

DOES NICHE CONSERVATISM PROMOTE SPECIATION? A CASE STUDY IN NORTH AMERICAN SALAMANDERS

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Abstract.—Recent speciation research has generally focused on how lineages that originate in allopatry evolve intrinsic reproductive isolation, or how ecological divergence promotes nonallopatric speciation. However, the ecological basis of allopatric isolation, which underlies the most common geographic mode of speciation, remains poorly understood and largely unstudied. Here, we explore the ecological and evolutionary factors that promote speciation in *Desmognathus* and *Plethodon* salamanders from temperate eastern North America. Based on published molecular phylogenetic estimates and the degree of geographic range overlap among extant species, we find strong evidence for a role for geographic isolation in speciation. We then examine the relationship between climatic variation and speciation in 16 sister-taxon pairs using geographic information system maps of climatic variables, new methods for modeling species' potential geographic distributions, and data on geographic patterns of genetic variation. In contrast to recent studies in tropical montane regions, we found no evidence for parapatric speciation along climatic gradients. Instead, many montane sister taxa in the Appalachian Highlands inhabit similar climatic niches and seemingly are allopatric because they are unable to tolerate the climatic conditions in the intervening lowlands. This temporal and spatial-ecological pattern suggests that niche conservatism, rather than niche divergence, plays the primary role in promoting allopatric speciation and montane endemism in this species-rich group of vertebrates. Our results demonstrate that even the relatively subtle climatic differences between montane and lowland habitats in eastern North America may play a key role in the origin of new species.

Key words.—Amphibia, Appalachians, biodiversity informatics, climate, diversification, montane endemism, niche modeling, Plethodontidae, speciation.

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Understanding the processes that lead to the origin of new species is a fundamental goal of evolutionary biology (Darwin 1859; Mayr 1963; Coyne and Orr 2004; Futuyma 2005). In recent years there has been a renewed interest in the role of adaptive processes in speciation (Orr and Smith 1998; Schluter 2000; Smith et al. 2001; Funk et al. 2006). Empirical studies of a wide variety of taxa have demonstrated an important role for ecological divergence in sympatric (Feder 1998; Lu and Bernatchez 1999; Rundle et al. 2000; Schlieven et al. 2001; Via 2001) and parapatric speciation (Knox and Palmer 1995; Smith et al. 1997, 2001; Schneider et al. 1999; Ogden and Thorpe 2002; Richmond and Reeder 2002). Yet, despite an expanding literature on the role of ecology in nonallopatric speciation, the relationship between ecology, geographic isolation, and allopatric speciation remains poorly understood.

Given that allopatric speciation is widely considered to be the most common geographic mode (e.g., Barraclough and Vogler 2000; Turelli et al. 2000; Coyne and Orr 2004), it is surprising that its ecological and evolutionary causes have been neglected in speciation research. For example, vicariance may often occur when environmental change in the geographic space between two sets of populations (i.e., development of a geographic barrier) occurs more rapidly than adaptation to these new ecological conditions, resulting in the fragmentation of a species' ancestral geographic range (Wiens 2004a). Thus, in contrast to ecological models of nonallopatric speciation, incipient species may become geographically isolated because of the ecological similarity of their populations, rather than their ecological differences. The general tendency for species to retain similar ecological characteristics over evolutionary time scales is known as "phy-

logenetic niche conservatism" (Ricklefs and Latham 1992; Peterson et al. 1999; Webb et al. 2002; see review in Wiens and Graham 2005) and may play a key role in promoting vicariant isolation and speciation in landscapes where favorable habitats alternate with unfavorable habitats, such as in montane regions (Wiens 2004a,b; Kozak et al. 2006).

To date, few studies have adequately explored the relationship between niche conservatism and allopatric speciation (Wiens and Graham 2005). Recent studies on birds, mammals, butterflies, and frogs in tropical montane regions have found evidence both for and against niche conservatism (Peterson et al. 1999; Graham et al. 2004). However, these studies did not address whether the geographic isolation of sister lineages was actually caused by evolutionary conservatism in climatic niche characteristics. Graham et al. (2004) noted differences in the climatic niche space of sister species, but they did not consider whether these differences were the cause of lineage splitting or developed after speciation. For example, species on different mesic mountaintops may have adapted to somewhat different climatic conditions, but their initial isolation might have been caused primarily by a dry valley between them (Wiens and Graham 2005). Here, we take advantage of recently developed methods for estimating species' potential geographic distributions based on climatic variables (Guisan and Zimmerman 2000; Elith et al. 2006; Phillips et al. 2006) to more thoroughly investigate whether niche conservatism promotes allopatric speciation, using North American salamanders (*Desmognathus* and *Plethodon*) as our model system.

A variety of methods exist for modeling species' potential distributions from presence-only data and digital maps of environmental data (Guisan and Zimmerman 2000; Elith et

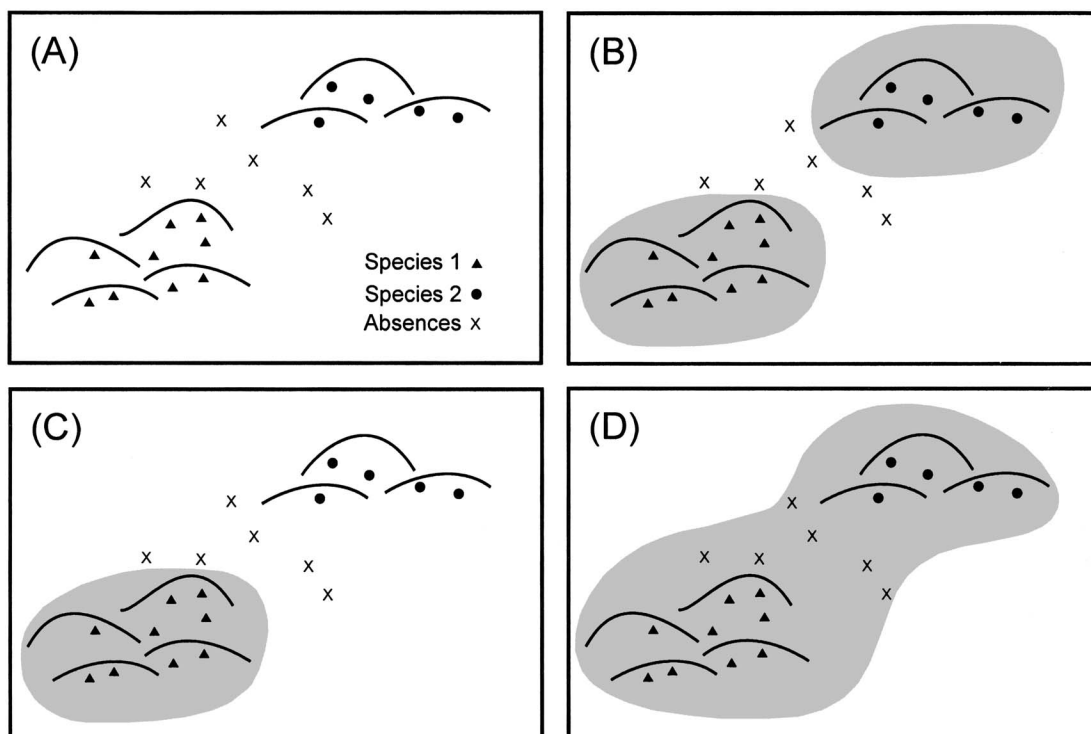


FIG. 1. Hypothetical example illustrating three spatial patterns of predicted climatic suitability and their potential implications for speciation mechanisms. (A) Extant sister species (species 1 and 2) presently are isolated in separate mountain ranges that are separated by intervening lowlands where other closely related species are found, but where species 1 and 2 are known to be absent. (B) Ecological niche modeling shows that the two species have similar climatic niches that do not encompass the intervening absence locations in the lowlands. This spatial pattern suggests that niche conservatism prevents dispersal of these species into the lowlands and may have caused their initial vicariance. (C) Species 1 and 2 do not have similar climatic niches, and the environmental conditions in the lowlands are outside of their climatic tolerances. This spatial pattern is consistent with the hypothesis that divergence in their climatic niches was involved in the origination and maintenance of their current range limits, although this scenario could also arise from niche divergence following allopatric speciation through niche conservatism (as in B). (D) Species 1 and 2 have similar climatic niches that encompass their intervening lowland absence localities. This spatial pattern suggests that factors other than climatic niche conservatism were involved in their origination and set their current range limits (e.g., other geographic and/or environmental factors not included in the models, competitors, predators, lack of time for secondary contact following vicariance). Illustration modified from Wiens and Graham (2005).

al. 2006). Referred to as ecological niche or distribution modeling, this approach uses geographic information system (GIS) methods to extract the environmental data associated with georeferenced sampling locations. The environmental data are then used to generate a model of habitat suitability in environmental space, which is then projected into geographic space to identify other grid cells with similar environmental characteristics. The resulting niche model can then be used to generate a map of the predicted potential distribution for the species based on climatic variables.

What is the expected empirical pattern if climatic niche conservatism drives allopatric isolation of lineages? Consider a pair of sister species that are distributed in two geographically isolated montane regions (Fig. 1A). Ecological niche modeling of their potential geographic distributions based on climatic variables is expected to produce one of three spatial patterns of habitat suitability (Wiens and Graham 2005). If niche conservatism promotes vicariance and maintains the species' present-day range limits, then their predicted distributions are expected to spatially overlap with each other but not with intervening locations where both species are absent (Fig. 1B). Alternatively, the species' potential distributions may exhibit no spatial overlap with each other or

with the intervening absence locations; this outcome is consistent with the hypothesis that niche divergence was important in their initial geographic isolation and maintains their current range limits (Fig. 1C). Finally, the species' potential distributions may exhibit spatial overlap with each other and also with the intervening absence locations (Fig. 1D). Such an outcome is expected if nonclimatic factors caused vicariance and set the contemporary range limits of species, including riverine barriers (for terrestrial species), competitors, predators, environmental variables not included in the model, and lack of time for secondary contact following vicariance.

