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Niche Conservatism Drives Elevational Diversity Patterns in Appalachian Salamanders

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ABSTRACT: Many biodiversity hotspots are in montane regions, and many plant and animal groups have their highest species richness at intermediate elevations. Yet, the explanation for this hump-shaped diversity pattern has remained unclear because no studies have addressed both the ecological and evolutionary causes. Here, we address these causes in North American plethodontid salamanders, using a near-comprehensive phylogeny and environmental data. We develop a null model for assessing the relationship between the time that an area has been occupied and its species richness, and we apply a new approach that tests whether clades exhibit long-term stasis in their climatic niches (niche conservatism). Evolutionarily, the midelevation peak in species richness is explained by the time-for-speciation effect, with intermediate-elevation habitats seemingly being inhabited longest and accumulating more species. We find that this pattern is associated with evolutionary stasis in species' climatic niches, driving the midelevation peak by constraining the dispersal of lineages to environments at lower and higher elevations. These processes may help explain elevational diversity patterns in many montane regions around the world. The results also suggest that montane biotas may harbor high levels of both species diversity and phylogenetic diversity but may be particularly susceptible to rapid climate change.

Keywords: climate, elevation, niche conservatism, phylogeny, speciation, species richness.

Introduction

Understanding the underlying causes of species diversity patterns across the globe is afundamental goal in ecology. Elevational variation in species richness is ubiquitous (Lomolino et al. 2006), and many of the world's biodiversity hotspots are associated with montane regions (e.g., Myers et al. 2000). Numerous studies have identified environmental correlates of elevational variation in species richness (e.g., McCain 2005, 2007, 2009; Oömmen and Shanker 2005). However, it remains unclear how these environmental variables influence the biogeographic and evolutionary processes (speciation, extinction, and dispersal) that ultimately increase or decrease species diversity at different elevations (Ricklefs 2004; Wiens and Donoghue 2004).

In montane regions across the world, many diverse taxa reach their greatest species richness at intermediate elevations (e.g., Rahbek 1995, 1997; McCain 2005, 2007, 2009; Oömmen and Shanker 2005; Li et al. 2009). Although not all taxa share this pattern (e.g., many taxa also show declining diversity with increasing elevation), it is nevertheless widespread and is particularly interesting because intermediate elevations typically have smaller areas and lower temperatures than surrounding lowlands. One potential explanation for the midelevation peak is that it is simply the result of placing species ranges within a bounded geographic space (i.e., the elevational mid-domain effect; McCain 2004). However, recent studies suggest that the mid-domain effect is generally a poor predictor of elevational diversity patterns (e.g., McCain 2005, 2007, 2009; Smith et al. 2007; Wiens et al. 2007; Li et al. 2009).

Recently, two evolutionary explanations have been proposed for this hump-shaped pattern: the "montane species-pump" hypothesis and the "montane museum" hypothesis (Smith et al. 2007; Wiens et al. 2007). The montane species-pump hypothesis predicts that montane regions have high species richness because their topographic complexity and climatic zonation increase opportunities for allopatric and parapatric speciation (e.g., Moritz et al. 2000; Rahbek and Graves 2001; Hall 2005; Fjeldså and Rahbek 2006; Kozak and Wiens 2007). This could then lead to faster speciation and greater diversity at intermediate elevations than in lowlands or at the highest elevations. In contrast, under the montane museum hypothesis, more species occur at intermediate elevations simply because these elevations were occupied the longest (or the habitats that currently occur at these elevations were inhabited the longest) and there has been more time

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for speciation and the accumulation of species in these habitats relative to those at lower and higher elevations (i.e., the time-for-speciation effect; Stephens and Wiens 2003). Thus, it is not necessary to invoke elevational variation in diversification rates (i.e., rate of speciation − rate of extinction) under this model, unlike the montane species-pump hypothesis.

Recent evolutionary studies of elevational diversity patterns have found little support for either the mid-domain or montane species-pump hypotheses. Rather, they suggest that midelevation peaks in species richness may be caused primarily by the time-for-speciation effect (montane museum hypothesis). For example, the relative timing of colonization of different elevational bands strongly predicted elevational patterns of diversity in Mesoamerican salamanders (Wiens et al. 2007) and cyprinid fishes of the Tibetan Plateau (Li et al. 2009). In both groups, diversification rates of clades distributed in different elevational bands were similar, and the hump-shaped pattern of diversity could be explained by the early colonization of midelevation habitats and more recent dispersal of lineages to higher and lower elevations (resulting in less time for species to originate and accumulate, relative to intermediate elevations). Similarly, the midelevation peak for Mesoamerican hylid frogs is also correlated with time for speciation at intermediate elevations, although a tendency for montane clades to have higher diversification rates also contributes to this pattern (Smith et al. 2007).

Nevertheless, the ecological factors that underlie these evolutionary and biogeographic explanations for elevational variation in species diversity have not been studied. Recent studies posit that climatic specialization and retention of climatic tolerances over time (i.e., niche conservatism) might drive the time-for-speciation effect and the midelevation peak in species richness by limiting dispersal of ancestrally midelevation lineages to higher and lower elevations (Smith et al. 2007; Wiens et al. 2007; Li et al. 2009). Phylogenetic conservatism in climatic distributions might also interact with climate change to promote rapid allopatric speciation of montane endemics (e.g., Wiens 2004; Kozak and Wiens 2006), resulting in higher diversification rates in montane clades. However, the hypothesis that phylogenetic conservatism in the climatic niches of species underlies the midelevation peak in species richness has yet to be explicitly tested using phylogenetic, climatic, and distributional data.

Here, we address the underlying evolutionary and ecological causes of elevational species richness patterns in the Appalachian Highlands, a region that harbors exceptionally high diversity and endemism for many plant and animal groups (e.g., plants [Stein et al. 2000], crayfish [Crandall and Buhay 2008], mussels [Parmalee and Bogan 1998], spiders [Hedin 1997], freshwater fishes [Lundberg et al. 2000], and amphibians [Duellman and Sweet 1999; Kozak et al. 2009]). We focus on the plethodontid salamander fauna of the Appalachians and adjacent regions. This group is well suited for studying the ecological, evolutionary, and biogeographic causes of elevational diversity patterns, given the many previous molecular studies of their species limits (e.g., Tilley and Mahoney 1996; Highton and Peabody 2000; Kozak et al. 2005, 2006; Weisrock and Larson 2006), a time-calibrated phylogeny for nearly all species in eastern North America (Kozak et al. 2009), and the ability to rigorously quantify the elevational and climatic distributions of these species on the basis of thousands of georeferenced specimen localities in natural history museum collections.

In this study, we quantify how plethodontid diversity changes with elevation and show that regional species richness peaks at intermediate elevations (a widespread pattern in many groups and regions). We test for a relationship between an index of the relative timing of colonization of each elevational band and its current species richness (as predicted by the montane museum hypothesis and the time-for-speciation effect). We evaluate the significance of the observed time-for-speciation effect by using a null model in which species ranges are randomly placed along the available elevational gradient in eastern North America and are analyzed phylogenetically. Next, we test for a relationship between diversification rates and elevational distributions of lineages (as predicted by the montane species-pump hypothesis). We then test which climatic variables (if any) might set the elevational range limits of montane plethodontid species. Finally, we compare the fit of alternative evolutionary models of stasis and divergence in species' climatic distributions to test whether phylogenetic niche conservatism may be an important ecological factor shaping elevational variation in species richness.

