.

To date, most work on the replicated diversification of clades has focused on allopatric radiations that are separated on different islands, continents, or bodies of water. The replicated diversification we report here for plethodontids in ENA, along with a growing number of theoretical (e.g., Abrams and Matsuda 1994; Leibold 1998; Abrams 2000; Scheffer and van Nes 2006) and empirical studies (e.g., Knouft 2003; Cavender-Bares et al. 2004; Stoks and McPeek 2006; Matocq and Murphy 2007) suggest that parallel evolution of ecologically important traits might also occur in sympatric clades. Thus, it is possible that the pattern of parallel diversification that we document here might be a common feature of sympatric clades. Given this, understanding how replicated diversification can occur in sympatry (i.e., without one clade constraining the evolution of another) will be an important challenge to future workers in evolution and ecology. Key questions to address in future studies include: (1) whether clades are truly sympatric when similar phenotypes evolve, (2) why similar species in different clades coexist without competitive exclusion, especially when competition seems to limit coexistence of similar species within clades, and (3) to what extent evolution on multiple niche axes facilitates replicated diversification in sympatry.

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Supporting Information

The following supporting information is available for this article:

Appendix S1. Taxon and gene sampling for phylogenetic analysis of plethodontid salamanders.

Appendix S2. (A) Maximum-likelihood phylogram obtained from the combined analysis of the three nuclear genes (RAG-1, BDNF, and POMC); the tree has a ln-likelihood of -17993.182957. (B) Maximum-likelihood pylogram obtained from the combined analysis of the three mitochondrial genes (cyt-b, ND2, and ND4); the tree has a ln-likelihood of -90034.32079.

Appendix S3. Mean adult body-sizes (SVL) and sample sizes (*n*) for all species included in the morphological analyses.

Appendix S4. Geographic coordinates and species composition of the eight local communities included in the analyses of body-size variation and community structure.

Supporting Information may be found in the online version of this article.

(This link will take you to the article abstract).

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