Plethodontid salamanders of the genera *Desmognathus* and *Plethodon* are an excellent study system for examining the relationship between ecology and speciation. They generally occupy terrestrial (*Plethodon*, *Desmognathus*) to aquatic (streams, *Desmognathus*) microhabitats in moist, forested regions (Petranka 1998). Many closely related species are endemic to geographically disjunct montane areas in the eastern North American temperate zone (Highton 1995; Tilley and Mahoney 1996; Crespi et al. 2003), where there has been a dramatic history of climate-driven forest contraction, fragmentation, and expansion since the Miocene (Delcourt and

Delcourt 1988; Webb and Bartlein 1992; Webb et al. 1995; Jansson and Dynesius 2002). Geographic patterns of genetic variation have been described across hundreds of populations and geographic boundaries of many cryptic species have been studied (e.g., Tilley and Schwertdfeger 1981; Karlin and Guttman 1986; Highton et al. 1989; Highton 1995, 1997, 1999; Tilley and Mahoney 1996; Bonett 2000; Highton and Peabody 2000; Tilley 2000; Mead et al. 2001; Anderson and Tilley 2003; Weisrock et al. 2005; Weisrock and Larson 2006). In addition, recent phylogenetic analyses provide a robust framework for identifying extant sister species (Mahoney 2001; Rissler and Taylor 2003; Chippindale et al. 2004; Kozak et al. 2005, 2006; Wiens et al. 2006). Finally, the availability of thousands of georeferenced sampling locations in natural history museums permits rigorous quantification of potential climatic factors limiting species' geographic distributions.

In this study, we examine the relationship between climatic variation, geographic isolation, and speciation. We use published molecular phylogenetic estimates to identify sister taxa. Then we combine information on the geographic presence and absence of sister taxa, patterns of genetic variation, digital maps of climate variation, and methods for estimating species' potential distributions. Together, these approaches allow us to test rigorously the role of niche conservatism in promoting allopatric isolation and speciation.

MATERIALS AND METHODS

Selection of Sister Taxa

We used published phylogenetic analyses for *Desmognathus* (Kozak et al. 2005) and *Plethodon* (Kozak et al. 2006) to identify 16 pairs of extant sister lineages for niche modeling (13 sister-species pairs and three pairs of geographic isolates of genetically divergent conspecifics; Table 1). These phylogenies are relatively complete in terms of sampling of described species within these genera, suggesting that we have identified actual sister taxa (but see below). We chose to focus only on extant sister taxa to avoid the difficulties associated with ancestral state reconstruction at deeper nodes in phylogenies (e.g., Cunningham et al. 1998) and reduce the possibility that the geographic ranges of sister species and their modeled climatic niches differ substantially from the conditions during the time frame of lineage splitting (Losos and Glor 2003). We also included three pairs of geographically isolated conspecific populations to represent incipient stages of the allopatric speciation process (i.e., before lineages have sufficiently differentiated to be recognized as distinct). However, at least one of these pairs clearly represents two distinct species that have not yet been formally described (*D. wrightii*; Crespi et al. 2003). Additional potential pairs of morphologically cryptic sister species exist within *Desmognathus* (Kozak et al. 2005) that were not included. Most of these species are endemic to different mountaintops in the southern Appalachians and Allegheny Plateau. However, limited numbers of sampled populations for these species preclude rigorous analysis and modeling of their potential distributions.

The phylogenies used were based on mitochondrial data alone. Thus, there is potential for discordance between the

mitochondrial and species phylogeny (Funk and Omland 2003). However, most pairs of sister taxa identified here are concordant with those identified from analyses including nuclear DNA sequences (*Desmognathus*, K. H. Kozak and J. J. Wiens, unpubl. data; *Plethodon*, Wiens et al. 2006). Furthermore, in cases where there may be discordance between the gene trees and species trees due to historical hybridization, alternative phylogenetic arrangements always place different pairs of montane endemics as sister taxa (separated by known, absence localities). Thus, our analyses of climatic niches are insensitive to these different phylogenetic hypotheses (K. H. Kozak, unpubl. data).

Geography of Speciation

To test the role of niche conservatism in allopatric speciation in a given group of organisms, allopatric speciation should be a common geographic mode within the group. As an initial test for the phylogenetic signature of allopatric speciation in *Desmognathus* and *Plethodon*, we used age-range correlations (ARCs) to quantify the degree of range overlap between species versus the age of their most recent common ancestor (MRCA). To estimate the geographic overlap of species, we mapped 5436 georeferenced collection localities available from natural history museum collections and the literature using ArcGIS version 8.3 (ESRI, Redland, CA). We estimated the range of a species by enclosing its sampling locations within a minimum convex polygon. To quantify the degree of overlap between species, we first used Lynch's (1989) range-merging procedure in which the geographic distributions of ancestral species are estimated by summing the ranges for all species in a clade. We also conducted ARCs using the nested averages of pairwise overlaps between all species in each clade (Fitzpatrick and Turelli 2006). For both sets of analyses, overlap between species was calculated as the ratio of the area of overlap to the area of the smaller of the two species ranges (Chesser and Zink 1994). Therefore, if one range is contained within another, the amount of overlap between those species is 100%. We then performed linear regressions of the degree of range overlap between species versus the age of their MRCA. The ages of the MRCAs of sister taxa were taken from published studies (Chippindale et al. 2004; Kozak et al. 2005, 2006) in which divergence times were estimated using penalized likelihood, a relaxed molecular clock method (Sanderson 2002). Significance of the regressions for the range-merging approach was determined using standard parametric statistical tables. We assessed the significance of ARCs that employed nested averages by randomizing species range overlaps and recalculating the least-squares regression for each of 1000 randomized datasets.

Provided that postspeciation range shifts have not randomized the relationship between cladogenetic history and geographic overlap of sister taxa (Barraclough and Vogler 2000; Losos and Glor 2003) and that speciation generally occurred via a single geographic mode, the y-intercept and slope of the ARC may be informative regarding the role of geographic isolation in speciation. Minimally, this method can be used to investigate whether sympatric speciation has been rare throughout a taxon's evolutionary history. Positive

TABLE 1. Sister taxa included in analyses of climatic niche space. Generalized geographic distribution taken from Duellman and Sweet (1999). Distribution types follow Highton et al. (1989), Tilley and Mahoney (1996), Highton (1997, 1999), Highton and Peabody (2000), and Crespi et al. (2003).

| Sister-species pair / geographic isolates | Geographic distribution | Distribution type |
|--|---|-------------------|
| <i>Plethodon amplus</i> , <i>P. meridianus</i> | southern Appalachians | allopatric |
| <i>P. cheoah</i> , <i>P. shermani</i> | southern Appalachians | allopatric |
| <i>P. chlorobryonis</i> , <i>P. variolatus</i> | southern Appalachians, Piedmont, Atlantic Coastal Plain | parapatric |
| <i>P. cylindraceus</i> , <i>P. teyahalee</i> | southern Appalachians, Piedmont, Atlantic Coastal Plain | parapatric |
| <i>P. fourchensis</i> , <i>P. ouachitae</i> | Interior Highlands | parapatric |
| <i>P. grobmani</i> , <i>P. ocmulgee</i> | Atlantic Coastal Plain, Gulf Coastal Plain, Piedmont | parapatric |
| <i>P. jordani</i> , <i>P. metcalfi</i> | southern Appalachians | parapatric |
| <i>P. montanus</i> , north and south of Asheville Basin | southern Appalachians | allopatric |
| <i>P. montanus</i> , Roan Mountains and Ridge and Valley | southern Appalachians | allopatric |
| <i>P. punctatus</i> , <i>P. wehrlei</i> | southern Appalachians, northern Appalachians, Allegheny Plateau | allopatric |
| <i>P. electromorphus</i> , <i>P. richmondi</i> | Allegheny Plateau, Interior Lowlands | parapatric |
| <i>P. hoffmani</i> , <i>P. virginia</i> | Allegheny Plateau | parapatric |
| <i>P. hubrichti</i> , <i>P. nettingi</i> | southern Appalachians | allopatric |
| <i>P. dorsalis</i> , <i>P. ventralis</i> | southern Appalachians, Allegheny Plateau, Piedmont | parapatric |
| <i>Desmognathus ochropheaus</i> , <i>D. orestes</i> | southern Appalachians, northern Appalachians, Allegheny Plateau | allopatric |
| <i>D. wrighti</i> , north and south of Asheville Basin | southern Appalachians | allopatric |

slopes and y-intercepts < 0.5 are suggestive of a history of allopatric and/or parapatric speciation; negative slopes and y-intercepts > 0.5 are expected when speciation has been predominately sympatric (Barracough and Vogler 2000). To discriminate between allopatric and climate-associated parapatric speciation, we conducted additional analyses that compare the climatic space occupied by allopatric and parapatric sister taxa (see below for a description of these methods).

Niche Modeling

The hypothesis that niche conservatism promotes geographic isolation and speciation predicts that allopatric sister taxa should occupy climatic niches that are similar to each other but that do not encompass geographic regions separating their distributions (see Fig. 1B). To test this prediction, we modeled spatial patterns of habitat suitability and the potential distributions of sister taxa using ecological niche modeling.

To estimate spatial patterns of environmental suitability for the 16 pairs of sister taxa, we obtained georeferenced locality data from natural history museum collections ($n = 5\text{--}888$ locations per species; average of 119 localities per species) and employed recently developed applications of maximum entropy methods (Maxent) to estimate their potential geographic distributions (Phillips et al. 2004, 2006). Many museum localities were obtained from the collection of R. Highton, now deposited at the U.S. National Museum. These specimens were assigned to species based on the presence of diagnostic allozyme alleles at three blood-protein loci (R. Highton, pers. comm.) and geographic patterns of variation in coloration and/or body size (Highton et al. 1989; Highton 1997, 1999; Highton and Peabody 2000). Any species identification labeled as questionable in the database was excluded from analysis. A full list of georeferenced localities is available from K. H. Kozak.