Material and Methods

Elevational Variation in Species Richness

To quantify the elevational distributions of species, we used DIVA-GIS, version 5.2 (http://diva-gis.org), to map 9,780 georeferenced collection localities obtained from the U.S. National Museum of Natural History, the Museum of Vertebrate Zoology (University of California, Berkeley), and published systematic studies with georeferenced collection localities. These localities summarize the distributions of 66 of the 73 described plethodontid species of eastern North America (east of the Mississippi River) and 18 distinct phylogeographic lineages not yet formally described as separate species (for species and sample sizes, see the appendix in the online edition of the *American Naturalist*). These phylogeographic lineages were recognized on the basis of extensive analyses of the genera *Eurycea* and *Desmognathus* using explicit methods for species delimitation (e.g., Wiens and Penkrot 2002) based on the integration of mitochondrial DNA genealogies, geographic information, patterns of allozyme variation, and morphological distinctness (see Kozak et al. 2005, 2006). Many of these phylogeographic lineages are also distinct, based on phylogenetic analysis of nuclear genes (e.g., Kozak et al. 2009). Most of the missing species (e.g., *Desmognathus abditus*, *Eurycea wallacei*, *Gyrinophilus gulolineatus*, *Gyrinophilus palleucus*, *Gyrinophilus subterraneus*, *Urspelerpes brucei*, and *Plethodon sherando*) have very restricted geographic and elevational distributions (Anderson and Tilley 2003; Highton 2004; Camp et al. 2009; C. Camp and M. Niemiller, personal communication). Importantly, these missing species are concordant with the overall elevational diversity patterns for their respective clades (low-elevation spelerpines, midelevation *Desmognathus* and *Plethodon*).

We extracted the elevation of each locality from the WorldClim database, with 1-km^2 spatial resolution (Hijmans et al. 2005). Elevational variation in species richness at the regional scale (the focus of most previous studies) was summarized using elevation bands of 200-m width (ranging from 0 to 2,000 m) and counting the number of species with collection locations distributed within each band. We plotted elevational diversity including all species and then separately for each of the three major clades of plethodontid salamanders in eastern North America (*Desmognathus*, *Plethodon*, and Spelerpinae: *Eurycea* + *Gyrinophilus* + *Pseudotriton* + *Stereochilus* + *Urspelerpes*). We also tested for a correlation between log area and log species richness of each 200-m elevational band, using linear regression in JMP, version 7.0 (defining the region as the United States east of the Mississippi). To explore the potential impact of using a somewhat coarse digital elevation model (DEM), we compared elevations from WorldClim with those from the U.S. Geological Survey (DEM) with 30-m spatial resolution for a subset of collection locations ($n = 375$) in a region of extreme topographic heterogeneity (Great Smoky Mountains National Park). We found that using a 30-m DEM does not result in significantly different estimates of topographic variance or mean elevation for collection localities (Kolmogrov-Smirnov test, $P = .424$). We therefore used the coarser-resolution DEM to provide data with matching spatial resolution for elevation and climate.

According to some authors, the midelevation diversity peak may reflect random placement of species ranges along an elevational gradient (i.e., mid-domain effect; Colwell and Hurtt 1994; but see McCain 2005, 2007, 2009; Smith et al. 2007; Wiens et al. 2007; Li et al. 2009). To address this issue for our data, we used the program Mid-Domain Null (McCain 2004) to shuffle the elevational ranges of species between the minimum and maximum elevations possible in eastern North America. Ten thousand randomizations were used to generate the 95% confidence limits for the expected richness of each elevational band, given the random placement of elevational midpoints within the two elevational extremes. We tested the fit between the observed values and the expected values under the mid-domain model by using linear regression in JMP, version 7.0.

Time-for-Speciation and Species Richness

The time-for-speciation hypothesis predicts that more species will occur in regions and habitats (e.g., elevational bands) that have been colonized for longer periods of time (Stephens and Wiens 2003). To test this prediction, we reconstructed ancestral elevational distributions for each node on a strongly supported, time-calibrated phylogeny for Plethodontidae (Kozak et al. 2009) and tested for a relationship between an index for the relative time that a given elevational band has been occupied (based on elevational midpoints of species) and the number of species currently occurring in that band. The phylogeny includes 66 of the 73 known plethodontid species in eastern North America (all but the cave-dwelling species *G. gulolineatus*, *G. palleucus*, *G. subterraneus*, and *E. wallacei* and the more recently described species *D. abditus*, *P. sherando*, and *U. brucei*). As mentioned above, these missing species occur at elevations concordant with the overall elevational diversity patterns for their respective clades. We acknowledge that there is some uncertainty in estimating absolute ages of clades. However, our focus here is on relative ages of clades, and these relative ages appear to be largely insensitive to different assumptions regarding the absolute age of plethodontids (Wiens 2007).

We used the elevational midpoints of species to estimate the approximate ancestral elevational distribution for each node in the chronogram, using phylogenetic generalized least squares (PGLS) and assuming a single-peak Ornstein-Uhlenbeck (OU) model, which fitted the elevational data significantly better than a Brownian motion model. To reconstruct the elevational midpoints of ancestral nodes using the OU model, we set the strength of the restraining force, α, to its maximum likelihood value (Martins 2004). We focused on species' elevational midpoints, given the strong positive relationship between the species richness of elevational bands and the number of species with elevational midpoints that occur in that band ($r^2 = 0.65$, $P = .01$) but not the number of species with minimum ($r^2 = 0.04$, $P = .55$) or maximum ($r^2 = 0.05$, $P = .52$) elevations that occur in each band. In other words, only elevational midpoints of species seem to parallel the pattern of elevational diversity we seek to explain. We also conducted the analyses using species' mean elevations.

As a rough index of the relative amount of time that each elevational band has been occupied (see caveats below), we used the age of the oldest node with a reconstructed elevational midpoint (or mean elevation) occurring in a given elevational band. For example, for the elevational band 0–200 m, we inspected the tree for the oldest clade reconstructed as having an elevational midpoint within this band and then recorded the crown group age of this clade. Only nodes that could unambiguously be assigned as occurring in eastern North America were counted; plethodontids are widely distributed, but almost all species in eastern North America belong to three clades that are largely endemic to the region (*Desmognathus*, eastern *Plethodon*, Spelerpinae). In the few cases where an extant species appeared to be the first occupant of an elevational band, we arbitrarily assumed that the time of colonization occurred halfway between the present day and the estimated time of origin of the species (i.e., the time of the split with its sister species).

Next, we used linear regression to examine the relationship between our index for the relative time that an elevational band has been occupied (calculated as the age of the oldest elevational midpoint event for an elevational band) and the number of species currently in that band. This analysis included elevational bands from 0 to 1,600 m, given that no species have elevational midpoints (or mean elevations) higher than 1,600 m. We log transformed the number of species (given that species richness within a clade increases exponentially over time; Magallón and Sanderson 2001) and time (given the nonlinear relationship between log species richness and time). Because an elevational band may acquire species through multiple colonization events, we also performed an analysis using the summed ages of each inferred colonization of each band (again, using elevational midpoints) rather than the oldest age. Given the different patterns of elevational diversity among the three clades (fig. 1), we also conducted analyses separately for *Desmognathus* + *Plethodon* (hump shaped) and Spelerpinae (monotonic decline).

We make several caveats about this set of analyses. First, given that montane habitats have shifted up and down mountain slopes over time, use of reconstructed elevational midpoints (or means) may not reflect the actual time at which each elevation was colonized. Nevertheless, they can potentially indicate, for example, that lineages that are today predominately montane gave rise to those that today occur predominately in the lowlands, leaving less time for speciation to occur and species to accumulate in lowland habitats. Previous analyses (e.g., Kozak and Wiens 2006) also suggest that although species move up and down in elevation over time, they may still remain confined to a given elevational climatic zone over millions of years (see also "Results").

