Evolution, 59(9), 2005, pp. 2000–2016

PHYLOGENETIC ANALYSIS OF ECOMORPHOLOGICAL DIVERGENCE, COMMUNITY STRUCTURE, AND DIVERSIFICATION RATES IN DUSKY SALAMANDERS (PLETHODONTIDAE: *DESMOGNATHUS*)

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Abstract.—An important dimension of adaptive radiation is the degree to which diversification rates fluctuate or remain constant through time. Focusing on plethodontid salamanders of the genus *Desmognathus*, we present a novel synthetic analysis of phylogeographic history, rates of ecomorphological evolution and species accumulation, and community assembly in an adaptive radiation. Dusky salamanders are highly variable in life history, body size, and ecology, with many endemic lineages in the southern Appalachian Highlands of eastern North America. Our results show that life-history evolution had important consequences for the buildup of plethodontid-salamander species richness and phenotypic disparity in eastern North America, a global hot spot of salamander biodiversity. The origin of *Desmognathus* species with aquatic larvae was followed by a high rate of lineage accumulation, which then gradually decreased toward the present time. The peak period of lineage accumulation in the group coincides with evolutionary partitioning of lineages with aquatic larvae into seepage, stream-edge, and stream microhabitats. Phylogenetic simulations demonstrate a strong correlation between morphology and microhabitat ecology independent of phylogenetic effects and suggest that ecomorphological changes are concentrated early in the radiation of *Desmognathus*. Deep phylogeographic fragmentation within many codistributed ecomorph clades suggests long-term persistence of ecomorphological features and stability of endemic lineages and communities through multiple climatic cycles. Phylogenetic analyses of community structure show that ecomorphological divergence promotes the coexistence of lineages and that repeated, independent evolution of microhabitat-associated ecomorphs has a limited role in the evolutionary assembly of *Desmognathus* communities. Comparing and contrasting our results to other adaptive radiations having different biogeographic histories, our results suggest that rates of diversification during adaptive radiation are intimately linked to the degree to which community structure persists over evolutionary time.

Key words.—Adaptive radiation, Appalachian Highlands, community ecology, comparative method, North America, phylogeography, speciation.

Received January 12, 2005. Accepted July 3, 2005.

Adaptive radiations, clades that exhibit rapid divergence into an exceptional variety of adaptively disparate forms (Schluter 2000; Losos and Miles 2002), have served for over a century as model systems for investigating the processes that contribute to the origin and maintenance of biodiversity (Osborne 1902; Simpson 1953; Schluter 2000). Classic models of adaptive radiation link the buildup and maintenance of species richness with the evolution of phenotypic disparity. For example, evolution of key traits and/or colonization of competitor-free environments appear to promote diversification by giving lineages access to new adaptive zones (Simpson 1953; Schluter 2000). Similarly, ecomorphological divergence may influence diversification, long-term persistence, and coexistence of lineages by reducing competitive interactions (Barraclough et al. 1999).

An important, but less-studied dimension of adaptive radiation is to what degree diversification rates fluctuate or remain constant through time. Both paleontological and neontological studies of adaptive radiation suggest that the accumulation of lineages and ecomorphological disparity may be intimately linked to the persistence of species associations over evolutionary time. For example, rates of diversification may decline in taxa whose lineages show extensive long-term coexistence because ecological interactions restrict opportunities for diversification once available geographical and niche spaces are occupied (Stanley 1973; Schluter 2000; Harmon et al. 2003). A thorough understanding of the processes that influence diversification rates therefore requires simultaneous consideration of morphological evolution, species accumulation, phylogeographic history, and community structure. Recent studies have explored one or a few of these aspects of adaptive radiation; however, simultaneous analysis of the relationship between diversification rates, phylogeographic history, and community assembly is a new approach. Here we examine the temporal and geographical accumulation of species richness and ecomorphological disparity in a widespread and ecologically diverse clade of eastern North American plethodontid salamanders, genus *Desmognathus*.

In the southern Appalachian Highlands and adjacent physiographic provinces of eastern North America, plethodontid salamanders have undergone extensive evolutionary diversification. The southern Appalachian plethodontid fauna combines high species richness with great disparity in habitat use and life history. Species occupy fully aquatic to terrestrial habitats and exhibit developmental strategies ranging from long aquatic larval periods to direct development (Petranka 1998). The highlands of Central America represent another global hot spot of salamander species richness; however, diversity patterns in this region differ in two important respects. First, although regional species richness is greater in Central America (168 vs. 57 species; Campbell 1999; Duellman and

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TABLE 1. Microhabitat, length of aquatic larval period, and geographic distribution of the 18 formally recognized species of *Desmognathus* included this study. Microhabitat categories follow Titus and Larson (1996). Larval periods are from Bernardo (2000), Camp et al. (2000), and Tilley and Bernardo (1993). Geographic distribution follows Duellman and Sweet (1999).

Species	Microhabitat	Larval period (months)	Geographic distribution
D. aeneus	terrestrial	direct development	Southern Appalachians, Piedmont
D. apalachicolae	seepage	9	Gulf Coastal Plain
D. auriculatus	stream edge	12	Southern Atlantic Coastal Plain, Gulf Coastal Plain, Mississippi Embayment Pine, Woodlands
D. brimleyorum	stream edge	12	Interior Highlands
D. carolinensis	seepage	9–10	Southern Appalachians
D. conanti	stream edge	12	Interior Lowlands, Alleghany Plateau, Southern Appalachians, Piedmont
D. folkertsi	stream	34	Southern Appalachians
D. fuscus	stream edge	8–12	Interior Lowlands, Alleghany Plateau, Northern Appalachians, Southern Appalachians, Northern Atlantic Coastal Plain
D. imitator	seepage	10	Southern Appalachians
D. marmoratus	stream	35	Southern Appalachians
D. monticola	stream edge	9.5–12.5	Alleghany Plateau, Southern Appalachians, Piedmont, Gulf Coastal Plain
D. ochrophaeus	seepage	8	Alleghany Plateau, Northern Appalachians, Piedmont
D. ocoee	seepage	9–10	Southern Appalachians
D. orestes	seepage	8–9	Southern Appalachians
D. quadramaculatus	stream	35–48	Southern Appalachians
D. santeetlah	stream edge	12	Southern Appalachians
D. welteri	stream edge	24	Alleghany Plateau, Southern Appalachians
D. wrighti	terrestrial	direct development	Southern Appalachians

Sweet 1999), fewer species coexist locally than in the southern Appalachians (maximum of 11 species vs. 18 species; Duellman and Sweet 1999; García-París et al. 2000). Second, tropical plethodontids lack aquatic larval periods and subdivide arboreal and terrestrial adaptive zones (Wake and Lynch 1976; García-París et al. 2000), except for a single stream-associated species now considered extinct (Wake and Campbell 2001).

Desmognathus is a distinctive element of the eastern North American plethodontid fauna. In contrast to most plethodontid genera (Larson 1984), this clade of 19 recognized species exhibits a wide range of variation in body size, life history, and microhabitat use. Up to seven species of *Desmognathus* occur in local sympatry in the southern Appalachians (Bruce 1991), where species partition microhabitats (Hairston 1949; Organ 1961; Tilley 1968) along an aquatic-to-terrestrial microhabitat gradient (Table 1). The largest species have long aquatic larval periods (two to four years) and occupy the channels of streams with nearly permanent flow regimes, whereas the smallest species have direct development and occupy terrestrial habitats. Species with intermediate body sizes and larval periods ($\leq$1 year) inhabit stream edges and interstream seepage areas. The ecological basis of local community structure has been studied extensively; both predation and competition among congeners maintain an association between body size and the spatial position that a species occupies along the stream-forest ecotone (Krzysik 1979; Keen 1982; Keen and Sharp 1984; Hairston 1986, 1987; Southerland 1986a,b; Roudebush and Taylor 1987).

The relationship between cladogenesis, ecomorphological divergence, and dusky-salamander community assembly is poorly understood. Evolutionary transitions in ecomorphology might be accomplished easily by changes in length of the larval period and age at maturity (Bruce 1996; Camp et al. 2000). If so, ecomorphs may have evolved repeatedly as lineages diversified to form local communities in which ecological interactions among species generated selective regimes promoting subdivision of the stream-forest ecotone (Tilley and Bernardo 1993). Alternatively, some evidence suggests that cladogenesis and ecomorphological divergence are concentrated early in the history of the *Desmognathus* radiation and that communities have formed by spatial overlapping of stable, ecomorphologically disparate lineages (Titus and Larson 1996). These alternative hypotheses of evolutionary radiation have important implications for understanding the role of phylogenetic history in *Desmognathus* community assembly. This first hypothesis predicts recurring in situ evolution of ecomorphological divergence between codistributed lineages, whereas the second hypothesis predicts a strong phylogenetic inertia for ecomorphological characteristics and community structure (Webb et al. 2002).

Distinguishing these hypotheses of evolutionary diversification and community assembly requires a phylogenetic analysis that includes comprehensive geographic sampling of genetic and morphological variation across the range of the group. We present the first combined analysis of phylogeographic history, morphometric evolution, and community assembly in an adaptive radiation. This integrative approach permits us to examine (1) constancy versus fluctuation in accumulation of lineages and ecomorphological disparity throughout the phylogenetic history of dusky salamanders, and (2) the relationship between phylogeny, ecomorphological divergence, and coexistence of lineages.