Maxent is a general approach for characterizing probability distributions from incomplete information (Phillips et al. 2006). It has recently been applied to the problem of estimating the geographic distributions of poorly known species where some presence data are available, but reliable information on their absence is lacking (Elith et al. 2006; Phillips et al. 2006). When applied to estimating a species potential geographic distribution, Maxent computes a probability distribution of habitat suitability over all the grid cells in a study area. Maxent formalizes the general principle that the modeled distribution should agree with everything that is known about the environment at the sample locations, but should avoid placing any unfounded constraints. The probability distribution that is chosen as the best description of the species potential geographic range is the one that is closest to uniform (i.e., closest to having equal probabilities of occurrence in all map grid cells), subject to the constraint that the expectation for each environmental variable included in the modeled probability distribution must match its empirical average over the presence locations. Thus, the Maxent probability distribution expresses the suitability of each grid cell as a function of the environmental variables at all the known occurrence locations, and when projected into geographic spaces it produces a map of the species' potential geographic distribution. For a detailed mathematical formulation of Maxent, see Phillips et al. (2006). Although application of Maxent to estimating species' distributions is relatively new, studies show that it performs better at discriminating between suitable and unsuitable areas (Elith et al. 2006; Phillips et al. 2006) than the commonly used genetic algorithm for ruleset prediction (GARP; Stockwell and Peters 1999) and Bioclim (Nix 1986) methods.

To estimate the climatic niches of sister taxa, we used Maxent version 2.3 (<http://www.cs.princeton.edu/~schapire/maxent>), which employs a machine-learning algorithm (Dudík et al. 2004) to converge on the Maxent probability dis-

tribution. Following the recommendation of Phillips et al. (2006), we used the default modeling parameters (convergence threshold = 10^{-5} , maximum iterations = 500, regularization value β = auto). Climatic niche models were developed using a raster coverage with 1-km² grid resolution clipped to include only eastern North America (western limit, -96.40° ; northern limit, 50.25°) and five temperature- and precipitation-based variables selected from the WORLDCLIM dataset (Hijmans et al. 2005a) with 1-km² spatial resolution. These variables are: Bio2, mean diurnal temperature range (mean of monthly [maximum temperature – minimum temperature]); Bio5, maximum temperature of the warmest month; Bio6, minimum temperature of the coldest month; Bio15, precipitation seasonality (calculated as the coefficient of variation in annual precipitation); and Bio17, precipitation of the driest quarter. Each variable selected represents one of a group of highly correlated variables (arbitrarily chosen using $r \geq 0.75$) that were identified by constructing a Pearson-product correlation matrix of the 19 bioclimatic variables (based on 5436 unique, georeferenced sampling locations) in JMP version 3.2.1 (SAS Institute, Cary, NC). We chose variables that quantify climatic stability and extremes of heat, cold, or aridity, rather than average temperature or precipitation. The former variables may be more likely to set the range limits of many plethodontid salamander species than differences in yearly averages of temperature or precipitation, as plethodontids seem to require cool, moist habitats that are climatically stable for cutaneous respiration (Feder 1983) and foraging (Jaeger 1978; Grover 1998; Petranka and Starnes 2001).

For each species distribution modeled, Maxent assigns a probability of occurrence in each grid cell in the study area. Because these probabilities must sum to 1.0, they are extremely small and a prediction value known as the cumulative probability (t) is calculated for each pixel in the study area (Phillips et al. 2006) and is used to facilitate model interpretation. The cumulative probability of each cell is the sum of that cell's probability along with other cells with equal or lower probability multiplied by 100 to give a percentage. Thus, the Maxent prediction for a given cell is an index of relative suitability that ranges from 0 to 100. To produce binary prediction maps of presence-absence for each species, we extracted the cumulative probabilities for all pixels with known species occurrences and then classified all remaining pixels with values below the 10th percentile as predicted absences. Choosing thresholds for converting continuous predictions into presence-absence maps is a topic of ongoing research (Liu et al. 2005). Our threshold appears to adequately capture spatial patterns of high habitat suitability in that it yields very few presence locations incorrectly being classified as absences.

To produce maps of the spatial pattern of overlap between the Maxent niche models of sister taxa, we imported these niche models into DIVA-GIS version 5.2 (Hijmans et al. 2005b) and used the grid overlay function. For allopatric species, we also examined the degree of overlap between the niche-models of sister species and between the sister species and the absence locations separating their geographic ranges, where neither species is present. Absence localities for a given pair of sister taxa were selected as follows. First, we

enclosed the sampling locations of each individual species (or conspecific, geographic isolate) within a minimum convex polygon (MCP). Next, we selected as absence locations those localities between the MCPs of sister taxa where neither taxon has been collected, but where other closely related species in the same genus are known to occur. We chose to focus specifically on absence locations separating the ranges of sister taxa, because these absence localities should provide a more meaningful assessment of the environmental conditions that set the range limits of species than absence locations randomly selected from the study area (e.g., locations where no salamanders could occur are excluded, as are locations where the taxa in question may actually exist but have not yet been visited). Due to a lack of reliably georeferenced localities for *Desmognathus* species between the ranges of *D. ochrophaeus* and *D. orestes* and for the northern and southern lineages of *D. wrighti*, we used *Plethodon* localities instead for absence localities for these sister pairs. Overall, the number of absence locations separating allopatric sister taxa ranged from six to 94 (average = 37). A full list of the presence and absence locations is available from K. H. Kozak upon request.

We acknowledge that failure to find a species at a given location is not proof of its absence. However, we believe that the montane endemics truly are absent at all or most of these lowland localities. Many of these sites have been searched multiple times (mean visits per site = 2, range = 1–37) and despite considerable sampling effort (mean = 24 plethodontid specimens collected per site, range = 1–1158), the montane endemics in question have not been found. In addition, our Maxent models, which are based on presence-only data, assign very low occurrence probabilities to these absence locations.

Analysis of Climatic Niches

Allopatric sister taxa

If niche conservatism promotes allopatric isolation and speciation, then the climatic niche spaces of allopatric sister taxa should be more similar to each other than they are to the climatic niche space of the absence localities separating their geographic ranges. We tested this prediction in two different ways.

First, we mapped the georeferenced point locations of each species onto the Maxent prediction surface of its sister taxon and extracted the cumulative probability (t) for each point. This approach quantifies the probability with which each sampling location of a species is predicted by the niche model of its sister taxon and is termed the alloprediction probability (Peterson and Holt 2003). Similarly, we mapped the absence locations separating sister taxa onto the Maxent prediction surface for each species in the sister-taxon pair separately. This quantifies the probability with which the niche model of each species in a sister-taxon pair incorrectly predicts the absence locations intervening their geographic ranges and is termed the absence-prediction probability. To test whether allopatric sister taxa predict each other's distributions with greater probability than the absence locations separating their geographic ranges, we conducted a t -test using the prediction type (alloprediction or absence-prediction) as the treatment

effect and the corresponding Maxent cumulative probabilities as the dependent variable. Because the intervening absence locations were used in multiple comparisons (i.e., cumulative probabilities of individual absence locations were extracted from the Maxent prediction surfaces of both sister taxa), the assumption of independence among observations is violated. We therefore evaluated the significance of the *t*-statistic by randomizing the extracted cumulative probabilities among prediction types. If fewer than 5% of the 999 randomizations had a *t*-statistic greater than the observed one, we considered the results significant.

Second, we compared the similarity of the climatic niches of sister taxa by conducting a principal-components analysis (PCA) using climatic data extracted from the localities of both species of each of sister-taxon pair and including the intervening absence localities between them. PCA used the correlation matrix of the five bioclimatic variables included in the climatic niche models described above. For each pair of sister taxa and their absence locations (available for allopatric pairs only), we calculated the mean PC score along each axis and its 95% confidence limit using JMP version 3.2.1.

To test whether the climatic niches occupied by allopatric sister taxa are more similar to each other than expected by chance, we extracted the mean factor score along each (PC) axis for each species and its corresponding set of absence locations. We then used the factor scores to calculate the Euclidean (climatic) distance between sister species and their intervening absence locations. We then conducted a *t*-test using the climatic distances as the dependent variable and the distance type (i.e., between sister taxa or between sister taxa and their absence locations) as the treatment effect. Thus, there were a total of eight climate distances measured between sister taxa, and a total of 16 between the sister taxa and the absence locations (i.e., each species in a sister-taxon pair is compared to its corresponding absence locations). Because pairs of sister taxa and the intervening absence locations were used in multiple comparisons of climate distance, the assumption of independence among datapoints is violated. We therefore evaluated the significance of the *t*-statistic by randomizing the climate distances among treatments. If fewer than 5% of the 999 randomizations had a *t*-statistic greater than the observed one, we considered the results significant.

Parapatric sister taxa

Eight of the 16 sister-taxon pairs we considered are parapatric. Although it may be possible to demonstrate that sympatric speciation has been rare using age-range correlations, it may be difficult to distinguish allopatric from parapatric speciation using this method (Lynch 1989; Fitzpatrick and Turelli 2006). For example, sister taxa with geographically abutting ranges may have either originated via parapatric speciation, or they may have become parapatric secondarily through range expansion following allopatric speciation (Lynch 1989).

Given that all environmental variables show variation along latitudinal, longitudinal, and elevational gradients, it is expected that sister taxa with geographically abutting dis-

tributions will display at least some degree of divergence in the environmental niche spaces they occupy. Parapatric speciation is generally thought to require local adaptation along steep environmental gradients to overcome the homogenizing effects of gene exchange (Endler 1977; Rice and Hostert 1993; Gavrillets 2000; but see Gavrillets et al. 2000), and empirical work in a variety of organisms has demonstrated that gene flow between geographically adjacent populations can constrain adaptive divergence (e.g., Storfer et al. 1999; Hendry and Taylor 2004; Nosil and Crespi 2004; Rosenblum 2006). Therefore, under the standard model of parapatric speciation and given climatic and genetic data for populations from a pair of parapatric sister species, the deepest genetic breaks between populations should be concordant with sharp transitions in environmental variables (e.g., Ogden and Thorpe 2002). We acknowledge that such a relationship is necessary, but may not be sufficient, to demonstrate parapatric speciation via this model. Parapatric sister species could have originated in allopatry and come into secondary contact along a sharp environmental gradient. However, a lack of a relationship between genetic and environmental variation would argue against parapatric speciation via this standard model.