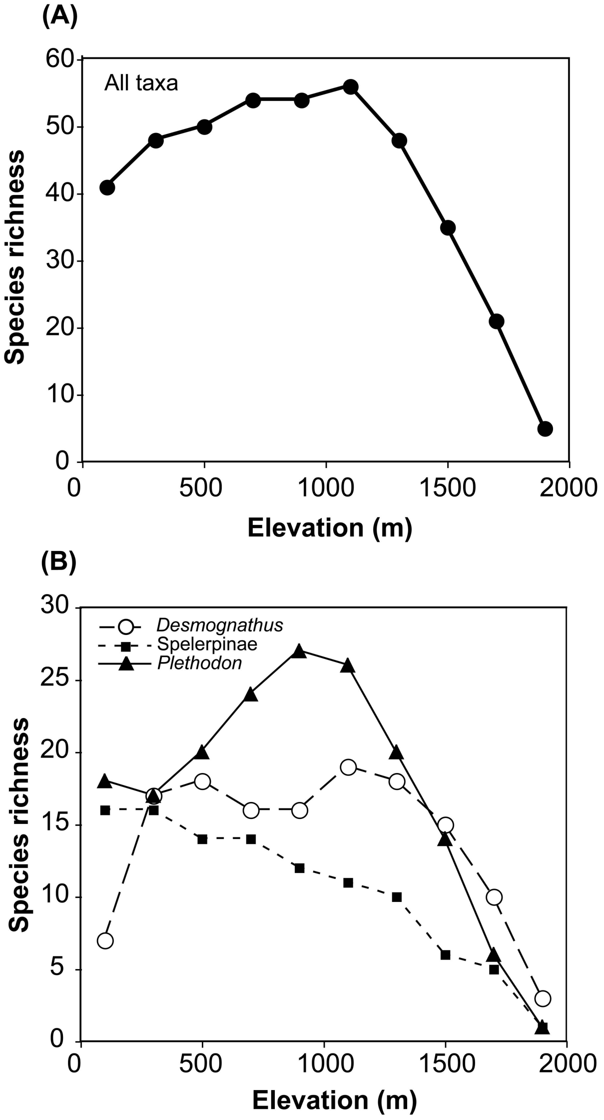


Figure 1: *A*, Elevational patterns of species richness for all eastern North American plethodontid salamanders. *B*, Elevational patterns of species richness plotted separately for each of the three major clades of plethodontids that occur in eastern North America.

Second, continuous-trait methods (such as PGLS) might reconstruct a clade as having a montane ancestor simply because the clade contains many montane species, not because the clade is ancestrally montane. Similarly, ancestral reconstruction methods might also be biased toward reconstructing intermediate elevations for the root of a clade, regardless of whether midelevation habitats are ancestral for the clade. We used two approaches to address these possibilities. First, we performed elevational reconstructions using a very different method (that should be robust to this issue) and compared the results to those from PGLS. We coded elevational distribution as a binary character instead (elevational midpoint >500 m arbitrarily considered montane) and reconstructed the ancestors of key clades using maximum likelihood (Mk model; Lewis 2001) in Mesquite, version 2.71 (Maddison and Maddison 2009). In general, results from discrete coding paralleled those from PGLS; clades reconstructed by PGLS as occurring at >500 m were supported as montane by discrete coding (i.e., much higher probability of montane than lowland for the ancestor of eastern *Plethodon* and *Desmognathus*, with significant support for a montane ancestor in the most species-rich clades within these two genera: the *cinereus* and *glutinosus* groups of *Plethodon* and the ancestor of all *Desmognathus* above *Desmognathus wrighti*). These observations suggest that the reconstruction of montane ancestors is not simply an artifact of PGLS reconstruction. Further, repeating this analysis using midpoints >1,000 m as montane indicates that the ancestors of the major clades had elevational midpoints >500 but <1,000 m, as shown by PGLS.

We also used a second, simulation-based approach to more directly test whether an apparent time-for-speciation effect might arise as a methodological artifact of continuous ancestral trait-reconstruction methods reconstructing midelevations as ancestral. We used the program Mid-Domain Null (McCain 2004) and the empirical range–size frequency distribution to simulate 1,000 data sets in which species ranges were randomly placed between the observed maximum and minimum elevations in eastern North America (i.e., no time-for-speciation effect). For each simulated data set, we extracted the elevational midpoint of each species' range and then reconstructed the ancestral elevational midpoint for each node on the chronogram by using PGLS. We also tallied the number of simulated species occurring in each elevational band. We then calculated the correlation between the time that an elevational band has been occupied (estimated from the reconstructed elevational midpoints) and its simulated species richness. If fewer than 5% of the simulated data sets had F values greater than that calculated for the actual data set, we rejected the hypothesis that the observed time-for-speciation effect for eastern North American plethodontid salamanders is simply an artifact of PGLS ancestral reconstruction. Ancestral reconstructions and linear regressions of the relationship between time and species richness for each of the 1,000 simulated data sets were conducted in R, version 2.7.1.

Finally, traits that influence speciation and extinction rates may also influence reconstructions for those traits (Maddison 2006). We address this possibility by testing whether elevational distribution influences rates of diversification.

Diversification Rates and Elevational Distributions of Clades

The montane species-pump hypothesis predicts that the midelevation peak in species richness is associated with increased rates of net diversification (speciation − extinction) in clades occurring in montane regions. New methods (BiSSE model; Maddison et al. 2007) now allow estimation of the influence of a trait on rates of speciation and extinction, at least for binary characters. We tested the influence of montane distribution on rates of speciation, extinction, and net diversification, using Mesquite, version 2.71. Montane distribution was coded in two ways. First, we used the elevational midpoint (species with midpoint >500 m considered montane). Second, we used the minimum elevation (minimum elevation >500 m considered montane). The two gave similar results, and only those for elevational midpoints are presented. For each variable (i.e., speciation rate, extinction rate, net diversification rate), we separately compared the overall likelihood when the parameter was constrained to be equal in montane and lowland lineages (e.g., a single speciation rate) and when it was allowed to differ (e.g., separate speciation rates for montane and lowland lineages). Following Maddison et al. (2007), we assumed that log likelihood differences (× 2) between constrained (one rate) and unconstrained (two rates) models follow a χ^2 distribution with 1 df (corresponding to the difference in number of parameters). For these analyses, we included only clades that occur primarily in eastern North America (88 species total). We also tested the effects of coding species with midpoints >1,000 m as montane, but this gave generally similar results (i.e., no significant effect of elevational distribution on speciation, extinction, or net diversification rates), and so we present only results using 500 m. We performed analyses including all species in eastern North America together and then performed analyses treating Spelerpinae, eastern *Plethodon*, and *Desmognathus-Phaeognathus* separately.

We also performed a set of analyses after adding six missing species (i.e., not in the phylogeny) to the tree. The three additional *Gyrinophilus* were placed with *Gyrinophilus porphyriticus* (Niemiller et al. 2008), *E. wallacei* was placed with the *Eurycea bislineata* complex (P. Chippindale, personal communication), *Urspelerpes* was placed as the sister taxon to *Eurycea* (Camp et al. 2009), and *D. abditus* was placed in a clade with *Desmognathus carolinensis* and *Desmognathus conanti* (Anderson and Tilley 2003). However, the branch lengths for these added taxa were unknown, and the tree was therefore re-ultrametri-

Table 1: Summary of plethodontid species richness, estimated timing of colonization (based on elevational midpoints), and area for 200-m elevational bands in eastern North America

Elevational band (m)	Area (km^2)	Species richness	Time first colonized (myr)	Summed ages of colonization events (myr)
0–200	2,216,510	40	10.35	45.43
200–400	1,743,974	48	18.45	30.81
400–600	539,701	50	37.57	63.37
600–800	112,027	54	23.14	87.79
800–1,000	42,307	54	36.90	111.81
1,000–1,200	13,754	56	15.59	42.95
1,200–1,400	4,034	45	11.94	39.18
1,400–1,600	1,056	33	7.21	7.21

Note: myr = millions of years before present. Area of each elevational band was calculated using DIVA GIS, version 5.2, and the digital elevation model in the WorldClim data set with ~1-km^2 spatial resolution.

cized using Mesquite. These results are preliminary because the placement of some of these species is uncertain (especially *D. abditus*) and the branch lengths may not be accurate. However, these analyses gave results very similar to those using only the species in the chronogram of Kozak et al. (2009).