MATERIALS AND METHODS

Phylogenetic Analyses

We sampled 113 specimens representing 18 formally recognized species of *Desmognathus* and its sister taxon,

Phaeognathus (Figure 1; Appendix 1, available online only at http://dx.doi.org/10.1554/05-024.1.s1). *Aneides flavipunctatus* and *Eurycea wilderae* were used as outgroups.

Genomic DNA was extracted from liver using the Qiagen (Valencia, CA) QIAamp tissue kit. Amplification of genomic DNA was conducted using primers L4437 (5′-AAGCTTTC GGGCCCATACC-3′) and H6159 (5′-GCTATGTCTGGGG CTCCAATTA-3′) (Macey et al. 1997; Weisrock et al. 2001) with denaturation at 94°C for 35 sec, annealing at 50°C for 35 sec, and extension at 70°C for 150 sec with 4 sec added to the extension time per cycle, for 30 cycles. Negative controls were run for all amplifications. Amplified products were purified with Qiagen gel-extraction kits. Primers L4882b (5′-TGACAAAAAATTGCNCC-3′) and H5617a (5′-AAAATR TCTGRGTTGCATTCAG-3′) were used as internal sequencing primers (Macey et al. 1997, 2000; Weisrock et al. 2001). Sequencing reactions were run using Big-Dye Terminator Ready-Reaction Kits (Perkin Elmer, Wellesley, MA) with an initial denaturation at 96°C for 2 min, then denaturation at 96°C for 15 sec, annealing at 50°C for 1 sec, and extension at 60°C for 4 min for 40 cycles. All sequences were then visualized on an ABITM 373A automated sequencer (PE Applied Biosystems, Foster City, CA).

Sequence data collected in this study included the genes encoding ND2 (NADH dehydrogenase subunit 2), $tRNA^{Trp}$, $tRNA^{Ala}$, $tRNA^{Asn}$, $tRNA^{Cys}$, and $tRNA^{Tyr}$, the origin for light-strand replication (O_L), and 154 base pairs of the CO1 (cytochrome oxidase subunit 1) gene. Sequences were aligned manually. Sequences encoding ND2 and CO1 were translated to amino acids analytically using MacClade (Maddison and Maddison 1992) to verify alignments and to check for premature stop codons. The five tRNA sequences were aligned manually based on models of secondary structure (Macey and Verma 1997).

Phylogenetic trees were estimated using parsimony and Bayesian methods. PAUP* 4.0b10 (Swofford 2002) was used to reconstruct phylogenetic relationships among haplotypes using maximum parsimony and 100 heuristic searches with random addition of sequences and tree bisection-reconnection branch swapping. The nonparametric bootstrap was conducted using 500 bootstrap pseudoreplicates with 10 random taxon-addition-sequence replicates per bootstrap pseudoreplicate. Decay indices (Bremer 1994) were calculated for all nodes using Treerot version 2a (Sorenson 1999). Following selection of the GTR + I + Γ model using MrModelTest 1.1b (http://www.ebc.uu.se/systzoo/staff/nylander.html), Bayesian phylogenetic analyses were implemented in MrBayes 3.01 (Huelsenbeck and Ronquist 2001). Flat priors were used for all parameter estimates, and random trees were used to begin each Markov chain. Four incrementally heated Markov chains were run for 2×10^6 generations, sampling every 2000 generations. To ensure that the Markov chains reached a stable equilibrium, ln-likelihood values for sampling points were plotted against generation time. All samples prior to the point at which ln-likelihood values achieved stationarity were discarded as burn-in; remaining samples estimated the posterior-probability distributions for the tree topology, branch lengths, and substitution parameters. To ensure that analyses were not trapped on local optima, five replicate searches were conducted; independent analyses were considered to have converged if their ln-likelihood scores approached similar mean values (Huelsenbeck and Bollback 2001).

We imported the post-burn-in sample of topologies from our Bayesian analysis into PAUP* and used the tree-filter option to count the number of topologies consistent with the monophyly of stream, stream-edge, seepage, or terrestrial haplotypes. Each microhabitat group was tested separately. The proportion of trees in which haplotypes from a given microhabitat category are monophyletic corresponds to the Bayesian posterior probability of that hypothesis. A hypothesis is rejected if its posterior probability is ≤ 0.05.

To test whether lineages accumulated nucleotide substitutions at significantly different rates we conducted relative-rate tests as implemented in the program RRTree (Robinson-Rechavi and Huchon 2000).

Delimitation of Independent Evolutionary Lineages

Our sampling of *Desmognathus* includes all recognized species except a single geographically restricted species described too recently to be included (Anderson and Tilley 2003); thus, our taxon sampling is nearly comprehensive at the level of described species. However, phylogeographic structure within recognized species suggests that phylogenetically and geographically distinct lineages that would qualify as species under the phylogenetic or evolutionary species concepts (Mayden 1997; de Queiroz 1998) are underestimated by current taxonomy.

To delineate boundaries of putatively independent evolutionary lineages, we employed the tree-based method of Wiens and Penkrot (2002), which uses geographic patterns of coalescence among DNA haplotypes to test for gene exchange among populations of a focal species and one or more closely related species. Two sampling-design components are required: inclusion of as many species as possible to test for exclusivity (monophyletic grouping of haplotypes within a population lineage) of the focal-species haplotypes, and inclusion of at least two individuals from as many localities as possible to evaluate gene flow among populations. Discordance between the phylogenetic relationships among haplotypes and the geographic areas from which they are sampled is interpreted as evidence for gene flow among populations of the focal species (Slatkin and Maddison 1989). Well-supported groupings of haplotype clades that are concordant with geography delimit independent populational lineages concealed by previous taxonomy. Inclusion of as many species as possible that are closely related to the focal species is crucial, because a focal species may be nonexclusive for haplotypic variation and may comprise multiple cryptic lineages that are not each other's closest relatives (e.g., Sinclair et al. 2004).

Application of this tree-based approach to mtDNA phylogenies has an important strength for delimiting independent evolutionary lineages. Mitochondrial genes have an effective population size one quarter that of nuclear genes. Thus, species become reciprocally monophyletic for mtDNA haplotypes more quickly than for those of neutral nuclear genes (Moore 1995; but see Hoelzer 1997). However, for species in which gene flow among populations is restricted by geographic distance (isolation by distance), haplotypes from pop-

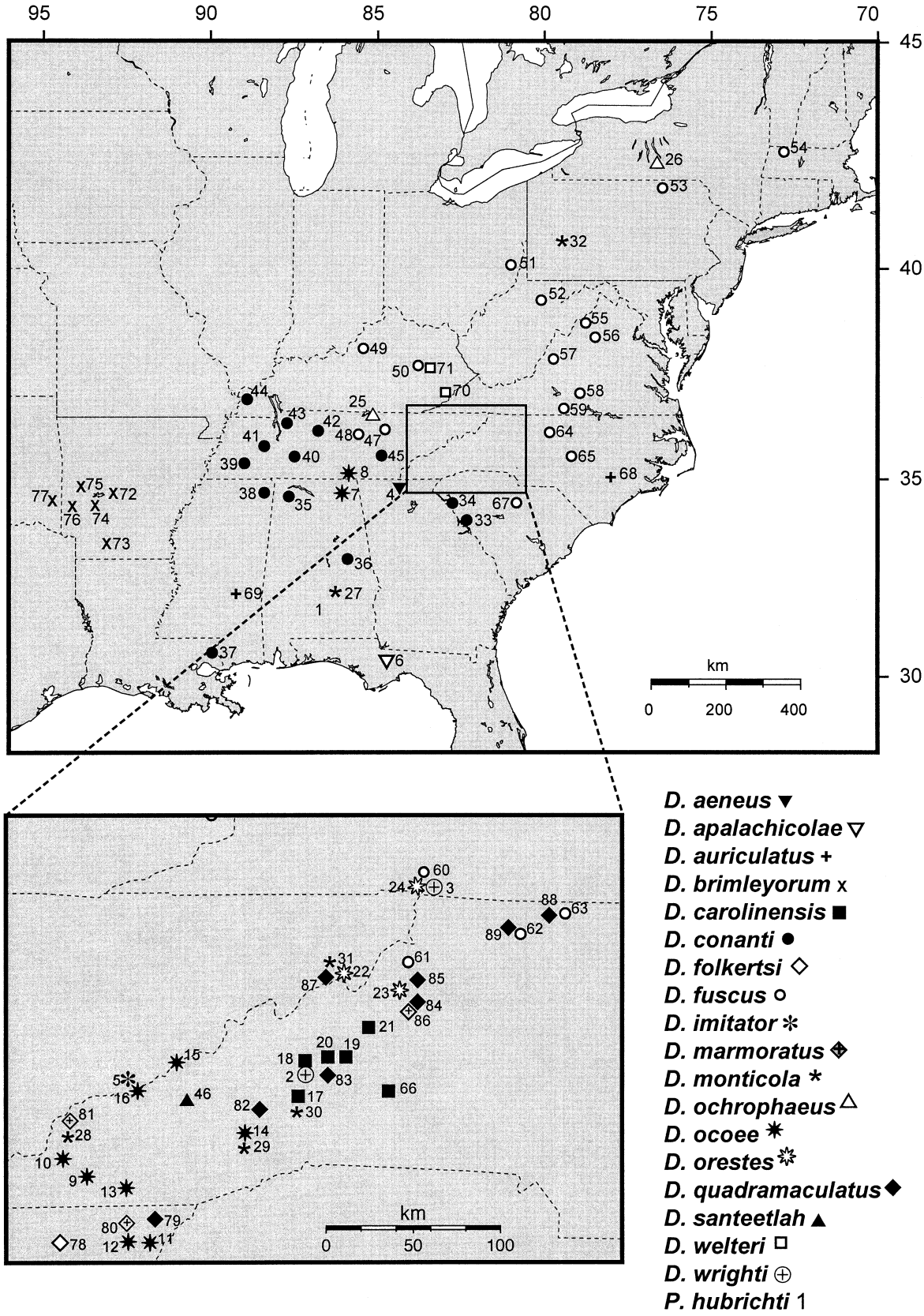

FIG. 1. Geographic sampling locations for the *Desmognathus* populations included in our phylogenetic study. Detailed locality information is listed in Appendix 1 (available online).