To test for congruence between geographic patterns of genetic divergence and climate variation, we combined published allozyme data on intra- and interspecific patterns of genetic variation (Duncan and Highton 1979; Highton et al. 1989; Highton 1997, 1999; Highton and Peabody 2000), along with the climate characteristics of those geographic sampling locations. Sample sizes for the parapatric species pairs ranged from 16 to 39 locations and genetic distance was calculated from allelic variation at 21–24 loci (Table 2).

For each pair of parapatric sister species, we first calculated the genetic, climatic, and geographic distances between all pairwise combinations of sampled populations. We then tested for a significant correlation between genetic distance and climate distance, while removing the effect of geographic distance on these variables using partial matrix correlation tests (Smouse et al. 1986). Many genetic comparisons of plethodontid salamander populations have found a strong pattern of isolation-by-distance (e.g., Jackman and Wake 1994; Tilley and Mahoney 1996; Weisrock and Larson 2006), and climatic differences are typically correlated with the geographic distance separating localities (K. H. Kozak, unpubl. data). Therefore, removal of geographic effects is necessary to detect a unique contribution of climatic gradients to patterns of genetic divergence. Significant, positive partial correlations indicate strong congruence between genetic breaks and climatic gradients.

Genetic divergence was calculated as the pairwise Nei's (1972) genetic distance between populations in PHYLIP version 3.57c (Felsenstein 1995). Geographic distance was calculated as the great-circle distance separating populations (unlike Euclidean distances, great-circle distances account for the curvature of the Earth and therefore are a more accurate measure of the distance between points spaced around the globe). To quantify climatic distance, we first took each location for which allozyme data were available, extracted the associated values for the five bioclimatic variables, and entered them into a PCA using the correlation matrix. We then

TABLE 2. Results of statistical analysis testing whether allopatric sister taxa predict each other's geographic distributions with greater probability than absence locations separating their ranges. The alloprediction value is the mean cumulative probability with which the niche model for each species (or conspecific, geographic isolate) predicts the sampling locations of its sister taxon. The absence prediction value is the mean cumulative probability with which the niche model of each species predicts the absence locations separating their geographic ranges. Mean prediction values are followed by their standard errors. t is the t -test statistic, P is the probability that the difference between the mean alloprediction and absence-prediction values is significantly different than zero.

| Sister-species pair | Alloprediction value | Absence-prediction value | t | P |
|--|----------------------|--------------------------|------|-------|
| <i>Plethodon amplus</i> , <i>P. meridianus</i> | 60.6 ± 4.8 | 40.4 ± 4.4 | 3.1 | 0.004 |
| <i>P. cheoah</i> , <i>P. shermani</i> | 66.8 ± 1.9 | 33.7 ± 2.5 | 8.3 | 0.001 |
| <i>P. hubrichti</i> , <i>P. nettingi</i> | 6.0 ± 3.3 | 17.2 ± 2.2 | 2.8 | 0.017 |
| <i>P. montanus</i> , north and south of Asheville Basin | 61.2 ± 2.7 | 35.6 ± 2.4 | 7.1 | 0.003 |
| <i>P. montanus</i> , Roan Mountains and Ridge and Valley | 60.5 ± 2.3 | 6.2 ± 2.7 | 15.6 | 0.001 |
| <i>P. punctatus</i> , <i>P. wehrlei</i> | 22.1 ± 2.1 | 49.4 ± 3.4 | 3.4 | 0.001 |
| <i>Desmognathus ochrophaeus</i> , <i>D. orestes</i> | 64.6 ± 5.3 | 31.2 ± 2.4 | 5.7 | 0.009 |
| <i>D. wrighti</i> , north and south of Asheville Basin | 46.3 ± 2.8 | 23.3 ± 1.3 | 4.1 | 0.011 |

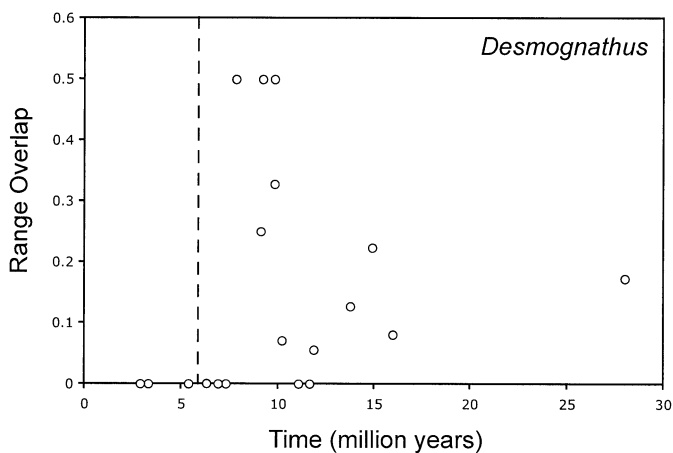
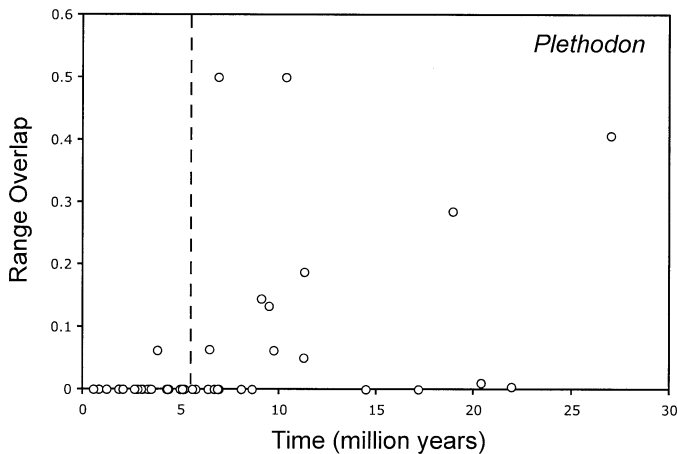


FIG. 2. Age-range correlation plots of the relationship between species range overlap and time since divergence in *Plethodon* and *Desmognathus*. Range overlap for each node was calculated as the nested-average overlap (following Fitzpatrick and Turelli 2006) among all species contained in that clade. The dashed vertical lines in each plot show the time period encompassing the origins of sister-taxon pairs for which inferences were made regarding the ecology and geography of speciation.

used the factor scores along the five PC axes to calculate the pairwise Euclidean distance between populations in multivariate climate space. We evaluated whether the correlation between the matrices was significant using 999 Monte Carlo randomizations. All matrix correlation tests were conducted in the software package PASSAGE (Rosenberg 2002).

As a final assessment of the role of climatic variation in genetic divergence between parapatric sister species, we examined whether parapatric sister taxa exhibit any greater divergence in climatic space than allopatric sister species. For each sister-taxon pair, we extracted the mean factor scores along each PC axis and used them to calculate the Euclidean (climate) distance between sister taxa. We then conducted a t -test using the distance type (i.e., between parapatric or allopatric sister taxa) as the treatment effect and the climate distances as the dependent variable.

RESULTS

Geography of Speciation

Analyses of the degree of geographic overlap between sister taxa provide evidence against a predominance of sympatric speciation in *Plethodon* and *Desmognathus*. Only four cases of sympatry between extant sister-species pairs (of 32 total pairs) in these clades are known (*P. cinereus* and *P. shenandoah*; *P. aureolus* and *P. glutinosus*; *D. folkertsi* and *D. quadramaculatus*; *D. ocoee* and *D. monticola*). Using the range-merging approach, both clades exhibit positive relationships between range overlap and node age and y -intercepts < 0.50 (*Plethodon*: $m = 0.049$, $b = 0.098$, $P < 0.009$; *Desmognathus*: $m = 0.102$, $b = -0.072$, $P < 0.006$). The ARCs were qualitatively similar when nested-average overlaps were employed (*Plethodon*: $m = 0.009$, $b = -0.012$; *Desmognathus*: $m = 0.005$, $b = 0.099$), but were not statistically significant. Nonetheless, ARC plots using the latter method show that the vast majority of species (91%) that diverged less than 7 million years ago show no range overlap, whereas those with longer histories of evolutionary separation show great variance in the degree to which their ranges overlap (Fig. 2). Thus, overall the ARC results appear to support a history of allopatric speciation followed by range movements and randomization of range overlaps at deeper levels of divergence (Barracough and Vogler 2000). Fur-

thermore, given the large proportion of sister species with entirely nonoverlapping distributions, and the low dispersal of plethodontid salamanders in general (Larson et al. 1984), it seems unlikely that their lack of range overlap could have arisen by chance alone.

Allopatric Sister Taxa

Most (six of eight) of the allopatric sister species and geographic isolates of conspecifics fit the pattern expected for vicariant isolation caused by niche conservatism (Fig. 1B). For these six sister-taxon pairs, niche models predict occurrence of that species in at least some portion of its sister taxon's geographic range, demonstrating the similarity of their climatic niches (Fig. 3). Conversely, the species are not predicted to occur in any or most of the geographically intervening absence locations in the lowlands separating the mountain ranges where they are found today, which supports the idea that limited climatic tolerances to ecological conditions that presently occur in the lowlands underlies the past and present geographic isolation of these montane sister taxa (Fig. 3). The degree to which some sister taxa predict each other's ranges is asymmetric (e.g., *D. ochrophaeus* and *D. orestes*, Fig. 3C; *D. wrighti* north and south of the Asheville Basin, Fig. 3D), implying greater climatic specialization of some species relative to their sister taxa.