A disadvantage of the BiSSE approach is that it requires treating continuous elevational distributions as a binary character. Therefore, we also tested whether the elevational distributions of species influence rates of diversification, using a new method for continuous traits (Freckleton et al. 2008). This approach examines whether the number of nodes (*N*) linking extant species to the root of a phylogeny (i.e., net diversification rate of each root-to-tip lineage) is correlated with variation in a continuous trait. Following Freckleton et al. (2008), we used PGLS (with COMPARE) to calculate the correlation between log *N* and species' elevational midpoints (using minimum elevations gave similar results; not shown). As in the BiSSE analyses, we performed these tests including all species in eastern North America together and then on the Spelerpinae, eastern *Plethodon*, and *Desmognathus-Phaeognathus* clades separately. All previous studies of diversification rates and elevational diversity used clade-level estimates of diversification rates (i.e., using clade ages and known species numbers), given that the available phylogenies included only some of the species in each clade (Smith et al. 2007; Wiens et al. 2007; Li et al. 2009). However, this approach requires arbitrarily delimiting clades and may ignore important variation within clades.

Testing for Phylogenetic Niche Conservatism in the Climatic Distributions of Species

To address the hypothesis that limited evolution of climatic tolerances helps drive the midelevation peak in species richness by restricting dispersal between elevational zones, we tested for phylogenetic conservatism in the climatic distributions of species. Our goals were threefold: (1) to identify climatic variables that may set the elevational range limits of plethodontid species, (2) to explore whether these potentially limiting variables are conserved among closely related species across the phylogeny, and (3) to test whether evolutionary stasis associated with tolerance to climatic conditions at only intermediate elevations might drive the midelevation peak in species richness by constraining the dispersal of lineages to other climatic zones. We focus on factors limiting dispersal of montane species into lowlands, given that the major alternative to the hump-shaped pattern is to have higher diversity in lower elevations (e.g., the lowlands are also the elevational zone with the greatest area; table 1).

To identify climatic niche variables that may limit dispersal of montane endemics into lowlands, we used logistic regression (JMP, ver. 7.0). Specifically, we tested the extent to which five temperature and precipitation variables from the WorldClim data set with ~1-km^2 spatial resolution (Hijmans et al. 2005; see appendix) could correctly discriminate between known presence locations of 13 montane endemic species and nearby absence locations (lowland localities where plethodontids have been found but not the montane endemics in question). These 13 species were selected because they are montane (minimum elevation >500 m) and had absence localities within 25 km of their range limits. The five climatic variables were chosen from a set of 19, based on a previous study on Appalachian plethodontids, which suggested that these five variables limit dispersal of montane endemics into adjacent lowland habitats (Kozak and Wiens 2006).

Our results in this study show that three climatic variables (Bio2 = mean diurnal temperature range [mean of

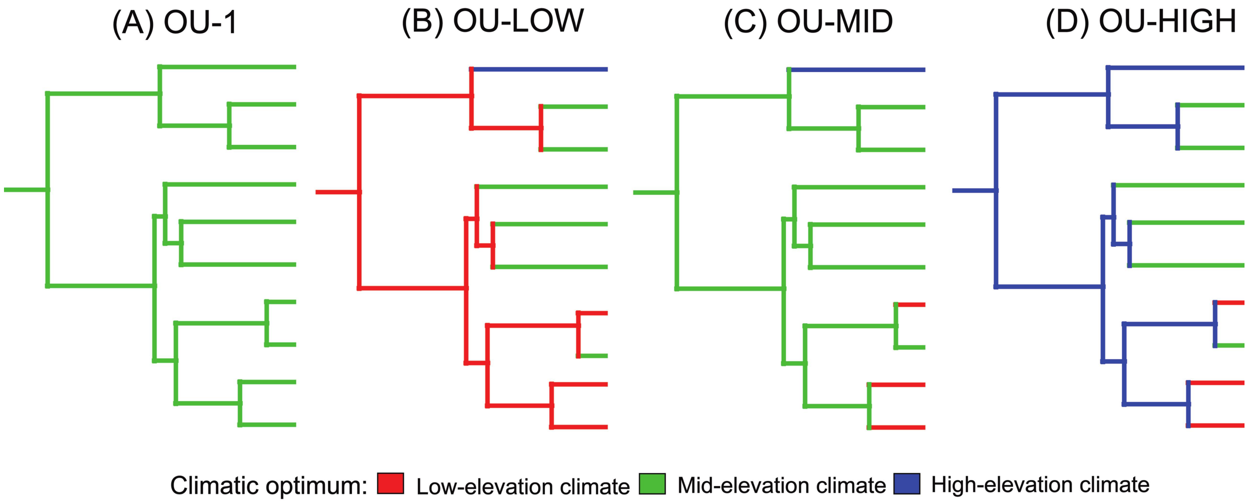


Figure 2: General representation of the Ornstein-Uhlenbeck models. *A*, Single (global) climatic optimum; *B–D*, separate climatic optima for species inhabiting low-, mid-, or high-elevation habitats. The latter three models also test whether the ancestral adaptive optimum for the clade was a low-, mid-, or high-elevation climatic regime, respectively.