ulations may appear exclusive when sampling is sparse relative to the dispersal abilities of the species (Hedin and Wood 2002; Irwin 2002; Templeton 2004). In these cases, failure to detect gene flow among populations will cause overresolution of species limits. Mitochondrial DNA gene trees and species trees may also be discordant as a result of introgression or lateral transfer mediated by interspecific hybridization (Tegelström 1987; Good et al. 2003; Weisrock et al. 2005). Another potential problem is that mtDNA gene trees and species trees may be discordant because of differences in male and female-mediated gene flow (e.g., Jockusch and Wake 2002). We therefore conducted our lineage-accumulation rate analyses under minimum and maximum estimates of the number of putatively independent evolutionary lineages. Because concordance of species limits inferred from unlinked markers often provides the strongest evidence of species status (Avise and Ball 1990; Sites and Crandall 1997; Wiens and Penkrot 2002), we also examined whether independent evolutionary lineages inferred from the mtDNA data are confirmed by published morphological and nuclear-encoded allozyme data.

Estimates of Lineage-Accumulation Rates

To test whether lineage-accumulation rates (speciation minus extinction) remained constant, we transformed the Bayesian consensus phylogram using penalized likelihood (Sanderson 2002) and plotted the natural logarithm of the number of lineages against the branch-length distance from the root node, with the total tree depth scaled to a value of 1.0. We tested for a significant departure from the null hypothesis of a constant rate of lineage accumulation by analyzing the distribution of relative divergence times among species using survivorship models described by Paradis (1997, 1998). DIVERSI version 0.20.0 (Paradis 2000) was used to test three alternative models of lineage accumulation in a maximum-likelihood framework. Model A specifies a constant rate of lineage accumulation, δ, and represents the null hypothesis of no heterogeneity in rates through time. Model B specifies a gradual change in the rate of lineage accumulation through time by estimating an additional parameter, β. Values of $\beta > 1$ indicate that δ has decreased through time; values of $\beta < 1$ indicate that δ has increased through time. Model C specifies two different rates of lineage accumulation before (δ_1) and after (δ_2) a specified break point in time. Because model A is nested within models B and C, a likelihood-ratio test is used to ask whether the data reject the constant-rates model in favor of an alternative in which the rate of lineage accumulation changes over time. In the case of models B and C, which are not nested, the Akaike information criterion (AIC; Akaike 1973) is used to ask whether one model is significantly better than the other. The model with the lowest AIC is the one that best describes temporal variation in relative divergence times.

An interpretation that lineage-accumulation rates represent instantaneous rates of diversification at past times requires that speciation and extinction occur equally among lineages during a given time interval, and that all extant species have been sampled. We used the relative-cladogenesis statistic (Purvis et al. 1995; Rambaut et al. 1997) to test the assumption of equal speciation and extinction among lineages. Incomplete taxon sampling at the species level tends to underestimate the number of nodes toward the present and may cause an apparent slowdown in the diversification rate (Nee et al. 1992; Pybus and Harvey 2000). Our use of geographic patterns of haplotype coalescence to discriminate tokogenetic (within species) and hierarchical branching patterns (between species) in mtDNA phylogenies permitted a rigorous delimitation of putatively independent population-level lineages included in our analyses.

Comparative Analyses

We measured 16 morphological variables of the head, trunk, limbs, and tail, from 300 adult salamanders representing 24 species/geographic lineages of *Desmognathus*. We entered the ln-transformed morphological variables into principal component analyses using the covariance matrix. To test whether lineages could be discriminated in morphological space according to a priori categories of microhabitat use (Table 1), we conducted a multivariate analysis of variance (MANOVA). For each lineage we used the mean factor scores along each principal component axis as dependent variables and microhabitat category as the treatment effect. Lineages showed highly significant morphological differences based on their microhabitat use (Wilks' $\lambda = 0.0000151$, $F_{48,9.7167} = 14.9250$, $P < 0.0007$). Discriminant-function analysis assigned all lineages to their designated categories of microhabitat use except for *D. brimleyorum*, which was assigned to the stream microhabitat category. The range of this stream-edge species is geographically disjunct from all other desmognathines, suggesting that its large body size may reflect release from competition with other congeners. Exclusion of this species does not alter the ability of the analyses to discriminate all the categories of microhabitat use.

Because overall body size (measured as the distance in millimeters from the tip of the snout to the posterior end of the cloaca) explained 94% of the morphological variation (Fig. 2), we used ln-transformed snout-vent length (ln-SVL; Table 2) as a proxy for overall morphological divergence in all subsequent analyses. The mean ln-SVL for each lineage was used because it is not possible to incorporate variance around the mean values with current comparative phylogenetic methods.

Phylogenetic tests of ecomorphological divergence

Because lineages share parts of their evolutionary histories, they cannot be considered independent datapoints; therefore, comparative methods must correct for phylogenetic nonindependence in statistical analyses (Felsenstein 1985). Due to a lack of morphological data for some lineages, it was not possible to include all the lineages in the lineage-accumulation rate analyses; however, because all missing lineages are morphologically cryptic forms, our sampling captures the major trends of morphological evolution in *Desmognathus*. We used phylogenetic analyses of variance (Garland et al. 1993) to test the hypothesis that morphological divergence among 24 lineages is correlated with microhabitat use independent of phylogenetic effects (Glor et al. 2003). For these analyses, we simulated body-size evolution under a gradual

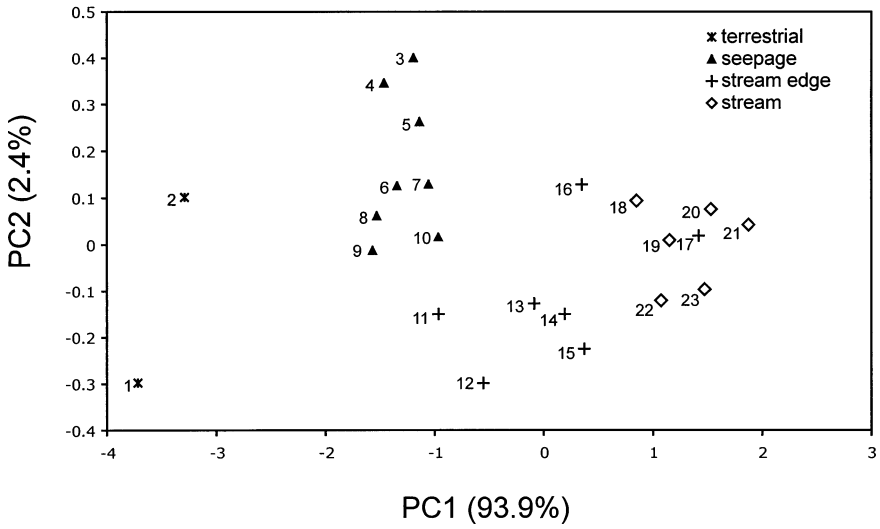

FIG. 2. Scatterplot of principal component axes 1 and 2. Shapes indicate groups of microhabitat use. Mean principal component scores for each species/geographic lineage in Table 2 are depicted (excluding *Desmognathus apalachicolae*, which is included in all subsequent analyses based on the mean snout-vent length [SVL] reported in Titus and Larson 1996): 1, *D. aeneus*; 2, *D. wrighti*; 3, *D. ocoee* A; 4, *D. ocoee* F; 5, *D. carolinensis*; 6, *D. imitator*; 7, *D. ocoee* G; 8, *D. ochrophaeus*; 9, *D. orestes*; 10, *D. ocoee* C; 11, *D. santeetlah*; 12, *D. auriculatus*; 13, *D. conanti* D; 14, *D. fuscus* B; 15, *D. fuscus* A; 16, *D. monticola*; 17, *D. brimleyorum*; 18, *D. welteri*; 19, *D. quadramaculatus/marmoratus* A; 20; *D. quadramaculatus/marmoratus* C; 21, *D. quadramaculatus/marmoratus* B; 22, *D. quadramaculatus/marmoratus* E; 23, *D. folkertsi*.

model (Brownian motion) of evolutionary change with the constraint that the mean and the variance of the simulated body sizes equal those observed for the real data. To account for uncertainty in the topology and branch lengths, we simulated 100 datasets along each of the 950 post-burn-in phylogenies sampled from the Bayesian posterior distribution. We then grouped the 24 sampled lineages into stream, stream-edge, seepage, and terrestrial categories of microhabitat use and conducted an ANOVA on each of the 95,000 simulated datasets. Using this procedure, we generated a null distribution for the *F*-statistic. If fewer than 5% of the simulated datasets had *F*-values greater than or equal to the *F*-value of the real dataset, we considered the results statistically significant. To determine whether all groups of microhabitat use could be discriminated from each other, we repeated the procedure for each possible pair of microhabitat categories. These tests were implemented in software developed by L. J. Harmon (available upon request).