Multivariate analyses of the climatic niche space occupied by sister taxa reveal the dimensions of the climatic variation among samples. PC1 explains 63–93% of climatic variation among sampling locations, depending on the sister taxon pair (Fig. 4). Inspection of the climatic variable loadings and the elevations, latitudes, longitudes, and factor scores of the individual sampling locations indicates that this axis separate samples along elevation, temperature, and precipitation gradients. Specifically, allopatric montane sister taxa generally occupy habitats that are cooler, wetter, and have less fluctuation in temperature than adjacent absence localities in the lowlands (as indicated by high and similar loadings of Bio2, Bio5, and Bio17 on PC1). In contrast, patterns of variation along PC2 reflect either latitudinal or longitudinal gradients in temperature and precipitation. Because many of the allopatric sister taxa analyzed have latitudinally and/or longitudinally nonoverlapping distributions, they show significant separation along this axis (Fig. 4A–H). Overall, PC2 accounts for little of the climate variation (range = 7–28%, depending upon the sister taxon pair) among sampling locations relative to PC1.

Among the six pairs of sister taxa that support the predictions of the vicariant niche conservatism hypothesis, we find that three sister-species pairs (*Plethodon amplus* and *P. meridianus*, Fig. 4A; *P. cheoah* and *P. shermani*, Fig. 4B; *D. ochrophaeus* and *D. orestes*, Fig. 4C) are significantly different from their intervening absence locations along PC1, but are not significantly different from each other. For all three of these sister-species pairs, alloprediction values are significantly greater than absence-prediction values (Table 2). Similarly, the three evolutionarily distinct, geographic isolates of conspecifics show some evidence of divergence from each other along PC1 (*D. wrighti* samples separated by the Asheville Basin, Fig. 4D; *P. montanus* samples separated

by the Asheville Basin, Fig. 4E; *P. montanus* from the Roan Mountains and Ridge and Valley in southwestern Virginia, Fig. 4F). However, the climatic niche spaces they occupy are more similar to each other than to their associated intervening absence localities, as evidenced by the higher alloprediction than absence-prediction values (Table 2).

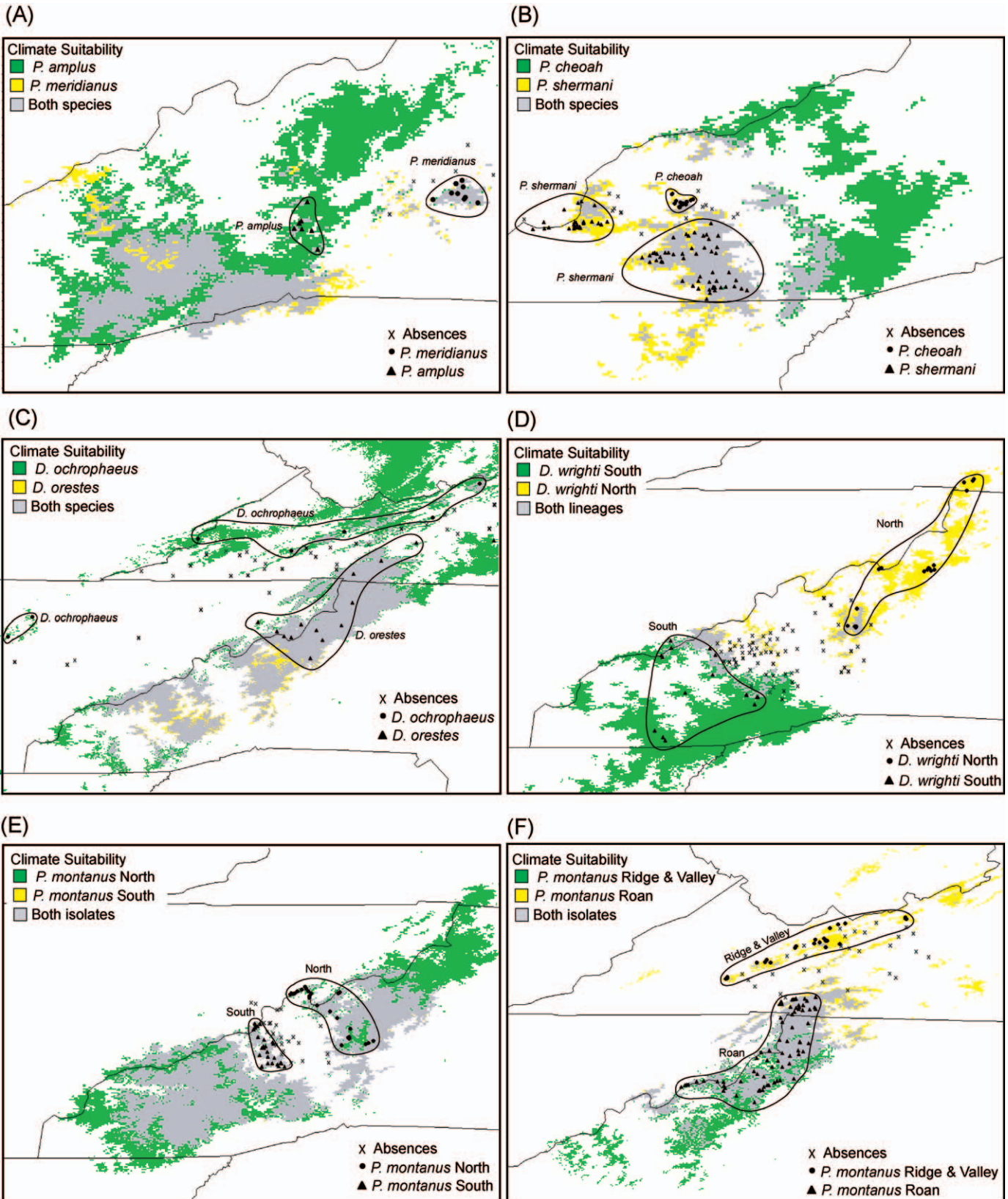
Two of the eight pairs of allopatric sister taxa do not match the predictions of the niche conservatism model. *Plethodon hubrichti* and *P. nettingi* (Figs. 3G, 4G) show evidence of strong divergence in climatic niche space, from both their absence localities and from each other (as in Fig. 1C). In fact, *P. nettingi* predicts some intervening absence locations with greater probability (albeit very low) than its sister species' localities (Table 2). It is possible that their geographic isolation is associated with niche conservatism and niche evolution after speciation, niche divergence alone, or neither. *Plethodon punctatus* and *P. wehrlei* (Figs. 3H, 4H) do not show significant niche divergence. However, their lack of geographic contact is apparently not related to climatic factors; the climatic niche space occupied by *P. wehrlei* overlaps with that of the geographically adjacent absence locations (as in Fig. 1D), and consequently, the overall absence-prediction rate is greater than the alloprediction rate for this sister-taxon pair (Table 2).

Overall, our results support the hypothesis that niche conservatism plays an important role in allopatric isolation in the majority of allopatric pairs of sister taxa. Randomization tests show that the climatic spaces occupied by allopatric sister taxa are, on average, more similar to each other than to the absence locations separating their geographic ranges ($t = 4.46$, $P = 0.002$; Fig. 5A).

Parapatric Sister Taxa

Niche models for the eight pairs of parapatric sister taxa show no evidence that their distributions were generated or are maintained by sharp climatic gradients (e.g., *P. richmondi* and *P. electromorphus*, Fig. 3I; *P. cylindraceus* and *P. teyahalee*, Fig. 3J). Although PCA (Figs. 4I, 4J) shows that these taxa exhibit statistically significant differences in the climatic niche spaces that they occupy (implying some variation in climatic regimes across their geographic ranges), the climatic niche models show that the neighboring borders of the ranges of these species appear to be broadly suitable for both species (Figs. 3I, 3J). One potential interpretation of this pattern is that the range limits of some of these species may be set by interactions with their sister species rather than by climatic tolerances.

Matrix correlation tests of the relationships among genetic divergence, geographic distance, and climatic distance provide no evidence for a history of parapatric speciation along climatic gradients (Table 3). For all eight parapatric species pairs, the genetic divergence among populations was more strongly correlated with geographic distance than with climatic distance. After controlling for geography, genetic distance was actually negatively correlated with climatic distance in one sister-taxon pair. Such an association is expected when deeply divergent lineages geographically contact each other in areas that are environmentally homogeneous. Three sister-species pairs showed no significant relationship be-