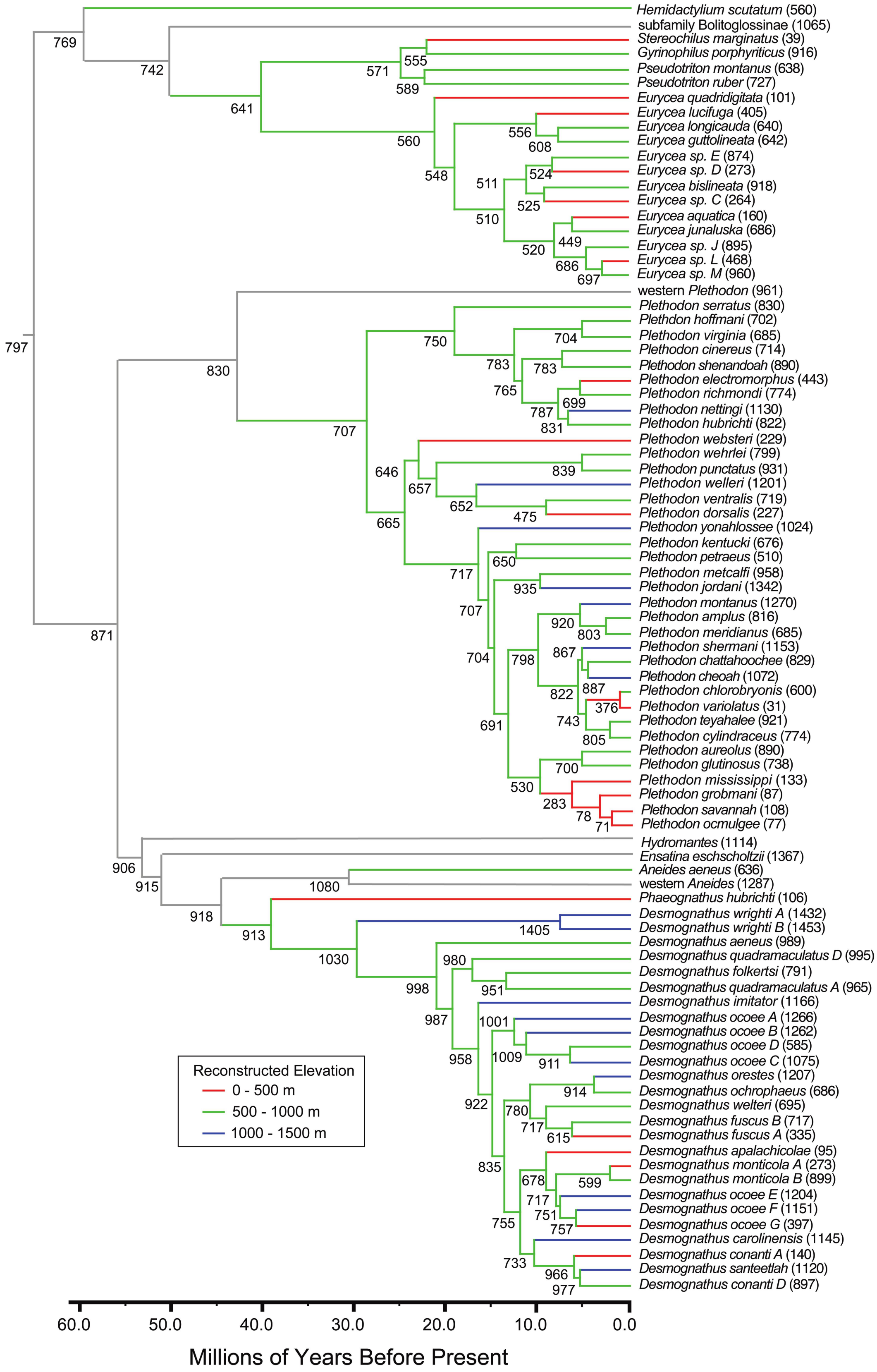
monthly max. temp. − min. temp.], Bio5 = maximum temperature of warmest month, and Bio17 = precipitation in driest quarter) consistently predicted the lower-elevational limits of all montane endemics and discriminated among the absence and presence locations most strongly (as evidenced by χ^2 values and the area under the receiving operator characteristic; see appendix). We therefore calculated the mean values for Bio2, Bio5, and Bio17 for each species in eastern North America, which we then used in all subsequent analyses.

If climatic niche conservatism underlies the time-for-speciation effect and the midelevation peak in species richness by constraining the dispersal of ancestrally midelevation lineages to lower and higher elevations, then one might expect significant phylogenetic signal for climatic variables that limit species' elevational distributions (as in a model of gradual evolutionary divergence under Brownian motion). Alternatively, species might exhibit evolutionary stasis or stabilizing selection for an ancestral climatic niche that is now located at midelevations (i.e., little or no change over time). This latter pattern would offer even stronger support for a role for climatic niche conservatism in generating and maintaining the midelevation peak in species richness. However, because evolutionary stasis erodes the relationship between phylogenetic and ecological divergence (Hansen 1997; Martins and Hansen 1997; Revell et al. 2008), failing to find phylogenetic signal may incorrectly suggest that there was no phylogenetic niche conservatism. Thus, an approach is needed that can distinguish between models of evolutionary stasis, phylogenetic signal, and the lack of either (random noise).

Here, we apply the general approach of Butler and King (2004) to test for phylogenetic niche conservatism. We calculated the log likelihoods of six different models of evolution for species climatic distributions, given their phylogenetic relationships and divergence times. The first two models are the simplest and do not explicitly incorporate adaptive constraints (i.e., evolutionary stasis associated with different climatic regimes). These were a white noise (WN) model of random variation, in which the similarity of species is independent of their phylogenetic relationships, and a Brownian motion (BM) model of gradual drift in species' climatic distributions.

To test for evolutionary stasis in species climatic distributions, we implemented four different OU models. OU models describe constrained character evolution in which traits are "pulled" toward an optimum value (or different optimum values in the case of multiple optima). The sim-

Figure 3: Time-calibrated phylogeny for eastern North American plethodontids from Kozak et al. (2009). Coloring of branches depicts the generalized elevational distribution of species and reconstructed ancestors (based on phylogenetic generalized least squares reconstruction, with the constraint parameter, α, set to its maximum likelihood estimate). The elevational midpoint of each species (m) is shown in parentheses. The reconstructed ancestral elevational midpoints are shown under each branch. Gray branches refer to taxa that do not occur in eastern North America or ancestral lineages that cannot be unambiguously reconstructed as occurring in eastern North America.


Hemidactylium scutatum (560)
subfamily Bolitoglossinae (1065)
Stereochilus marginatus (39)
Gyrinophilus porphyriticus (916)
Pseudotriton montanus (638)
Pseudotriton ruber (727)
Eurycea quadridigitata (101)
Eurycea lucifuga (405)
Eurycea longicauda (640)
Eurycea guttolineata (642)
Eurycea sp. E (874)
Eurycea sp. D (273)
Eurycea bislineata (918)
Eurycea sp. C (264)
Eurycea aquatica (160)
Eurycea junaluska (686)
Eurycea sp. J (895)
Eurycea sp. L (468)
Eurycea sp. M (960)
western Plethodon (961)
Plethodon serratus (830)
Plethdon hoffmani (702)
Plethodon virginia (685)
Plethodon cinereus (714)
Plethodon shenandoah (890)
Plethodon electromorphus (443)
Plethodon richmondi (774)
Plethodon nettingi (1130)
Plethodon hubrichti (822)
Plethodon websteri (229)
Plethodon wehrlei (799)
Plethodon punctatus (931)
Plethodon welleri (1201)
Plethodon ventralis (719)
Plethodon dorsalis (227)
Plethodon yonahlossee (1024)
Plethodon kentucki (676)
Plethodon petraeus (510)
Plethodon metcalfi (958)
Plethodon jordani (1342)
Plethodon montanus (1270)
Plethodon amplus (816)
Plethodon meridianus (685)
Plethodon shermani (1153)
Plethodon chattahoochee (829)
Plethodon cheoah (1072)
Plethodon chlorobryonis (600)
Plethodon variolatus (31)
Plethodon teyahalee (921)
Plethodon cylindraceus (774)
Plethodon aureolus (890)
Plethodon glutinosus (738)
Plethodon mississippi (133)
Plethodon grobmani (87)
Plethodon savannah (108)
Plethodon ocmulgee (77)
Hydromantes (1114)
Ensatina eschscholtzii (1367)
Aneides aeneus (636)
western Aneides (1287)
Phaeognathus hubrichti (106)
Desmognathus wrighti A (1432)
Desmognathus wrighti B (1453)
Desmognathus aeneus (989)
Desmognathus quadramaculatus D (995)
Desmognathus folkertsi (791)
Desmognathus quadramaculatus A (965)
Desmognathus imitator (1166)
Desmognathus ocoee A (1266)
Desmognathus ocoee B (1262)
Desmognathus ocoee D (585)
Desmognathus ocoee C (1075)
Desmognathus orestes (1207)
Desmognathus ochrophaeus (686)
Desmognathus welteri (695)
Desmognathus fuscus B (717)
Desmognathus fuscus A (335)
Desmognathus apalachicolae (95)
Desmognathus monticola A (273)
Desmognathus monticola B (899)
Desmognathus ocoee E (1204)
Desmognathus ocoee F (1151)
Desmognathus ocoee G (397)
Desmognathus carolinensis (1145)
Desmognathus conanti A (140)
Desmognathus santeetlah (1120)
Desmognathus conanti D (897)
769
742
641
571
555
589
560
548
556
608
511
524
525
510
520
449
686
697
797
830
707
750
704
783
783
765
787
699
831
646
657
839
652
475
665
717
650
935
707
920
803
704
798
867
822
887
376
743
805
691
700
530
283
78
71
871
906
915
1080
918
913
1405
1030
980
998
951
987
1001
958
1009
911
914
922
780
717
615
835
678
599
717
751
757
755
733
966
977
Reconstructed Elevation
0 - 500 m
500 - 1000 m
1000 - 1500 m
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Millions of Years Before Present