TABLE 2. Twenty-four lineages of *Desmognathus* sampled for morphometric analyses. Lineage designations are identified in Figure 3. See Appendix 2 (available online only at http://dx.doi.org/10.1554/05-024.1.s1) for exact geographic locations of specimens measured.

Species/geographic lineage sampled	*N*	Mean SVL (mm) ± S. E.
D. wrighti	10	26.4 ± 0.6
D. aeneus	7	25.8 ± 0.2
D. imitator	13	42.9 ± 1.2
D. ocoee A	5	42.9 ± 1.8
D. ocoee C	10	44.6 ± 0.8
D. ocoee F	7	40.1 ± 1.6
D. ocoee G	7	46.2 ± 0.8
D. carolinensis	12	45.6 ± 0.8
D. orestes	10	38.9 ± 1.2
D. ochrophaeus	12	40.4 ± 0.7
D. apalachicolae	*	42.7
D. fuscus B	28	59.0 ± 1.8
D. fuscus A	5	61.8 ± 2.3
D. conanti D	24	56.8 ± 1.4
D. santeetlah	10	45.2 ± 0.9
D. auriculatus	8	51.1 ± 0.3
D. monticola B	23	58.9 ± 1.7
D. brimleyorum	16	78.1 ± 1.0
D. welteri	11	66.4 ± 2.1
D. folkertsi	18	71.4 ± 1.0
D. quad/marm A	6	70.2 ± 2.6
D. quad/marm B	20	82.8 ± 1.4
D. quad/marm C	20	77.1 ± 1.4
D. quad/marm E	19	69.4 ± 1.4

* Data are from Titus and Larson 1996.

Accumulation of ecomorphological disparity

To quantify the tempo of ecomorphological divergence, we used relative disparity plots (Harmon et al. 2003). Disparity of a clade was calculated as the mean squared pairwise difference in body size between all pairs of terminal taxa. We first calculated the disparity for the entire *Desmognathus* clade and then for each subclade defined by a node in the phylogeny. Relative disparity of each subclade was standardized by dividing a subclade's disparity by the disparity of the entire clade. To investigate the partitioning of ecomorphological disparity among subclades of varying ages, we moved up the phylogeny from the root. At each depth in the phylogeny, we calculated the mean relative disparity for all subclades whose ancestral lineages were present at that time. We then plotted these mean morphological disparities across the full depth of the phylogenetic tree.

To evaluate how much the mean disparity differed from that expected under a null hypothesis of unconstrained Brownian motion, we conducted 1000 simulations on the ultrametric Bayesian consensus phylogram with the con-

straint that the mean and the variance of the simulated tip values be equal to those observed for the real data (Garland et al. 1993). We then used the morphological disparity index (MDI) to compare the overall difference in relative disparity of a clade compared with that expected under the null hypothesis (Harmon et al. 2003). The MDI calculates the area contained between the line connecting the actual relative disparity points calculated from the data and the line connecting median relative disparity points of the simulations. We used the median relative disparity because some simulations produce values that are not normally distributed and the median therefore provides a better measure of central tendency. Areas in which the observed values were greater than expected are assigned positive values; those below expected are given negative values. Positive MDI values indicate that the observed disparity is mainly partitioned within subclades, whereas negative values indicate that it is partitioned mostly among subclades. Our phylogenetic analyses of variance demonstrate that ecomorphological variation among lineages is greater than expected by chance (see Results section), and these relative disparity plots provide a visual depiction of the accumulation of ecomorphological disparity. All simulations and calculations were implemented in software developed by L. J. Harmon (available upon request).

Phylogeny, ecomorphology, and community assembly

We used Mantel tests to quantify the relationship between matrices representing phylogenetic distance, ecomorphological distance, and geographical coexistence among lineages. Ecomorphological distances were calculated as the pairwise difference in snout-vent length between lineages. Phylogenetic distances were calculated as the path length separating lineages along the ultrametric Bayesian consensus phylogram. To determine the pattern of spatial overlap among lineages, we used information on *Desmognathus* community composition from the literature (summarized in Petranka 1998) for nominal species that were monophyletic and/or considered a single lineage. For other species we mapped the geographic distributions of monophyletic phylogeographic subunits and determined whether their ranges overlapped. Although some geographically overlapping *Desmognathus* species differ in their mean elevational distributions, streamside communities at intermediate elevations contain both high- and low-elevation forms (Hairston 1949; Organ 1961; Bruce 1991; Tilley 2000; Dodd 2004), suggesting that the geographical overlap of phylogeographic subunits is an appropriate measure of the maximum number of species that coexist in local assemblages. The pattern of coexistence among all lineages was then represented as a binary design matrix (1, lineages coexist; 0, lineages do not coexist). This approach does not consider the magnitude of overlap between lineages but potentially gives a more sensitive test of the relationship between phylogeny, ecomorphology, and geographic coexistence by retaining information on which lineages coexist locally in at least some part of their geographical ranges (Barraclough et al. 1999).

The relationship between ecomorphological divergence and geographic overlap of lineages may follow three possible forms. First, a positive relationship is expected if ecomorphological divergence promotes the coexistence of lineages. Second, a negative relationship is expected if lineages must be ecomorphologically similar to coexist. Third, no relationship should occur if lineages have randomly assembled into communities with respect to ecomorphology. In groups showing phylogenetic niche conservatism, the degree of ecomorphological divergence among lineages is positively correlated with their phylogenetic divergence (Böhning-Gaese and Oberrath 1999; Webb et al. 2002). Under such circumstances, a significant relationship between phylogeny and coexistence is expected also if community assembly has been nonrandom with respect to patterns of ecomorphological divergence. Ecomorphological divergence also may be positively correlated with the geographic overlap of lineages independent of phylogenetic history. Such a relationship is expected if ecomorphs are evolutionarily labile and arose repeatedly during the assembly of ecological communities.

To explore these potential relationships, we conducted three-way Mantel tests, which calculate the partial Mantel correlation between two variables while holding the effect of a third variable constant (Legendre and Legendre 1998; Smouse et al. 1986). We evaluated the relationship between the morphological divergence matrix and the geographic coexistence matrix while holding the effect of phylogenetic distance on these variables constant. We evaluated whether the correlation between the matrices was statistically significant using 9999 Monte Carlo randomizations. If fewer than 5% of the randomizations had correlations greater than or equal to the correlation in the real dataset, the results were considered significant. All Mantel tests were implemented in the software package PASSAGE (Rosenberg 2002).

RESULTS

Phylogenetic Relationships

A total of 115 sequences representing 1581 aligned bases is reported for 19 species of desmognathine salamanders and two outgroup taxa. Absence of premature stop codons in the ND2 and CO1 protein-coding regions, functional stability of the five tRNA genes, and strong bias against guanine on the light strand indicate that the DNA sequences are from the mitochondrial genome and not nuclear-integrated copies of mitochondrial genes (Zhang and Hewitt 1996). An indel from positions 967–972 in the ND2 gene precludes unambiguous alignment of these sites in some taxa; these sites are therefore excluded from phylogenetic analyses. Length variation in the D-loop of the $tRNA^{Asn}$ gene (sites 1246–1259) and origin of light-strand replication (1289–1294) precludes unambiguous alignment of those sites, which are excluded from phylogenetic analyses.

Bayesian phylogenetic analysis using the GTR + I + Γ model produces a 50% majority-rule consensus tree with a mean ln-likelihood of $-20{,}323.54$ (SD = 13.73) following a burn-in of 100,000 generations (Fig. 3). Parsimony analysis of 667 informative characters (613 within the ingroup) produced 49 equally most parsimonious trees of 3788 steps. Because both analyses produced highly congruent phylogenetic estimates, with all topological differences restricted to nodes that are weakly supported (bootstraps $<$50% and posterior probabilities $<$95%), only the Bayesian tree is pre-