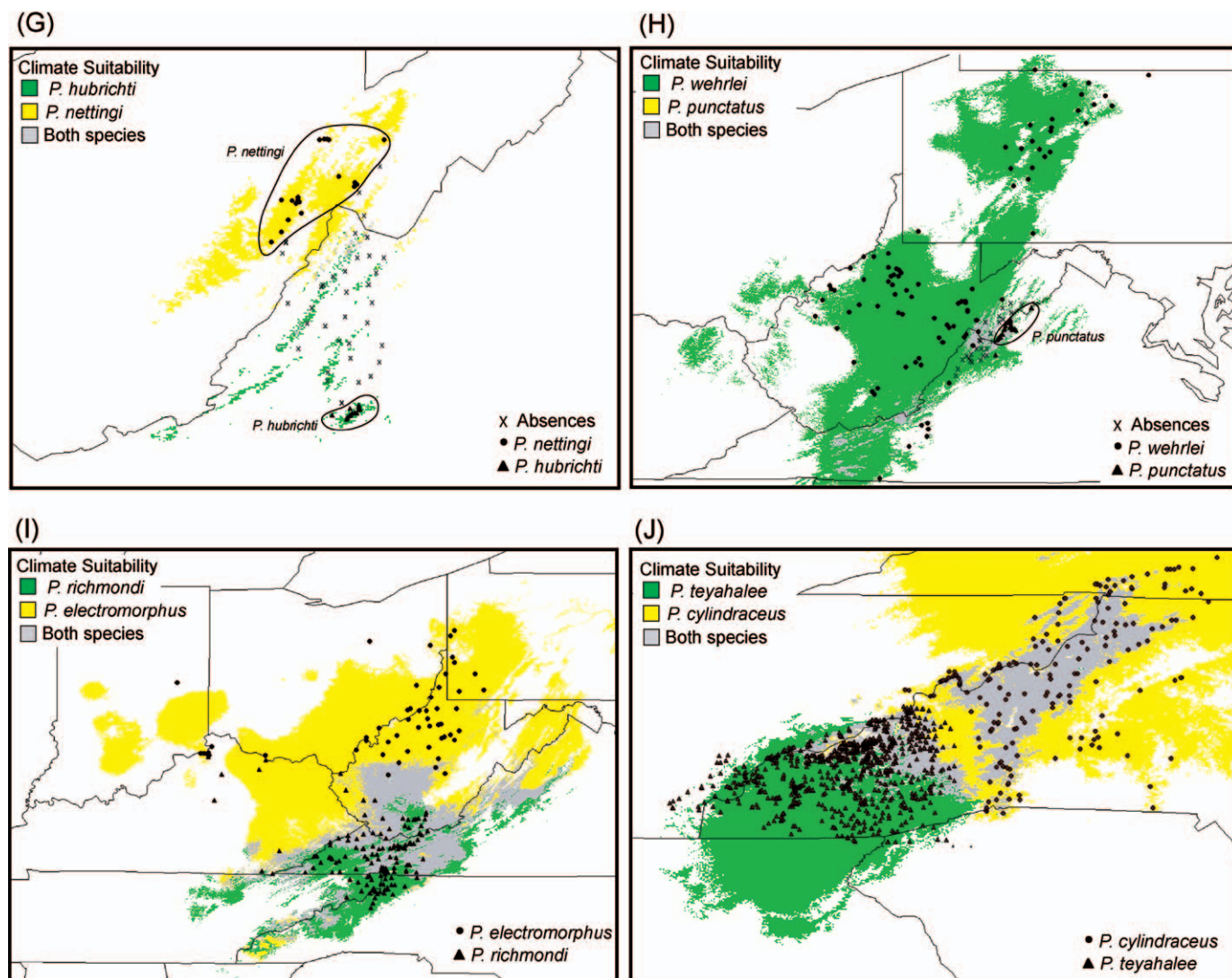


FIG. 3. Continued.

tween genetic divergence and climatic distance. For the remaining four pairs, climatic distance explained no more variation in genetic divergence than expected from the degree of geographic separation among populations. Parapatric sister-species also show no greater divergence in climatic space than allopatric sister-taxa (t -test for difference in climatic distances between allopatric sister species and parapatric sister species; $t = 0.568$, $P = 0.579$, Fig. 5B).

DISCUSSION

Recent speciation research generally has focused on how lineages that originate in allopatry evolve intrinsic reproductive isolating mechanisms (Coyne and Orr 2004; Futuyma 2005), or how ecological divergence promotes nonallopatric speciation (e.g., Knox and Palmer 1995; Feder 1998; Lu and Bernatchez 1999; Rundle et al. 2000; Schliewen et al. 2001;

←

FIG. 3. Predicted geographic distributions of select sister species based on ecological niche modeling. Grid cells that are classified as suitable for one or both species follow the legend of climatic suitability on the top left of each presence-absence map. Georeferenced presence locations from which the models were constructed, along with known absence locations (if available), follow the key on the bottom right of each presence-absence map. Allopatric sister species or genetically divergent geographic isolates: (A) *Plethodon amplus*, *P. meridianus*; (B) *P. cheoah*, *P. shermani*; (C) *Desmognathus ochrophaeus*, *D. orestes*; (D) *D. wrighti* isolates north and south of the Asheville Basin; (E) *P. montanus* isolates north and south of the Asheville Basin; (F) *P. montanus* isolates from the Ridge and Valley and Roan Mountains, respectively; (G) *P. hubrichti*, *P. nettingi*; (H) *P. punctatus*, *P. wehrlei*. Representative parapatric sister species: (I) *P. electromorphus*, *P. richmondi*; (J) *P. cylindraceus*, *P. teyahalee*. Niche models for the remaining six parapatric sister-species pairs (*P. chlorobryonis* and *P. variolatus*; *P. fourchensis* and *P. ouachitae*; *P. grobmani* and *P. ocmulgee*; *P. jordani* and *P. metcalfei*; *P. hoffmani* and *P. virginia*; *P. dorsalis* and *P. ventralis*) show patterns of overlap as in (I) and (J) and are available from K. H. Kozak upon request.

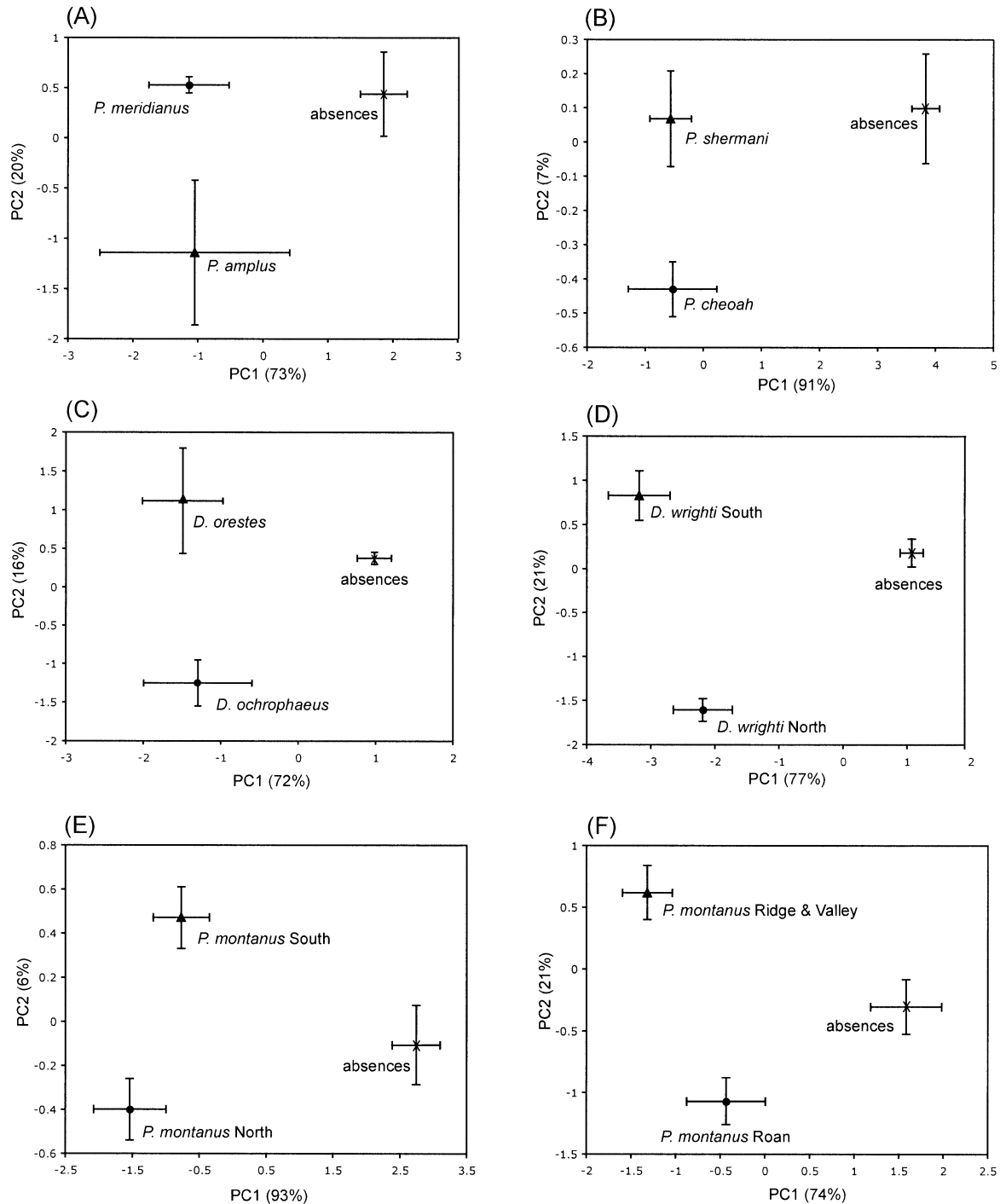


FIG. 4. Principal component analyses (PCAs) of the climate niche space occupied by sister taxa and absence locations separating their geographic ranges (if available) as in Figure 3. The mean principal component scores and 95% confidence limits are shown for each species and the known absence locations. PCAs for the remaining six parapatric sister species are available from K. H. Kozak upon request.

Smith et al. 2001; Via 2001; Ogden and Thorpe 2002; Richmond and Reeder 2002). Yet, the ecological and evolutionary basis of geographic isolation, which drives the most common geographic mode of speciation, has rarely been addressed in the modern speciation literature (Wiens 2004a,b). In this study, we have combined information from phylogeny, geo-

graphic patterns of genetic variation, and climatic niche models to explore the factors that promote the geographic isolation of populations and speciation.