plest model (OU-1) tested whether all species in the clade occupy a single (global) climatic optimum. We also implemented OU models that had separate climatic optima for species inhabiting low-, mid-, or high-elevation habitats. In the case of OU-1, all branches (i.e., extant and ancestral species) were coded as belonging to single midelevation climatic optimum (fig. 2). For the three models with multiple climatic optima, terminal branches (i.e., extant species) were coded as occurring in one of three different optima based on whether the species occurred in a low-elevation (elevational midpoint = 0–500 m), midelevation (elevational midpoint = 500–1,000 m), or high-elevation (elevational midpoint = 1,000–1,500 m) climatic regime. All internal branches (i.e., ancestral species) were then assumed to occur in either the low-elevation (OU-LOW), midelevation (OU-MID), or high-elevation (OU-HIGH) optimum (fig. 2). This latter assumption allowed us to test whether the climatic distributions of species in the entire clade tend to be constrained to an ancestral climatic optimum that is currently located in low-elevation (OU-LOW), midelevation (OU-MID), or high-elevation (OU-HIGH) habitats.

We compared the fit of each of the six models to species mean values for Bio2, Bio5, and Bio17. If evolutionary stasis associated with an ancestral climatic niche that is currently at midelevations drives the midelevation peak by constraining the dispersal of lineages to lower and higher elevations, one would expect either OU-1 or OU-MID to have the highest log likelihood. We used the OUCH (Butler and King 2004) and GEIGER (Harmon et al. 2008) packages in R to calculate the log likelihood of each model and to compare the fit of the WN, BM, and OU models of evolution to the climatic data. Given that we were interested in explaining high plethodontid species richness at midelevations in the Appalachians, we focused on the *Desmognathus* and eastern *Plethodon* clades (spelerpines exhibit low species richness at midelevations and high elevations; fig. 1). We compared the log likelihoods of all models using the sample size–corrected Akaike Information Criterion ($\mathrm{AIC_c}$). We note that for these analyses, we assume that there is a relationship between climatic distributions and climatic tolerances of species and that the trait that actually evolves is the physiology that underlies these climatic tolerances and distributions.

Results

Plethodontid salamanders in eastern North America exhibit the widespread hump-shaped pattern of species richness, with the greatest number of species occurring at intermediate elevations (fig. 1*A*). Two major clades drive this pattern (fig. 1*B*); *Desmognathus* and *Plethodon* both show strong midelevation peaks, whereas Spelerpinae exhibits a monotonically declining pattern with elevation. There is a poor fit between the number of species observed in each elevational band and the expected values under the middomain model ($r^2 = 0.30$, $P = .10$). Further, there is no relationship between the area of elevational bands and their species richness ($r^2 = 0.05$, $P = .35$).

The species richness of each elevational band and our index for the relative timing of colonization of each band are both shown in table 1. Reconstruction of ancestral elevational midpoints on the chronogram using PGLS and assuming the maximum likelihood estimate of the stabilizing selection parameter, α, suggest that clades have been present and diversifying longer at intermediate elevations than at higher and lower elevations (fig. 3). There is a strong relationship between our index for the relative time that an elevational band has been occupied (based on elevational midpoints) and the total number of extant species occurring in each band (fig. 4*A*). The relationship between time and species richness holds within *Desmognathus* and *Plethodon* (fig. 4*C*, 4*D*), which have midelevational peaks in diversity (fig. 1), but not in Spelerpinae (fig. 4*E*, 4*F*). This relationship is also strong when we use our index for the summed ages of colonization events (fig. 4*B*) and when the species' mean elevations are used instead of elevational midpoints ($R^2 = 0.73$, $P = .007$). The observed correlation between time and the species richness of each elevational band is much stronger than expected, given a null model of randomly placed elevational ranges ($P = .028$). Thus, the time-for-speciation effect does not appear to be a methodological artifact of PGLS ancestral reconstruction.

Analyses using the BiSSE model showed no significant differences in rates of speciation, extinction, or net diversification between lowland lineages (midpoint <500 m) and montane lineages, with $P > .10$ in all cases (but with a trend toward higher diversification rates in lowland species). Results were also nonsignificant when each of the three clades was analyzed separately and when six missing species were added to the tree. Similarly, the test of Freckleton et al. (2008) showed no significant relationship between rates of diversification and elevational midpoints of lineages whether the three clades were analyzed together or separately ($P > .60$ in all cases). Overall, these results suggest that high montane diversity is not generated by higher diversification rates in montane lineages (especially given that montane lineages actually have somewhat lower diversification rates based on the BiSSE analyses) and that reconstruction of montane ancestors for most plethodontid clades in eastern North America is not an artifact of elevational variation in rates of speciation and extinction (i.e., BiSSE analyses suggest that the estimated diversification rates would bias methods to reconstruct a lowland ancestor, not a montane ancestor).