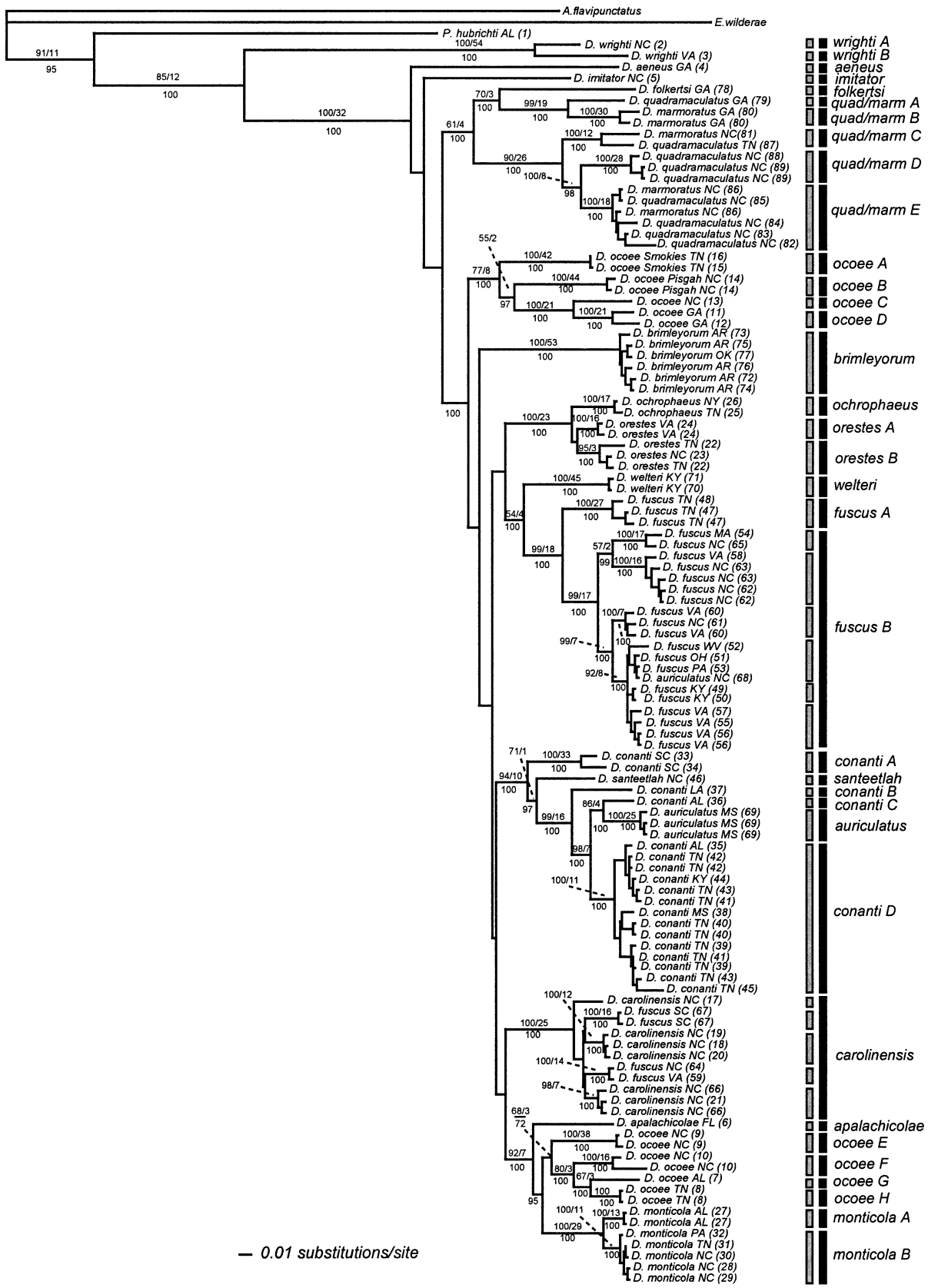

FIG. 3. Bayesian 50% majority-rule consensus phylogram. Posterior probabilities based on 950 post-burn-in trees (which had a mean likelihood score of −20,323.54; SD = 13.73) are shown below the branches and nonparametric bootstrap/decay indices for the parsimony analysis above. Branch lengths represent means from the post-burn-in sample of trees. Black versus gray vertical bars designate alternative diagnoses of independent evolutionary lineages that were considered in the diversification-rate analyses.

sented, with nonparametric bootstrap and decay indices from the parsimony analyses included for shared branches.

Our phylogenetic analyses confirm that *Phaeognathus hubrichti* is the sister taxon to *Desmognathus*; *D. wrighti* is the sister taxon to a clade containing all other species of *Desmognathus*, and the lineage leading to *D. aeneus* diverged early in the latter clade (Titus and Larson 1996; Rissler and Taylor 2003). The exact phylogenetic relationships among *D. aeneus*, *D. imitator*, and the *D. folkertsi-marmoratus-quadramaculatus* clade are not well supported. These lineages are outside a clade containing all other species with aquatic larval periods, although the latter clade receives strong support only in the Bayesian analysis. Within the latter clade, comprehensive inter- and intraspecific sampling identifies seven major evolutionary branches separated by short and poorly supported internodes (Fig. 3). These branches are (1) *D. ocoee* (populations 11–16) from the Great Smoky Mountains, Pisgah Ridge, Nantahala Mountains, and Tugaloo River; (2) *D. brimleyorum*; (3) *D. orestes* + *D. ochrophaeus*; (4) *D. welteri* + *D. fuscus* + *D. auriculatus*; (5) *D. conanti* + *D. santeetlah* + *D. auriculatus*; (6) *D. carolinensis* + *D. fuscus*; and (7) *D. apalachicolae* + *D. monticola* + *D. ocoee* (populations 7–10) from the Tusquittee Mountains, Unicoi Mountains, and lowland tributaries of the Tennessee River. Phylogenetic relationships corroborated by analyses of nuclear-encoded allozymic loci and additional mitochondrial gene sequences include the grouping of *D. ochrophaeus* and *D. orestes* as a clade (Mead et al. 2001); the close relationship between *D. conanti* and *D. santeetlah* (Tilley and Schwerdtfeger 1981; Titus and Larson 1996); the close relationship between *D. welteri* and *D. fuscus* (Karlin and Guttman 1986); the close relationship between some populations of *D. ocoee* and *D. apalachicolae* (Means and Karlin 1989); and the grouping of *D. folkertsi*, *D. marmoratus*, and *D. quadramaculatus* as a clade (Titus and Larson 1996; Camp et al. 2002).

No topologies sampled from the Bayesian posterior distribution were compatible with monophyly of the stream, stream-edge, seepage, or terrestrial groupings of microhabitat use.

Relative-rate tests demonstrate that a single lineage, *P. hubrichti*, has accumulated nucleotide substitutions at a significantly different rate than the remaining samples ($P = 0.029$).

Intraspecific Phylogeny and Delimitation of Independent Evolutionary Lineages

Haplotypes from *D. brimleyorum*, *D. ochrophaeus*, and *D. welteri* form strongly supported monophyletic groups, and these three lineages are diagnosable using available morphological and/or allozyme data. These species exhibit shallow haplotypic divergences throughout their geographic ranges, suggesting that they do not harbor cryptic species. However, our comprehensive geographic sampling suggests that other nominal species of *Desmognathus* comprise phylogenetically and geographically distinct groups of haplotypes diagnosing multiple, independent evolutionary lineages. Below we discuss diagnoses of 35 putatively independent lineages. A more extreme set of alternative diagnoses that likely exceed the maximum number of independent evolutionary lineages is also included to test the robustness of our lineage-accumulation rate analyses to possible errors of incomplete taxon sampling. The groupings of haplotypes corresponding to both schemes are denoted in Figure 3.

Haplotypes sampled from *D. folkertsi*, *D. marmoratus*, and *D. quadramaculatus* form exclusive groups that are concordant with geography rather than current taxonomy. *Desmognathus folkertsi* is known only from a single locality in the Georgia Piedmont where it shows complete reproductive isolation from syntopic *D. quadramaculatus* (Camp et al. 2002). Populations of *D. marmoratus* in the northern Atlantic Slope, southern Atlantic Slope, and Tennessee River drainages are grouped with *D. quadramaculatus* haplotypes from the same drainage basins. These results are at least partly concordant with allozyme variation showing seven fixed differences between populations of *D. marmoratus* on opposite sides of the eastern Continental Divide (Voss et al. 1995). An independent study of the *D. quadramaculatus-marmoratus* complex also reveals phylogeographic patterns corresponding to drainage basins rather than to named species (Jones et al. 2005). We recognize *D. folkertsi* and five strongly supported lineages corresponding to major drainage basins (*quad/marm* A, B, C, D, and E).

Desmognathus fuscus haplotypes are nonexclusive with respect to *D. auriculatus* and *D. carolinensis*. Geographic patterns of haplotype coalescence diagnose at least two independent evolutionary lineages. Haplotypes from populations in the Cumberland Plateau (*fuscus* A), and Ohio River Basin plus Atlantic Slope (*fuscus* B) form exclusive groups concordant with geography. Six groups of haplotypes within the latter clade are also exclusive, but more inclusive groups of these haplotypes have geographic ranges that overlap. This spatial and temporal phylogenetic pattern is suggestive of restricted gene flow with isolation by distance (Wiens and Penkrot 2002; Templeton 2004).

A small subset of *D. fuscus* haplotypes sampled from the Atlantic Slope (populations 59, 64, 67) interdigitate with haplotypes from sampled *D. carolinensis*. These haplotypes are sampled from within the range of *D. fuscus*, but they are geographically disjunct from the range of *D. carolinensis*, making contemporary gene flow an unlikely explanation for the discordance between the gene tree and current taxonomy. One explanation for this pattern is that *D. carolinensis* is composed of multiple independent evolutionary lineages that extend beyond its currently known range in the Blue Ridge Mountains. However, a fine-scale study of allozyme variation in *D. carolinensis* suggests that isolation by distance has shaped geographic patterns of genetic variation within this species (Tilley 1997), but that study did not include geographically proximate populations of *D. fuscus*. We therefore hypothesize that *D. carolinensis* is a single lineage, and that discordance between the gene tree and species tree results from mtDNA introgression facilitated by past geographic contact with *D. fuscus*.

Desmognathus conanti haplotypes are nonexclusive with respect to *D. santeetlah* and *D. auriculatus* from the Gulf Coastal Plain. Geographic patterns of haplotype coalescence diagnose four phylogenetically and geographically distinct evolutionary lineages. The phylogenetic split separating haplotypes of *D. conanti* populations in Atlantic Coastal Plain

drainages (*conanti* A) and Gulf Coastal Plain plus Lower Tennessee River drainages (*conanti* B + C + *auriculatus*) is concordant with the allozymic results of Bonett (2002) in diagnosing separate evolutionary lineages. Our results also suggest that populations of *D. conanti* from the Lower Tennessee River Basin (*conanti* D) are phylogenetically distinct from samples of *D. conanti* (*conanti* C) and *D. auriculatus* in Gulf Coastal Plain drainages. Our sampling of single haplotypes from the Mississippi Embayment (*conanti* B) and Gulf Coastal Plain (*conanti* C) limits application of the Wiens and Penkrot (2002) method. We anticipate that further fine-scale study of genetic variation in these regions will corroborate these phylogeographic patterns and uncover cryptic species corresponding to major drainage basins. We therefore recognize *conanti* A, B, C, and D as independent evolutionary lineages.