Reconstructing the geographic and ecological context of speciation can be difficult (e.g., Barraclough and Vogler 2000; Losos and Glor 2003; Graham et al. 2004; Fitzpatrick

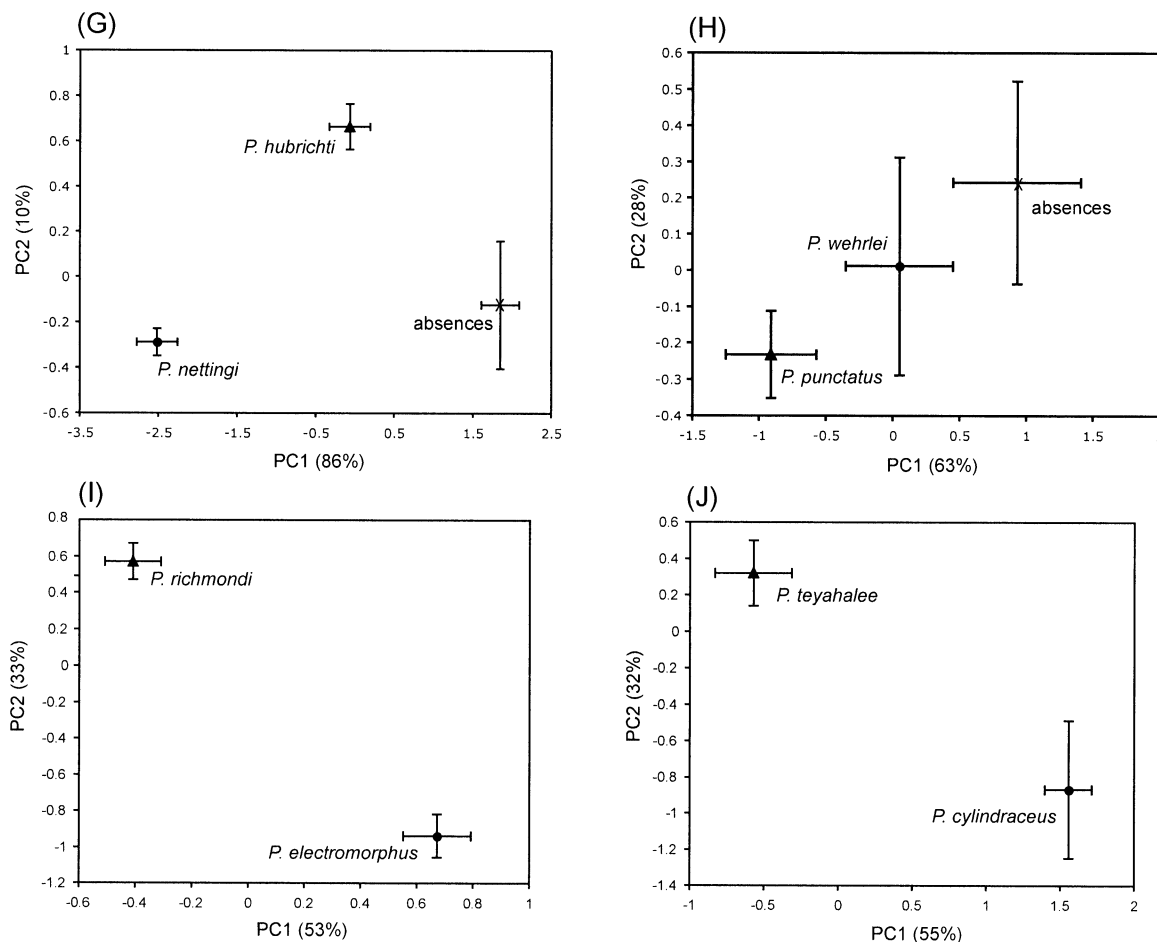


FIG. 4. Continued.

and Turelli 2006). We acknowledge that there may be reasonable alternate explanations for the causes of almost any specific speciation event (e.g., postspeciation dispersal). However, several lines of evidence support our interpretation that climatic niche conservatism promotes allopatric isolation and speciation among these montane endemics. First, it seems unlikely that patterns of postspeciation dispersal have somehow converged to create a similar pattern in six of the eight allopatric sister taxa in this study. The climatic niche models also show that there are many isolated patches of suitable habitat for montane species at higher elevations (Fig. 3). In many cases, these patches are inhabited by other closely related montane endemics (e.g., *D. imitator*, *D. ocoee*, *D. carolinensis*, *P. jordani*, and *P. metcalfi*). Thus, the biogeographic pattern of montane vicariance we observe may be considerably more widespread. As described below, we have also found broad concordance between the timing of the speciation events considered here (Kozak et al. 2005, 2006) based on penalized-likelihood analyses (Sanderson 2002) and the timing of expansion and contraction of montane, forested habitats (Webb and Bartlein 1992; Webb et al. 1995). We postulate that these contractions, coupled with niche conservatism, drive the vicariance of montane endemics. Finally, we have found little evidence to support the other major modes of speciation. For example, the ARCs do not support

widespread sympatric speciation, and our analyses of parapatric sister species suggest that sharp environmental gradients are unlikely to have given rise to these species or to maintain their current distributions.

Climatic Niches and Allopatric Speciation

Our analyses are consistent with the hypothesis that niche conservatism plays an important role in promoting geographic isolation in two species-rich clades of North American salamanders. Many allopatric sister lineages (species and genetically divergent, geographic isolates of conspecifics) in the Appalachian Highlands have seemingly originated through vicariance events associated with evolutionary conservatism in their climatic tolerances. Despite their geographic isolation, the climatic niche spaces occupied by these sister taxa generally show striking similarity to each other, but not to nearby absence locations separating their geographic ranges. Unlike other montane regions that have been studied extensively by evolutionary biologists, such as the Australian Wet Tropics, Mexican Highlands, Andes, and the spruce-fir sky islands of western North America, the temperature and precipitation differences between mountaintops and valleys in the Appalachians are not extreme (i.e., both highlands and lowlands are covered predominantly by temperate deciduous

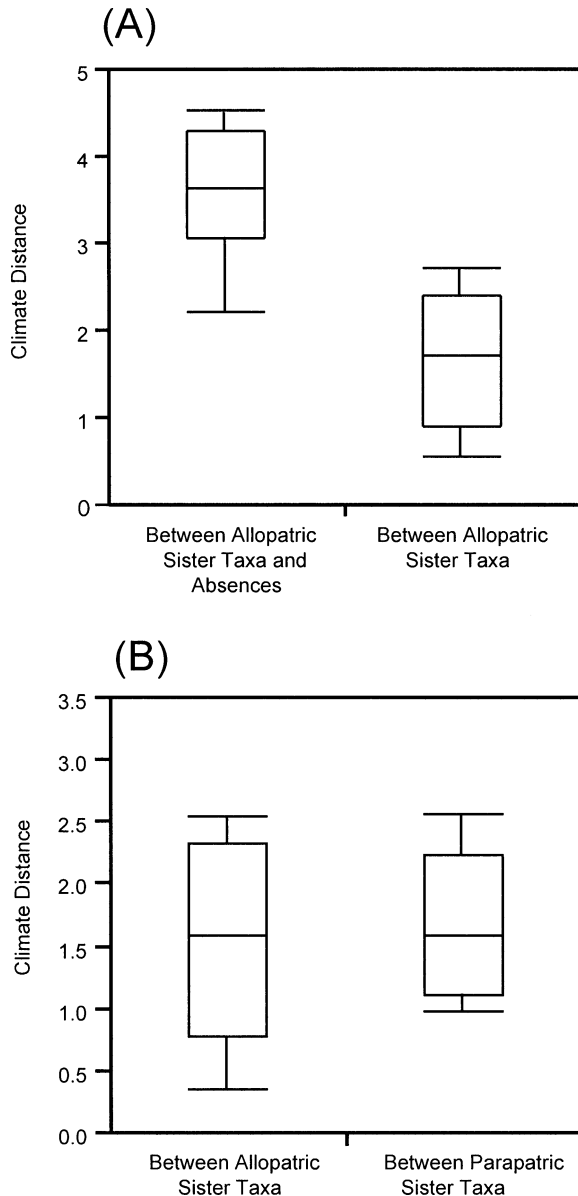


FIG. 5. (A) Climatic distances between allopatric sister taxa and their known absence locations and between allopatric sister species. (B) Climatic distances between allopatric sister taxa and between parapatric sister taxa. Boxes show the median and the 25th and 75th percentiles. Whiskers show minimum and maximum values.

forest). Our results suggest that even relatively subtle climatic gradients between montane and lowland habitats may promote the geographic fragmentation of populations into evolutionarily distinct, allopatric lineages. Furthermore, we find that this occurs even in temperate regions, where climatic zonation and habitat differentiation between elevations is thought to be relatively weak (Janzen 1967; Hawkins and Diniz-Filho 2006).

Although recent studies have tested for the phylogenetic signature of allopatric speciation (e.g., Barraclough and Vogler 2000; Near and Benard 2004; Fitzpatrick and Turelli 2006), few studies have addressed its evolutionary and ecological causes. Our analyses provide phylogenetic and eco-

logical support for a role of niche conservatism and climate change in allopatric isolation. We hypothesize that the ancestors of the sister taxa that are currently confined to higher elevations were distributed continuously at lower elevations when climates were cooler and wetter. As climates became warmer and drier, the high-elevation habitats contracted toward their present locations, and these sister taxa were geographically isolated by their inability to tolerate and adapt to the conditions at lower elevations. In support of this hypothesis, all 16 sister lineages considered in this study appear to have originated during a time period (2–5 million years ago) for which there is a signature of strictly allopatric speciation and palynological and paleontological evidence for elevational shifts of climatic conditions that are currently confined to montane regions (Delcourt and Delcourt 1988; Webb and Bartlein 1992; Webb et al. 2005). Furthermore, recent ecophysiological studies on two species of montane *Desmognathus* demonstrated that limited physiological tolerance to warm temperatures prevents the persistence of these species at low elevations, precluding contemporary dispersal between mountain ranges (Bernardo and Spotila 2006).

Biotic factors may also interact with limited physiological tolerances to prevent the dispersal of montane endemics through lowland habitats. For example, the elevational range limits of populations of *P. jordani* and *P. teyahalee* in the Smoky Mountains, *P. metcalfi* and *P. teyahalee* in the Balsam Mountains, and *P. cinereus* and *P. shenandoah* in the Ridge and Valley are influenced by competitive interactions (Jaeger 1971; Hairston 1980; Nishikawa 1985; Adams 2004). However, competition with congeneric species that inhabit the lowlands separating mountain ranges does not appear to drive the allopatric splitting of montane sister taxa, as the closely related species of *Plethodon* and *Desmognathus* that inhabit the lowlands are also broadly sympatric with the allopatric, montane endemic sister species we considered (e.g., *P. amplus*, *P. meridianus*, and *P. cylindraceus*; *D. ochrophaeus*, *D. orestes*, and *D. fuscus*; *P. cheoah*, *P. shermani*, and *P. teyahalee*).