We compared the fit of six alternative models for the

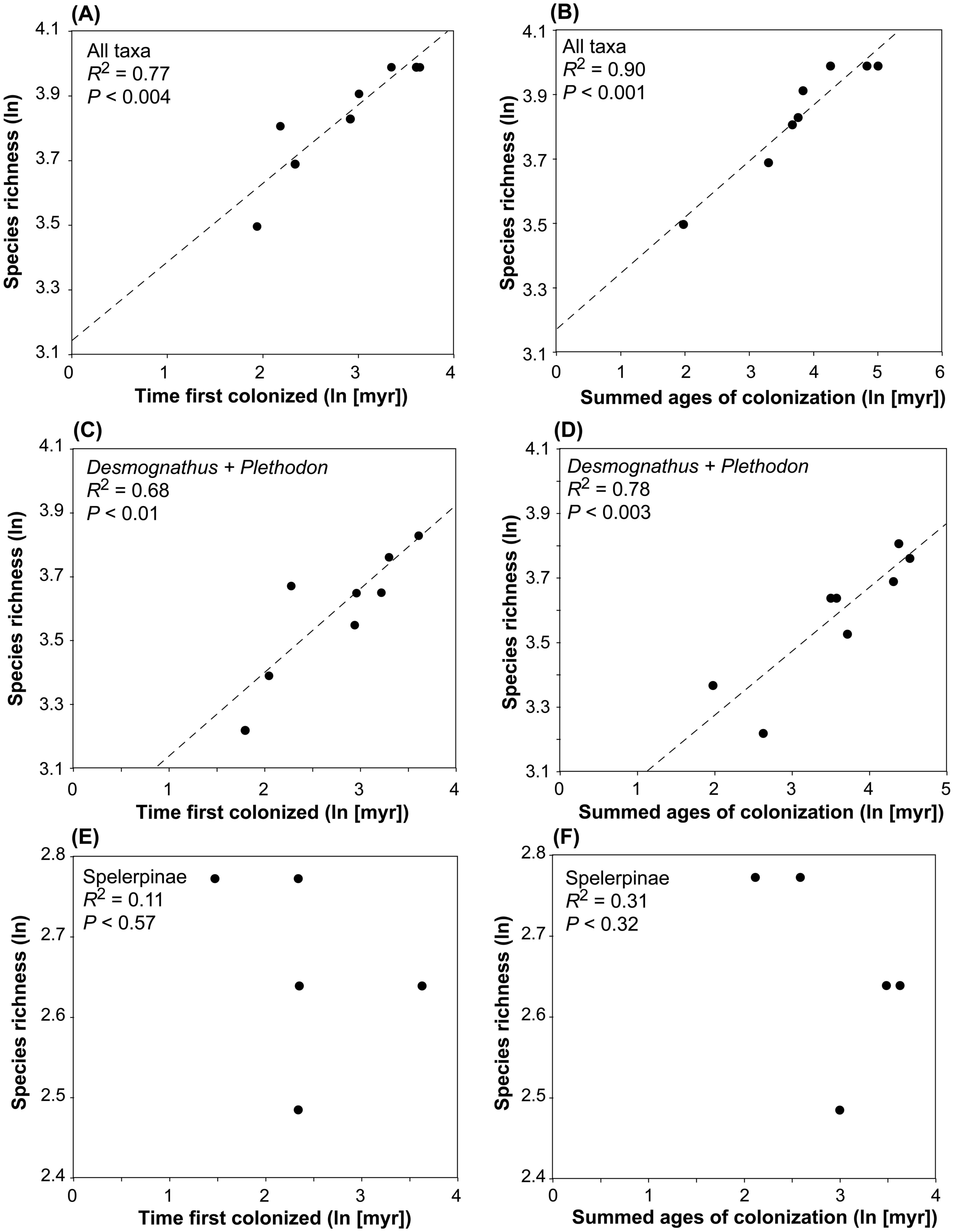


Figure 4: Relationship between the species richness of 200-m elevational bands and our index for the relative length of time that each band has been occupied, based on elevational midpoints (myr = millions of years before present). *A*, *C*, *E*, Species richness versus the relative time at which each band was first colonized (i.e., age of the oldest colonization event) for all taxa in eastern North America (*A*), for *Desmognathus* + *Plethodon* (*C*), and for Spelerpinae (*E*). *B*, *D*, *F*, Species richness versus the summed ages of colonizations of each elevational band for all taxa (*B*), for *Desmognathus* + *Plethodon* (*D*), and for Spelerpinae (*F*).

Table 2: Fit of alternative models of divergence and stasis in species' climatic distributions

Clade/variable	WN	BM	OU-1	OU-LOW	OU-MID	OU-HIGH
Desmognathus:						
Bio2	.00	.00	.00	.06	**.90**	.04
Bio5	.00	.00	.00	.00	**.89**	.11
Bio17	.00	.00	.05	.10	**.46**	.39
Eastern *Plethodon*:						
Bio2	.00	.00	.03	**.61**	.33	.01
Bio5	.00	.00	.00	.00	**.99**	.01
Bio17	.00	.00	.03	.21	**.70**	.05

Note: Sample size–corrected AIC weights (AIC_c) are reported for each model and for each variable. WN = white noise; BM = Brownian motion; OU = Ornstein-Uhlenbeck model. OU-1 tests for evolutionary stasis associated with a single (global) climatic optimum; OU-LOW, OU-MID, and OU-HIGH test for evolutionary stasis associated with separate climatic optima for species inhabiting low-, mid-, or high-elevation habitats, respectively. The last three models also test whether the ancestral climatic optimum for the entire clade was a low-, mid-, or high-elevation climatic regime, respectively. See "Material and Methods" for details on OU model construction. Bold indicates the best-fitting model.

evolution of species' climatic distributions in the *Desmognathus* and eastern *Plethodon* clades (table 2). We found no support for the WN, BM, or OU-1 models of evolution. Instead, OU models that assume separate adaptive optima for climatic regimes located at low, mid-, and high elevations have higher log likelihoods. In particular, models that assume evolutionary stasis associated with an ancestral climatic niche that is currently at midelevations (OU-MID) receive very strong support (table 2). This pattern is especially evident for Bio5 (maximum temperature), which receives AIC_c weights >0.90. The only exceptions to the generally strong support for the OU-MID model include Bio17 (precipitation in the driest quarter) for *Desmognathus* (OU-MID and OU-HIGH fit the data equally well) and Bio2 (mean diurnal temperature range) for eastern *Plethodon* (OU-LOW receives somewhat greater support). Overall, these results support the hypothesis that conservatism in species' climatic niches underlies the time-for-speciation effect and the midelevation peak in species richness.

Discussion

A major goal of ecology is to explain patterns of species richness, yet explaining these patterns generally requires considering both ecological and evolutionary processes (e.g., Ricklefs 2004; Wiens and Donoghue 2004). For example, even though species diversity often shows strong correlations with environmental variables, the only factors that can directly change species richness within a region are speciation, extinction, and dispersal. But simply quantifying geographic variation in these three processes is not sufficient, either. The challenge is to understand how variation in environmental variables acts on these three processes to create patterns of species diversity. In this article, we integrate ecological and evolutionary explanations for elevational diversity patterns.

Many previous studies have found a hump-shaped pattern in elevational diversity in various clades and regions, with higher regional diversity at intermediate elevations than at lower or higher elevations (e.g., Rahbek 1995; McCain 2009). Three recent studies have addressed the evolutionary causes of this pattern, and all three suggested that the time-for-speciation effect plays a major role (Smith et al. 2007; Wiens et al. 2007; Li et al. 2009). However, the underlying ecological causes of this montane museum hypothesis have not been explicitly addressed.

Our analyses of North American salamanders reveal a similar pattern and provide insights into its underlying evolutionary and ecological causes. Specifically, our reconstructions of elevational midpoints of species on the phylogeny (fig. 3) suggest that the ancestor of all extant plethodontids in eastern North America occurred predominately at intermediate elevations (or in habitats that today occur at intermediate elevations) and that species and clades occurring predominately at lower or higher elevations have colonized them more recently. This evolutionary pattern results in a strong positive correlation between the relative time that an elevational band has been occupied (based on elevational midpoints) and the species richness of each elevational band (i.e., a time-for-speciation effect). We also showed that the observed time-richness relationship is much stronger than expected, given a null model in which species ranges are randomly placed along the elevational gradient. Thus, the time-for-speciation effect observed for Appalachian plethodontids is not simply an artifact of current methods for ancestral reconstruction of continuous traits, such as PGLS. In contrast, we found no evidence that elevational patterns of species richness are caused by either higher speciation rates in montane regions or higher extinction rates in lowland habitats (e.g., Roy 1997; Moritz et al. 2000; Rahbek and Graves 2001; Fjeldså and Rahbek 2006). Together, these analyses suggest that the ecological question to address is not, What environmental variables promote greater net rates of diversification in montane habitats? Instead, the key question is, What prevents montane plethodontids from dispersing into lowland habitats?

Our results suggest that niche conservatism, the tendency of species to remain ecologically similar over time, limits dispersal of montane species into lowland habitats and creates and maintains the disparity between montane and lowland species richness over time. More specifically, we suggest that specialization of species to environmental conditions currently occurring at intermediate elevations limits their dispersal to other elevational zones and that conservatism in this climatic adaptation over time leads to a greater buildup of species in the ancestral environment (which is now present at intermediate elevations). We first showed that larger daily fluctuations in temperature (Bio2) and warmer and drier conditions in the lowlands (Bio5, Bio17) seemingly set the lower elevational limits of montane endemics (appendix). Our previous studies using ecological niche modeling on eight species pairs of montane endemics (Kozak and Wiens 2006) also support this hypothesis, as do physiological studies of montane species (Bernardo and Spotila 2006). We then showed that these climatic variables show a pattern consistent with evolutionary stasis in an ancestral climatic niche that is currently at midelevations, also the elevational zone with the greatest species richness. Evolutionary stasis is the macroevolutionary pattern that is the most consistent with the microevolutionary predictions of niche conservatism (e.g., Wiens and Graham 2005).