Geographic genetic variation in *D. auriculatus* requires much further study. An Atlantic Coastal haplotype of *D. auriculatus* groups with eastern and northern *D. fuscus* haplotypes, whereas a Gulf Coastal haplotype of *D. auriculatus* groups with haplotypes of *D. conanti* from this region. Geographic genetic variation in *D. auriculatus* has not been studied. Karlin and Guttman (1986) also reported deep genetic divergence between a Gulf Coastal Plain population of *D. auriculatus* and *D. conanti* from Tennessee, but they did not compare populations of these species from the same region. Our study is the first phylogenetic analysis to include geographically separated populations of this form. The Atlantic Coastal Plain haplotype is nearly identical to northern and eastern haplotypes of *D. fuscus*; we consider these populations conspecific until analyses demonstrate their evolutionary independence. The Gulf Coastal haplotypes are exclusive, and we consider these populations phylogenetically distinct from *D. conanti* for our analyses.

The range of *D. monticola* is fragmented into geographic isolates in the Alabama Piedmont (*monticola* A) and in the Appalachian Highlands plus Alleghany Plateau (*monticola* B), and these isolates correspond to exclusive groups of haplotypes. Haplotypes sampled throughout the range of this species are remarkably uniform and indicate that this species has recently expanded its range throughout the Appalachian Highlands and Alleghany Plateau. These patterns of phylogeographic divergence confirm results of a recent study of mtDNA variation (Rissler and Taylor 2003); we therefore treat these two groupings as independent evolutionary lineages.

Desmognathus ocoee is nonexclusive and comprises at least two independent evolutionary lineages. A recent allozyme study also shows *D. ocoee* to be nonmonophyletic (Anderson and Tilley 2003). Haplotypes sampled from populations in the Great Smoky Mountains (*ocoee* A), Pisgah Ridge (*ocoee* B), Nantahala Mountains (*ocoee* C), and lowland tributaries of the Tugaloo River in the Georgia Piedmont (*ocoee* D) form a strongly supported monophyletic group. The remaining haplotypes sampled from populations in the Tusquittee Mountains (*ocoee* E), Unicoi Mountains (*ocoee* F) and the Alleghany Plateau in Alabama (*ocoee* G) and Tennessee (*ocoee* H) are grouped in a clade with *D. apalachicolae* and *D. monticola*. Despite the highly restricted area from which *D. ocoee* populations are sampled, in all cases where we sample multiple individuals from a population those haplotypes always form exclusive groups. These results are consistent with the allozyme study of Tilley and Mahoney (1996) in showing that *D. ocoee* populations from each major mountain range in southwestern North Carolina might represent different evolutionary species.

Geographic patterns of haplotype coalescence diagnose two exclusive lineages within *D. orestes* (*orestes* A and B). These lineages are also diagnosed by patterns of geographic genetic variation in allozymes (Tilley and Mahoney 1996). Hybridization occurs across their contact zone in the Blue Ridge (Mead et al. 2001).

A recent analysis of mtDNA and allozyme variation in *D. wrighti* demonstrates that populations distributed north (*wrighti* A) and south (*wrighti* B) of the Asheville Basin are distinct evolutionary lineages, and that isolation by distance has shaped geographic patterns of genetic variation within each of these lineages (Crespi et al. 2003). We therefore treat *D. wrighti* haplotypes from these regions as independent evolutionary lineages.

Desmognathus apalachicolae, *D. imitator*, and *D. santeetlah* are diagnosable based on available morphological and allozyme data. These species have geographically restricted ranges and little geographic genetic variation (Tilley and Schwerdtfeger 1981; Karlin and Guttman 1986; Tilley 2000), so each is considered a single lineage. Genetic variation in *D. aeneus* has not been studied.

Diversification Rates

The lineages-through-time plot indicates a burst of lineage accumulation followed by deceleration in the accumulation of lineages (Fig. 4). To test whether the accumulation of *Desmognathus* lineages deviates from a constant-rates model of diversification, we fit three alternative models of diversification to our dataset using maximum likelihood. These tests assume that speciation and extinction are equally probable among all lineages.

The relative cladogenesis statistic shows significant differences in diversification rates among *Desmognathus* lineages. In particular, the ancestral node uniting lineages with aquatic larval periods has experienced a higher rate of diversification relative to the ancestral lineages leading to *D. wrighti* and *D. aeneus* ($P = 0.016$). At least some of this rate difference is likely attributed to a lack of data on geographic genetic structure within *D. aeneus*; however, this species would have to harbor many cryptic species for rates of lineage accumulation to be equal among direct-developing and metamorphosing lineages. This asymmetry in lineage-accumulation rates also remains significant in a pruned dataset that treats each of the formally recognized species of *Desmognathus* as a single lineage ($P = 0.02$). We therefore followed Pybus and Harvey (2000) and conducted these analyses on both the full dataset and a subset containing only species with aquatic larvae. Regardless of which dataset was used, the survivorship analyses reject a constant-rates model of lineage accumulation in favor of ones that specify declining rates of diversification (Table 3).

Overdispersed taxon sampling may cause spurious rejection of a constant-rates model of diversification in favor of

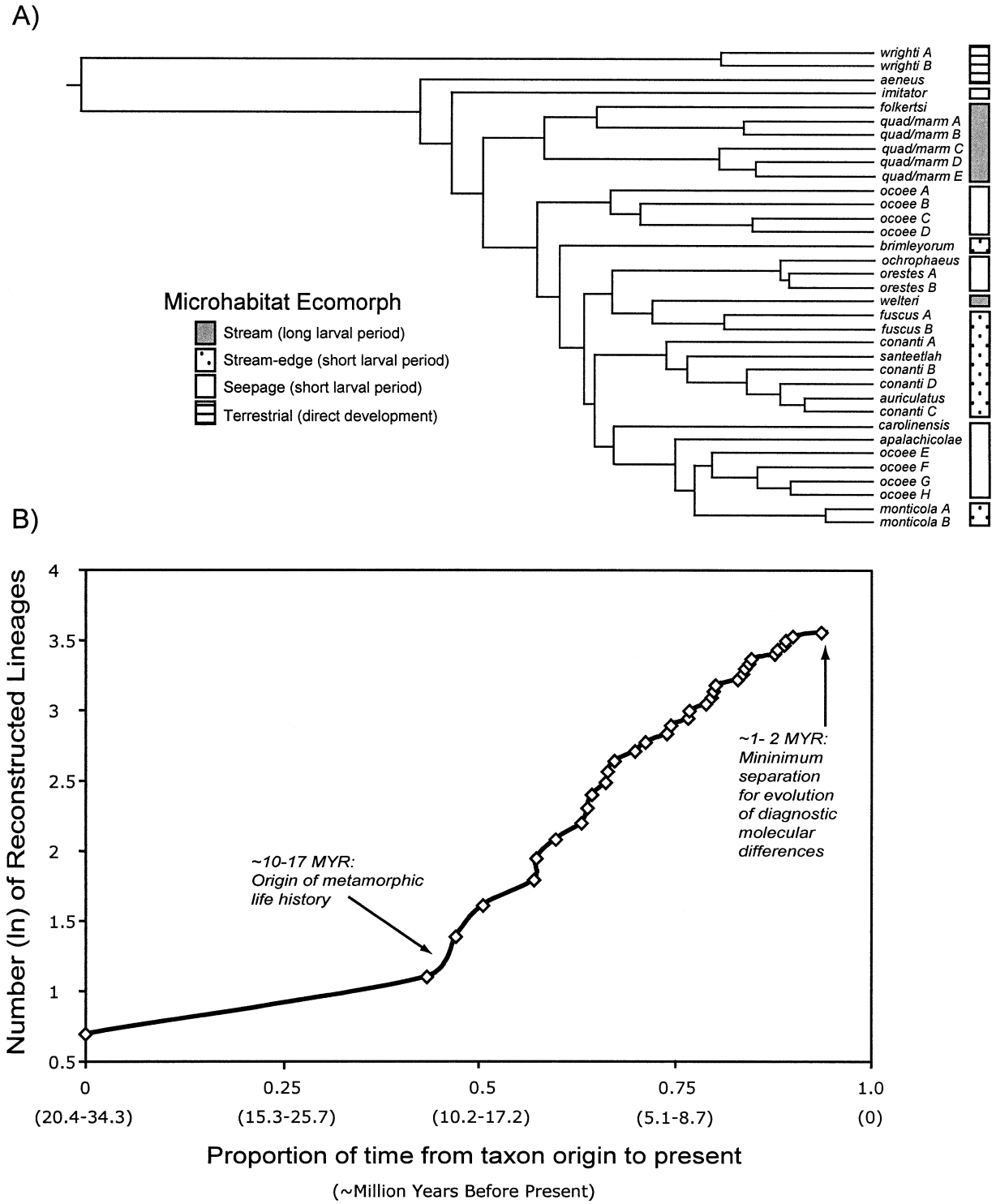

FIG. 4. (A) Ultrametric Bayesian consensus phylogram for the 35 species/geographic lineage dataset. (B) Semilogarithmic plot of lineages through time for the ultrametric tree shown in (A); time is expressed as a proportion of the total time after the first cladogenetic event inferred for the taxon; estimated absolute timing based on alternative fossil-based ages (28 vs. 47 million years; Chippindale et al. 2004) for the most recent common ancestor of *Phaeognathus* + *Desmognathus* is shown below in parentheses.