Does geographic isolation of lineages through niche conservatism lead to speciation? Answering this question depends on one's view of exactly what species are and what speciation is. For example, under the evolutionary species concept, the origin of these geographically isolated lineages is speciation (Wiens 2004b). In contrast, under the biological species concept, speciation may not be considered complete until sister lineages have evolved partial or complete intrinsic reproductive isolation from each other (Coyne and Orr 2004). Although gene flow is not currently possible between populations isolated in different mountain ranges, the environmental conditions during glacial maxima permitted a more continuous distribution of populations in the lowlands and the mixing of their gene pools (Weisrock and Larson 2006).

Several lines of evidence suggest that geographic isolation arising through niche conservatism has played an important role in promoting speciation in Appalachian plethodontids, even under the biological species concept. First, extensive studies of geographic patterns of genetic variation in nuclear-encoded allozymes and mitochondrial DNA demonstrate that the nuclear gene pools of many of these currently allopatric, montane endemics (e.g., *P. amplus*, *P. jordani*, *P. meridianus*,

TABLE 3. Matrix correlations of the effect of geographic distance and climate distance on genetic divergence within and among parapatric sister species. Sample size pertains to the number of populations included (pooled for both species) and the number of loci refers to allozyme loci. Residual climate distance is the correlation between genetic divergence and climate distance after removing the effect of geographic distance alone on genetic divergence. Significance of the correlations was evaluated using 9999 Monte Carlo randomizations.

| Sister-species pair | Sample size | Number of loci | Geographic distance | Climate distance | Residual climate distance |
|---|-------------|----------------|---------------------|------------------|---------------------------|
| <i>Plethodon chlorobryonis</i> , <i>P. variolatus</i> | 18 | 22 | 0.44*** | 0.22* | -0.02 |
| <i>P. cylindraceus</i> , <i>P. teyahalee</i> | 39 | 22 | 0.66*** | 0.39* | -0.15* |
| <i>P. grobmani</i> , <i>P. ocmulgee</i> | 13 | 22 | 0.50** | 0.16 | -0.10 |
| <i>P. jordani</i> , <i>P. metcalfi</i> | 21 | 22 | 0.68*** | 0.38* | 0.02 |
| <i>P. fourchensis</i> , <i>P. ouachitae</i> | 17 | 21 | 0.42** | 0.12 | -0.17 |
| <i>P. electromorphus</i> , <i>P. richmondi</i> | 35 | 21 | 0.47** | 0.27** | -0.01 |
| <i>P. hoffmani</i> , <i>P. virginia</i> | 21 | 21 | 0.27* | 0.12 | 0.01 |
| <i>P. dorsalis</i> , <i>P. ventralis</i> | 16 | 24 | 0.46** | 0.21* | -0.09 |

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

P. metcalfi, *P. montanus*, and *P. shermani*) have remained distinct, despite opportunities for geographic contact and hybridization with each other in the past (Highton and Peabody 2000; Weisrock et al. 2005; Weisrock and Larson 2006). Thus, they seem to have maintained separate evolutionary trajectories in the face of historical opportunities for gene flow.

Second, periods of allopatric isolation have been accompanied by the evolution of intrinsic reproductive isolation, at least in some lineages. In courtship encounters staged between sister species and genetically divergent populations of *Plethodon* and *Desmognathus*, those encounters between individuals from populations that are geographically isolated in different mountain ranges exhibit reduced insemination rates when compared to those involving populations from the same geographic isolate (Tilley et al. 1990; Reagan 1992; Mead et al. 2001). Sister species within *Plethodon* and *Desmognathus* also show divergence in male courtship behaviors and pheromones that contribute to mate recognition and successful courtship (Reagan 1992; Houck et al. 1998; Rollman et al. 1999, 2000; Verrell and Mabry 2000; Mead and Verrell 2002).

Finally, we show that populations whose geographic distributions have very recently become allopatrically fragmented (e.g., *P. montanus*) appear to have retained similar climatic niches and diverged from absence locations along the same climatic axes as lineages that have completed the biological speciation process (e.g., *D. ochrophaeus* and *D. orestes*; *P. cheoah* and *P. shermani*; *P. amplus* and *P. meridianus*). This finding suggests that the same ecological factors may promote geographic isolation throughout the speciation process.

Parapatric Speciation along Environmental Gradients

In contrast to recent studies on the ecology of speciation in tropical montane regions (Schneider et al. 1999; Ogden and Thorpe 2002; Graham et al. 2004; Hall 2005), we found no evidence that parapatric genetic divergence along environmental gradients plays a role in speciation in these temperate North American salamanders. Although many extant sister species of *Plethodon* and *Desmognathus* have parapatric distributions, few vertically replace each other across the climatic gradients that characterize the transition between the Appalachian Mountains and adjacent lowlands, as would be

expected under a standard model of montane parapatric speciation (Patton and Smith 1992; Hall 2005). For example, of a total of 13 extant sister-species pairs within *Desmognathus* (Kozak et al. 2005), only a single pair is parapatrically distributed along an elevational gradient (*D. conanti* and *D. santeetlah*, which were not included in niche modeling analyses due to a lack of fine-scale georeferenced sampling data). Similarly, in *Plethodon* only one sister species pair (*P. dorsalis* and *P. ventralis*) of 19 extant pairs (Kozak et al. 2006) exhibits a pattern of vertical replacement.

Our analyses of the relationships among genetic divergence, geographic distance, and climatic distance also demonstrate that parapatric sister species boundaries are not congruent with steep transitions in climate, despite some variation in the climatic niche spaces that these species occupy. Furthermore, we found that the climatic distances between parapatric sister species are no greater than those between the allopatric sister taxa that seemingly originated through niche conservatism. Thus, climatic gradients in the lowlands are apparently too shallow to promote parapatric speciation. Instead, our results and those of previous studies suggested that many plethodontid species that are presently parapatric originated in allopatry, and that their contemporary range limits are set by competition and hybridization, rather than climate per se (Means 1975; Hairston 1980; Nishikawa 1985; Highton 1995; Adams 2004; Kozak et al. 2005, 2006).

Methodological Implications for Studying the Geography and Ecology of Speciation

Niche modeling is a promising new approach for investigating large-scale ecological dimensions of the speciation process. Recently, Graham et al. (2004) proposed a novel methodological framework for elucidating speciation mechanisms by combining species-level phylogenies, information on the distributional overlap of species, and niche models. They suggested that a divergence-with-gene flow model of parapatric speciation can be regarded as the primary mode of speciation when many sister species with geographically abutting distributions are found to inhabit different climatic niches. Similarly, they suggested that divergent selection in isolation should be regarded as the predominant speciation mechanism when a clade is found to contain many allopatric sister species that inhabit different climatic niches. Our results suggest that it may be misleading to draw conclusions

on the role of natural selection in speciation based solely on comparisons of sister species' climatic niche models.

For example, our PCA showed significant differences in the climatic niche spaces occupied by parapatric sister species of *Plethodon*, a pattern that might be interpreted as supporting parapatric speciation along climatic gradients. However, our matrix correspondence analyses suggest that patterns of genetic divergence are not associated with climatic gradients. The general matrix correlation framework we have applied to parapatric sister species can be easily applied to any taxonomic group for which data are available on intra- and interspecific patterns of genetic variation, geographic distance among sampled populations, and climatic variation. This approach may be particularly useful for determining whether the apparent importance of parapatric speciation in tropical montane regions is a methodological artifact, whether this speciation mode may be more prevalent in tropical regions than in the North American temperate zone, or whether patterns simply differ between clades.

Similarly, three of the allopatric sister taxa we considered showed statistically significant divergence in the climatic spaces they occupy, a result that might be considered strong evidence for speciation being driven by divergent natural selection. The hypothesis that niche conservatism drives allopatric speciation does not predict that species have statistically indistinguishable niches (Wiens and Graham 2005). Rather, allopatric fragmentation of a species' ancestral geographic range will occur when climatic change in the geographic space between populations occurs more rapidly than the rate at which adaptation can occur to the new environmental conditions. We found that, despite some differentiation in their climatic niches, the climatic spaces occupied by these sister taxa are much more similar to each other than they are to the absence locations between them. This pattern suggests that niche conservatism, and the establishment of warm, dry conditions in the lowlands separating the geographic ranges of montane sister taxa, played the primary role in promoting allopatric range fragmentation. We are not claiming that the climatic differences experienced by these lineages are biologically insignificant. Instead, our study suggests that the role of niche conservatism in speciation may go undetected if the climatic niches of allopatric sister taxa are not compared to absence locations separating their geographic ranges (e.g., Graham et al. 2004).

It is also important to identify the specific role that climatic factors might play in divergence. We have focused on how climatic niche conservatism or evolution might drive geographic isolation and lineage splitting, rather than adaptive phenotypic divergence between these isolates. For example, differences in the climate regimes of major mountain ranges may promote adaptive divergence between some lineages whose initial isolation appears to have been associated with niche conservatism (e.g., northern and southern lineages of *D. wrighti*; Ridge and Valley and Roan isolates of *P. montanus*). However, in the absence of phenotypic data, concluding that climate-associated natural selection drives phenotypic divergence and speciation (e.g., Graham et al. 2004) seems problematic. Future studies should address not only how climatic niche conservatism promotes allopatric isola-

tion, but also how it may promote the divergence of phenotypes that contribute to speciation.

Conclusions

In this study we investigated the ecological and evolutionary basis of geographic isolation and speciation in two clades of North American salamanders. We found that allopatric sister taxa generally (six of eight pairs examined) inhabit similar climatic niches and that failure to adapt to warmer and drier conditions in the lowlands adjacent to their geographic ranges seemingly led to geographic range fragmentation and speciation. Furthermore, although half of the sister taxa we considered have geographically abutting distributions, we found no evidence for parapatric speciation along climatic gradients. Our study demonstrates how climatic niche conservatism and spatial climatic differences may interact to promote speciation. The general methodological approach described here can be readily applied to other taxonomic groups and geographic regions to determine how general a role niche conservatism plays in allopatric speciation and the origin of biodiversity.

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