Further work is clearly needed on the mechanistic underpinnings of these patterns. We speculate that limited physiological tolerances (e.g., to high temperatures) are the proximate constraint on dispersal from the ancestral montane environment into lowland habitats. Some experimental work on *Desmognathus* supports the idea that lower-elevational range limits in montane taxa are set by their physiological inability to tolerate high temperatures (Bernardo and Spotila 2006; Bernardo et al. 2007), but more extensive physiological comparisons within and between species are needed. Although some evidence points to the importance of physiology, the role of competition in limiting the dispersal of montane species into the lowlands seems less clear. For example, distributional data show that some species that inhabit the lowlands are broadly sympatric with montane species, rather than competitively excluding them (Kozak and Wiens 2006). In other cases, ecological studies suggest that montane species are more aggressive than lowland species and seemingly exclude them from high-elevation habitats through interference competition (e.g., Nishikawa 1985; Anthony et al. 1997; Arif et al. 2007). Thus, it seems unlikely that the lowland species limit the elevational ranges of montane species. Although additional ecological and physiological work is needed, our phylogeny-based analyses provide insights into the direction that this future work should take in order to explain the widespread mid-diversity peak in species richness.

We suggest that long-term occupation of certain habitats is critical to explaining present-day diversity patterns, but we do not assume that climatic conditions have been constant during the diversification of plethodontids in eastern North America. Our results from elevational reconstructions (fig. 3) and phylogeny-based analyses of climatic distributions (table 2) suggest that climatic regimes currently at midelevations have been inhabited the longest by plethodontids. But this climatic zone has almost certainly shifted in elevation over time. For example, many species occurring today at intermediate elevations have sister species at similar elevations in adjacent mountain ranges but are separated by lowland habitats (e.g., Kozak and Wiens 2006). Rather than assuming that each species of these montane pairs separately evolved from lowland-adapted ancestors that became extinct, a simpler explanation is that climatic conditions now at midelevations were present at lower elevations in the past, when the climate was cooler, allowing their ancestors to be continuously distributed in the lowlands separating these mountain ranges. Similarly, we do not assume that midelevation habitats in the Appalachians are reconstructed as ancestral because elevations >500 m were miraculously colonized first, without the ancestors ever passing through lower elevations. Instead, we assume that these midelevation habitats occurred at lower elevations when the region was first colonized by these plethodontid clades and that the habitats (and corresponding salamanders) subsequently shifted to their current elevations.

Our results do not address why climatic conditions that occur at midelevations should be ancestral for these North American plethodontid clades. One possible explanation is that these conditions represent those that are ancestral for the family Plethodontidae. The large-scale climatic distribution of all salamanders suggests that most are temperate (except for the tropical bolitoglossine clade of plethodontids), and even in the temperate zone, they tend to avoid warmer and drier conditions by being aquatic (e.g., amphiumids, cryptobranchids, proteids, salamandrids, sirenids), fossorial (e.g., many ambystomatids), or a combination of the two (Pough et al. 2004). Other terrestrial, surface-dwelling salamanders occur in cool, moist environments (e.g., temperate rainforests in the Pacific Northwest), at either high latitudes or high elevations (e.g., dicamptodontids, hynobiids, rhyacotritonids). A second nonexclusive explanation is that plethodontids at lower elevations in eastern North America were driven to extinction more frequently than those at intermediate elevations, possibly due to climatic changes (e.g., drought). We would expect such a process to be reflected in significantly lower net diversification rates in low-elevation lineages in our study (high extinction rates decrease net diversification), but it may be that the available tests and data are not powerful enough to detect such a pattern.

Overall, our results suggest that elevational variation in species richness may be explained by the combination of the time-for-speciation effect and niche conservatism in climatic tolerances. This combination of factors may explain many other species richness patterns as well (e.g., Wiens and Donoghue 2004), including the typical latitudinal diversity gradient and the reverse latitudinal gradient (high temperate diversity; e.g., Pyron and Burbrink 2009). At present, few other studies have tested the importance of both factors by combining phylogenetic analyses of biogeography, diversification rates, and climatic data (but see Wiens et al. 2006). Integrative studies in other clades and regions are clearly needed. However, we acknowledge that there is a great diversity of species richness patterns and that this combination of factors will not explain them all. For example, the time-for-speciation effect does not seem to explain elevational patterns of species richness in all the major clades of plethodontids in eastern North America. We found no evidence for a time-for-speciation effect in the spelerpine clade, which exhibits a declining pattern of species richness with increasing elevation and in which almost all ancestral nodes have similar reconstructed elevational midpoints (fig. 3).

More generally, our study reveals a counterintuitive and underappreciated phenomenon that has not been widely addressed in the burgeoning literature on niche evolution, namely, that strong niche conservatism (i.e., stasis) may be present even if there is no phylogenetic signal (see also Revell et al. 2008). For example, we found that models that assume phylogenetic signal (i.e., Brownian motion) are rejected in favor of alternative models in which the phylogenetic and ecological divergence of species is decoupled as a result of evolutionary stasis. Thus, it may be misleading to draw conclusions about the lability or conservatism of the niche based on tests of phylogenetic signal alone (contra Losos 2008). However, the general model-comparing approach we have employed here can address whether a clade exhibits evolutionary stasis for the climatic conditions that are currently within the zone of greatest species richness.

Beyond illustrating how ecological and evolutionary processes interact to generate elevational patterns of species richness, our findings may also have important conservation implications. If hump-shaped patterns of species richness are frequently caused by the time-for-speciation effect and niche conservatism, then midelevation habitats should receive special priority for conservation, given that they will harbor greater species diversity and greater average phylogenetic diversity per species (i.e., montane lineages tend to be older and therefore more divergent). Furthermore, our results suggest that limited evolution of climatic tolerances in montane species (over millions of years) may play an important role in generating elevational diversity patterns, which raises the disturbing possibility that many of the species in these areas of high phylogenetic and species diversity may be particularly susceptible to rapid anthropogenic climate change.

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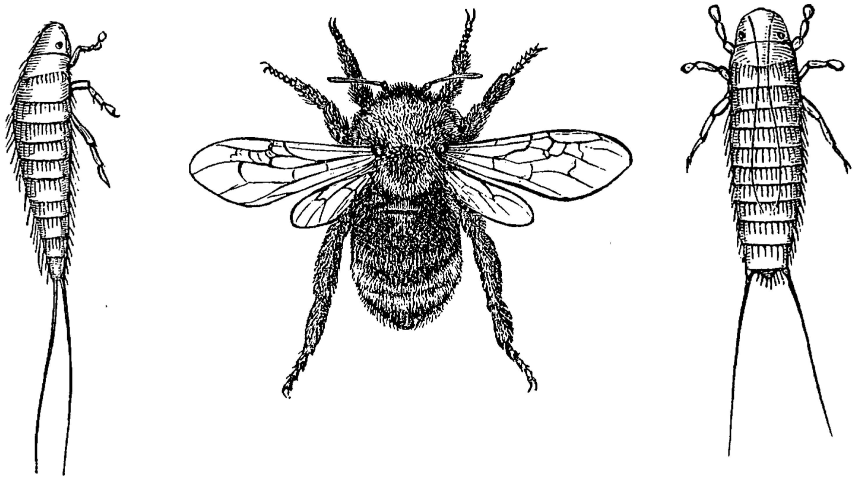

Center, *Apathus ashtoni*, which "lives cuckoo-like in the cells of the humble-bee, though we know not yet how injurious it really is." "Examining [*Stylops*] under a high power of microscope, we saw multitudes, at least several hundred, of very minute larvae." *Left*, side view. *Right*, as seen from above. From "The Parasites of the Honey-Bee" by A. S. Packard Jr. (*American Naturalist*, 1868, 2:195–205).