one showing lineage-accumulation rates to decrease over time (Pybus and Harvey 2000; Shaw et al. 2003). Our phylogeographic methods permit more precise delimitation of putatively independent population-level lineages; nevertheless, some lineages that have become evolutionarily independent too recently to have evolved diagnostic differences for our molecular markers could have been missed by our method of lineage delimitation. Model C specifies an abrupt shift in

TABLE 3. Tests of the null hypothesis that the rate of lineage accumulation (speciation minus extinction) was constant during the phylogenetic history of *Desmognathus*. Model A specifies a constant lineage-accumulation rate. Model B specifies a gradual decrease in the lineage-accumulation rate. Model C specifies different lineage-accumulation rates before and after the most recent split in the mtDNA phylogeny that was diagnosed as leading to the formation of independent evolutionary lineages. Model C is expected to fit the data best if a decrease in the lineage-accumulation rate is an artifact of the lag time needed for lineages to evolve diagnostic molecular differences.

Phylogeny	*n*	Model A lnL (AIC)	Model B lnL (AIC)	Model C lnL (AIC)
1. All *Desmognathus*	35	−148.08 (298.17)	−140.62[1] (285.25)	−142.79 (289.57)
	44	−179.40 (360.80)	−175.16[1] (354.32)	−176.42 (356.84)
2. Biophasic *Desmognathus* only	32	−131.69 (265.38)	−119.97[1] (243.93)	−126.17 (256.34)
	41	−162.23 (326.47)	−155.21[1] (314.43)	−159.00 (321.99)

[1] The data favor model B, which has the highest ln-likelihood. A likelihood-ratio test is used to compare model A versus B, and model A versus C because they are nested hypotheses (Paradis 1998). Models B and C are not nested, so the Akaike information criterion (AIC; Akaike 1973) is used to compare their ln-likelihoods (Paradis 1998). Models that specify decreasing lineage-accumulation rates fit the data significantly better than model A (model A vs. B: χ^2 = 8.48–14.92, df = 1, P = 0.01–0.001; model A vs. C: χ^2 = 5.96–10.58, df = 1, P = 0.025–0.01). For all datasets, model B has the lowest AIC score and fits the data better than model C.

the rate of diversification following the most recent lineage-splitting event that we diagnosed as leading to independent evolutionary lineages. This model would be expected to fit the data best if a constant-rate model is rejected purely as an artifact of the lag time for lineages to evolve diagnostic molecular differences. Model B, which specifies a gradual decline in the diversification rate, fits the data significantly better than Model C (Table 3), suggesting that rejection of the constant-rates model is not an artifact of the resolution of our molecular markers or methods of lineage delimitation.

Comparative Analyses

Phylogenetic analyses of variance strongly support the hypothesis that lineages form distinct groupings in morphological space according to their microhabitat use (Table 4). Because we conducted our trait simulations on samples from the Bayesian posterior distribution for the topology and branch lengths, our results are robust to uncertainty in these parameters.

Mantel tests demonstrate a positive correlation between morphological divergence and phylogenetic distance (Table 5). Lineages that coexist tend to show greater morphological divergence and phylogenetic divergence than parapatric or allopatric lineages. The partial Mantel test indicates that the degree of morphological divergence among coexisting lineages is no greater than would be expected from their phylogenetic relationships alone.

The relative disparity plot shows that subclades of *Desmognathus* exhibit lower average disparity than expected under the null hypothesis of unconstrained divergence by Brownian motion (Fig. 5). This result is corroborated by a MDI value of −0.18, which indicates that ecomorphological variation is partitioned mainly among older subclades of *Desmognathus*.

TABLE 4. Phylogenetic analyses of variance for body size between pairs of microhabitat categories (see Table 1). A significant result indicates that the paired categories differ in mean body size after correcting for phylogenetic relatedness.

Phylogenetic ANOVA	Observed *F*-statistic	Simulated *P*[1]
Full dataset	73.46	0.000011
Stream vs. terrestrial	218.69	0.000011
Stream vs. seepage	200.38	0.000011
Stream vs. stream-edge	11.73	0.003726
Stream edge vs. terrestrial	55.95	0.000074
Stream edge vs. seepage	36.43	0.000021
Seepage vs. terrestrial	124.41	0.000011

[1] *P* is the probability of obtaining an *F*-statistic equal to or greater than the observed value by chance.

DISCUSSION

Classic models of adaptive radiation link the buildup and maintenance of species richness with the evolution of ecomorphological disparity. Ecomorphological divergence is hypothesized to play a key role in the diversification, long-term persistence, and coexistence of lineages by reducing the strength of ecological interactions among species (Simpson 1953; Schluter 2000). As a corollary, the accumulation of lineages and ecomorphological disparity may be intimately linked to the persistence of species associations over evolutionary time, because ecological interactions among coexisting lineages influence opportunities for subsequent diversification (Stanley 1973; Schluter 2000; Harmon et al. 2003). We present a novel, synthetic analysis of phylogeographic history, rates of ecomorphological evolution and species accumulation, and community assembly in an adaptive radiation.

Methods that use relative branching times in molecular phylogenies to detect changes in diversification rates (Paradis 1997, 1998; Pybus and Harvey 2000) rely critically on a complete sampling of extant independent evolutionary lineages (Pybus and Harvey 2000; Pybus et al. 2002; Shaw et al. 2003). Most studies that have used molecular phylogenies of extant taxa to make inferences about diversification patterns consider only a single representative of each described species (Barraclough and Nee 2001). Given the ubiquity of historically isolated, cryptic phylogeographic units within many taxonomic species (Avise 2000), a rigorous characterization of geographic patterns of genetic variation among populations is necessary in many taxonomic groups to delimit the evolutionary units most appropriate for analysis. Our geographic sampling of genetic variation within many of the formally recognized species of *Desmognathus* suggests that the current taxonomy of the group underestimates extant species-level diversity. The geographic limits of many of these lineages are generally congruent with those diagnosed by

TABLE 5. Mantel tests for association between morphological distance, phylogenetic distance, and coexistence among 19 lineages (Appendices 3–5, available online only at http://dx.doi.org/10.1554/05-024.1.s1). Significance of Mantel correlation coefficients (r) is evaluated using 9999 Monte Carlo randomizations.

Matrices tested	t[1]	r	P[2]
Morphological distance, phylogenetic distance	3.45	0.52	0.0001
Phylogenetic distance, coexistence	1.69	0.26	0.0562
Morphological distance, coexistence	1.79	0.21	0.0367
[3]Phylogenetic distance, morphological distance, coexistence	0.89	0.08	0.1774

[1] Student's t.
[2] Probability that a value of t this large or larger would result from chance alone.
[3] Effect of phylogeny on morphological distance and coexistence was removed using a three-way Mantel test.

strong allelic-frequency differences in allozymes, although we emphasize that some of our diagnoses are based solely on mtDNA variation and should be confirmed using nuclear DNA markers before they receive formal taxonomic recognition (Glor et al. 2004).

Recent molecular phylogenetic analyses of the salamander family Plethodontidae provide strong support for the hypothesis that the ancestral life-history mode in desmognathines is direct development (Titus and Larson 1996) and suggest that aquatic larvae re-evolved relatively early in the evolutionary history of dusky salamanders (Chippindale et al. 2004; Mueller et al. 2004). Our diversification-rate analyses are based on relative divergence times; nevertheless, the absolute time course for the radiation of desmognathines can be roughly approximated. Based on molecular-rate calibrations (Larson et al. 2003) and fossil-based dating methods (Chippindale et al. 2004), the origin of a biphasic life history occurred 10–17 million years ago (Fig. 4). Our study shows that this major life-history shift was followed by a high rate of lineage accumulation that then gradually decreased until approximately 1–2 million years ago (the minimum separation needed for independent evolutionary lineages to accumulate diagnostic differences for our molecular markers).

The diversification of *Desmognathus* has important implications for understanding the high species richness and phenotypic disparity that characterize eastern North American plethodontid salamanders. Ecological access to terrestrial adaptive zones has likely been limited by competition from codistributed species of the genus *Plethodon*, which are among the most abundant vertebrates in terrestrial ecosystems (Davic and Welsh 2004), and have occupied and diversified within a terrestrial adaptive zone for approximately 30 million years (Highton 1995; Larson et al. 2003). Our inference of an increased rate of diversification associated with a shift from direct development to a biphasic life history provides strong support for the hypothesis that a major life-

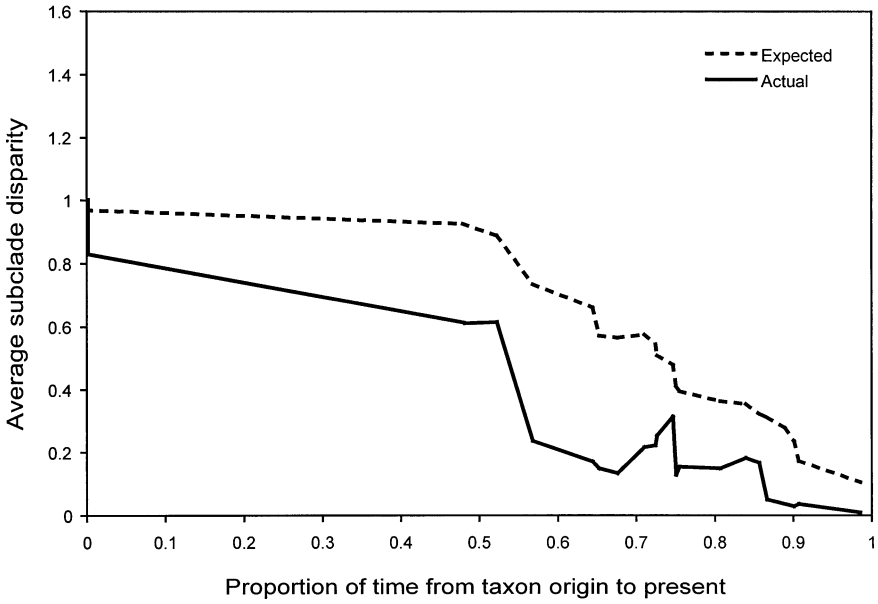

FIG. 5. Relative disparity plot for *Desmognathus* compared to the expected disparity from phylogenetic simulations of unconstrained, gradual morphological divergence. Average extant disparity at each depth in the tree is the average disparity of subclades whose ancestral lineages were present at that time relative to the disparity of the entire taxon. The higher the value of relative disparity, the greater the average volume of morphological space occupied by subclades relative to the morphological disparity of the taxon as a whole. Time is expressed as a proportion of the total time after the first cladogenetic event inferred for the taxon. The solid line is the actual disparity for the clade; the dashed line is the expected disparity derived from the phylogenetic simulations. At each depth in the phylogeny subsequent to the root, subclades show less average disparity than expected. This pattern suggests that ecomorphological divergence occurred early in the radiation of extant *Desmognathus* lineages. Because nested clades tend to contain less morphological disparity than the more inclusive clades to which they belong, average disparity generally decreases as time approaches the present.

history reversal promoted the radiation of *Desmognathus* in the southern Appalachians by allowing lineages to subdivide an aquatic adaptive zone (Chippindale et al. 2004).

The peak period of lineage accumulation in *Desmognathus* coincides with inferred evolutionary partitioning of lineages with aquatic larvae into seepage, stream-edge, and stream microhabitats. Our morphometric results demonstrate a strong correlation between morphology and microhabitat ecology independent of phylogenetic effects, which supports the hypothesis that natural selection played an important role in the buildup and maintenance of phenotypic disparity in *Desmognathus* (Miles and Dunham 1993; Schluter 2000). Variation in moisture predictability among microhabitats along the aquatic-terrestrial gradient probably elicited adaptive divergence in length of the larval and/or juvenile period, producing body-size divergence among *Desmognathus* species (Bruce 1990, 1996; Camp et al. 2000).

Although our molecular phylogenetic analyses reject hypotheses that ecomorphs form monophyletic groups, our morphological-disparity analysis suggests that ecomorphological changes occur disproportionately early in the radiation of *Desmognathus*. Thus, the overall evolutionary pattern is one of early adaptive divergence followed by long-term persistence of ecomorphologically distinct lineages (Barraclough et al. 1999). This temporal pattern of ecomorphological divergence is found in other ancient vertebrate adaptive radiations (Streelman et al. 2002; Rüber et al. 2003; Stephens and Wiens 2003) and contrasts with young adaptive radiations where most speciation events are associated with adaptive ecological divergence (Baldwin and Sanderson 1998; Sato et al. 1999). Therefore, the *Desmognathus* radiation lends support to a recent hypothesis that adaptive divergence becomes less important in driving speciation in older adaptive radiations where lineages have persisted long enough to fill available ecological and geographical space (Stephens and Wiens 2003).

Our analyses of *Desmognathus* community structure show that ecomorphological divergence promotes the coexistence of lineages, a result that provides evidence for strong ongoing ecological interactions among different ecomorph types in the evolutionary assembly of communities. We hypothesize that ecomorphological divergence promotes lineage coexistence in several ways. First, lineages with smaller body sizes have short larval periods (or direct development) and early maturation (Bruce 1991; Camp et al. 2000); these traits increase survivorship in semiterrestrial microhabitats that are unsuitable for long-term occupation by large, predatory congeners with protracted aquatic larval periods and delayed maturity (Werner 1986; Rowe and Ludwig 1991; Stearns 1992; Camp et al. 2000). Second, competition appears to limit the distributional overlap of similar-sized species (Means 1975; Hairston 1986). Furthermore, ecomorphological divergence may be associated with the evolution of reproductive isolation; disparity in body size reduces insemination success during plethodontid salamander courtship (Beachy 1996).

The lack of a significant relationship between ecomorphological divergence and lineage coexistence independent of phylogenetic history supports the hypothesis that desmognathine communities have formed almost entirely through the geographical overlapping of disparate ecomorphologically stable lineages. Therefore, independent evolution of ecomorphs, which is expected to erase the imprint of phylogenetic history on community structure (Webb et al. 2002), has a limited role in the evolutionary assembly of *Desmognathus* communities.

Concentration of lineage accumulation and morphological divergences disproportionately early in the history of the *Desmognathus* radiation suggests that ecomorphological diversification permitted coexistence of disparate forms as early as 6 million years ago, by which time ancestral lineages leading to clades of ecomorphologically stable terrestrial, seepage, stream-edge and stream ecomorphs appear to have originated (Fig. 4). Geographic isolation induced by climatic fluctuations (Highton 1995; Tilley and Mahoney 1996) and shifting drainage divides (Voss et al. 1995) likely prevailed in the multiplication of ecomorphologically similar species since that time. Although some populations of *Desmognathus* have undergone localized range expansions at northern latitudes (Rissler and Taylor 2003), the deep phylogeographic structure that we show within many codistributed ecomorph clades suggests long-term persistence and stability of endemic lineages and communities through multiple climatic cycles (Watts 1980; Webb and Bartlein 1992; Webb et al. 1995). More generally, *Desmognathus* contrasts with other continental evolutionary radiations where lineages diversified independently of each other in the absence of long-term evolutionary stability in community structure (Barraclough et al. 1999; Webb et al. 2002).

In a study of adaptive radiations in iguanian lizards Harmon et al. (2003) found that groups whose lineage accumulation occurs disproportionately early in their evolutionary histories partition ecomorphological changes among major clades rather than among closely related species. This pattern characterizes dusky salamanders, Caribbean *Anolis*, and Australian agamids, whereas the iguanid genus *Liolaemus* shows a steady increase in lineage accumulation through time, and ecomorphological disparity is common among relatively closely related species. Our inferred geographic history of desmognathine evolution confirms a prediction by Harmon et al. (2003) that early evolution of microhabitat structuring among species and geographic expansion of codistributed species throughout the group's long-term geographic range produces long-term persistence and stability of lineages and morphologies. The deep coregional history that we show for many desmognathine lineages and evidence that ecomorphological divergence promotes their coexistence supports the hypothesis that ecological interactions among lineages have restricted opportunities for diversification. The opposite pattern illustrated by *Liolaemus* appears when geographic expansion persists throughout most of a group's evolutionary history, and where microhabitat partitioning evolves independently after new areas are colonized by single lineages; *Liolaemus* is inferred to have experienced repeated episodes of dispersal and vicariance across the Andes throughout its evolutionary history (Schulte et al. 2000), in strong contrast to patterns of diversification in *Desmognathus*.

The *Desmognathus* pattern of early community assembly through ecomorphological divergence followed by long-term evolutionary stasis of ecological communities and their lin-

eages is probably a common one. We speculate that this community structure provides strong stabilizing selection on each of its component lineages. Although evolutionary transitions among ecomorphs in *Desmognathus* should be accomplished easily by changes in length of the larval period and age at maturity, these forms show enormous phylogenetic stability. We predict that the historical pattern of diversification shared by *Anolis*, Australian agamids, and dusky salamanders is common in adaptive radiations in which an evolutionarily stable community structure constrains diversification of the codistributed lineages. Continental refugial areas in southeastern North America and the large islands of the Greater Antilles provide two examples of such regions. Comparative studies of other evolutionary radiations using the phylogeographic, morphometric, and community-ecological analyses presented here for *Desmognathus* can test the generality of this evolutionary pattern and its ecological and biogeographic explanations.

Acknowledgments

We thank C. Camp, E. Casey, K. Irwin, L. Irwin, R. Makowsky, J. Marshall, L. Mead, D. B. Means, W. Savage, R. W. Van Devender, K. Zamudio, and the Museum of Vertebrate Zoology for help obtaining samples for genetic analysis. The Georgia Museum of Natural History, Kansas University Museum of Natural History, and the University of Texas at Arlington Collection of Vertebrates provided specimen loans for our morphometric analyses. R. Blaine, M. Gifford, R. Glor, D. Weisrock, J. Wiens, and two anonymous reviewers provided helpful comments that improved the manuscript. The American Society of Ichthyologists and Herpetologist's Gaige Fund, Highlands Biological Station's Bruce Family and Charles Ash Scholarships, National Science Foundation Doctoral Dissertation Improvement grant DEB-0206739, and the National Center for Ecological Analysis and Synthesis supported this research.

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Corresponding Editor: K. Schwenk