

LATITUDINAL VARIATION IN SPECIATION MECHANISMS IN FROGS

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Speciation often has a strong geographical and environmental component, but the ecological factors that potentially underlie allopatric and parapatric speciation remain understudied. Two ecological mechanisms by which speciation may occur on geographic scales are allopatric speciation through niche conservatism and parapatric or allopatric speciation through niche divergence. A previous study on salamanders found a strong latitudinal pattern in the prevalence of these mechanisms, with niche conservatism dominating in temperate regions and niche divergence dominating in the tropics, and related this pattern to Janzen's hypothesis of greater climatic zonation between different elevations in the tropics. Here, we test for latitudinal patterns in speciation in a related but more diverse group of amphibians, the anurans. Using data from up to 79 sister-species pairs, we test for latitudinal variation in elevational and climatic overlap between sister species, and evaluate the frequency of speciation via niche conservatism versus niche divergence in relation to latitude. In contrast to salamanders, we find no tendency for greater niche divergence in the tropics or for greater niche conservatism in temperate regions. Although our results support the idea of greater climatic zonation in tropical regions, they show that this climatic pattern does not lead to straightforward relationships between speciation, latitude, and niche evolution.

KEY WORDS: Amphibia, anura, climate, latitude, niche conservatism, niche divergence, speciation.

It is widely understood that geography is a critical aspect of speciation (e.g., allopatric, parapatric, and sympatric modes; Futuyma 1998; Coyne and Orr 2004), and that speciation may have a strong ecological component (see numerous recent papers on "ecological speciation"; e.g., Schluter 2001, 2009; Ogden and Thorpe 2002; Nosil et al. 2005; Rundle and Nosil 2005). However, the ecological basis for different geographic modes, particularly allopatric speciation, has not been as widely studied. For example, despite the general consensus that the allopatric mode seems to be the most common (e.g., Futuyma 1998; Barraclough and Vogler 2000; Coyne and Orr 2004; Phillimore et al. 2008), there is relatively little research on what ecological factors cause sister species to become geographically isolated from each other (e.g., Wiens 2004a,b).

The ecological niche is a crucial concept when considering the geographic ranges of species (Lomolino et al. 2006), and

therefore, the geography of speciation. The fundamental ecological niche of a species determines the biotic and abiotic conditions in which the species is able to persist and spread (Hutchinson 1957). There are two general models for speciation in terms of the niche: niche conservatism and niche divergence. Niche conservatism is the maintenance of ecological similarity among species or populations over time (e.g., Peterson et al. 1999; Wiens and Graham 2005). Niche conservatism may be an important driver of allopatric speciation, as it may be the initial cause of geographic isolation between two incipient species (e.g., Wiens 2004a,b; Kozak and Wiens 2006). A species may be split into two descendant species when a geographic barrier that consists of suboptimal environmental conditions for the species divides the species range, and niche conservatism limits adaptation to the ecological conditions at the geographic barrier, preventing gene flow between the two sets of populations (Wiens 2004a). For

example, many sister species that are endemic to montane habitats on adjacent mountain ranges presumably originated from an ancestral species that was more widely distributed in the lowlands during periods of cooler climate, and are now confined to higher elevations by climate change. These species presumably became geographically isolated because they were unable to adapt to climatic conditions in the lowlands separating their geographic ranges (Wiens 2004a,b; Wiens and Graham 2005; Kozak and Wiens 2006). This mechanism is also referred to as refuge speciation (Moritz et al. 2000). This model predicts that recently evolved sister species will occur in similar but allopatric habitats, which are separated by less similar habitat in between (Fig. 1A). This same basic model could apply to many different types of ecological barriers (Wiens 2004a,b), from the trivial (e.g., terrestrial species separated by oceans) to the more subtle (e.g., xeric-adapted species separated by mesic habitats, stream species separated by rivers).

Under the second model, new species may originate as populations adapt to new niches, through the process of niche divergence. Niche divergence is potentially important for both allopatric and parapatric speciation. For allopatric speciation, species may become allopatric through niche conservatism and subsequently diverge ecologically from their ancestral species' niches in allopatry, limiting any further gene flow between them. Under this model, environmental conditions in each sister species' geographic range are unsuitable for the other species, as is the area in between their ranges, even though the habitats of sister species could still be more similar to each other than they are to the barrier habitat that separates them (Fig. 1B). Similarly, species may become allopatric via factors other than climatic niche conservatism, and subsequent niche divergence may then limit further gene flow between them. Under this mechanism, we expect that allopatric sister species would occur in environments that are no more similar to each other than they are to the environment separating their geographic ranges (Fig. 1C). Thus, comparing habitats where species occur to where they do not occur is a critical aspect of distinguishing whether niche conservatism is involved in the initial isolation of species, even if the niches of sister species have diverged considerably (Wiens and Graham 2005). It is important to note that these two models of allopatric speciation refer to somewhat different portions of the speciation process: niche conservatism pertains to the initial isolation and origin of lineages whereas niche divergence pertains to the subsequent divergence of lineages that are already allopatric.

For parapatric speciation, niche divergence may be a common mechanism underlying the initial origin of parapatric lineages (Futuyma 1998; Coyne and Orr 2004). Under this model, incipient species experience divergent selection across a strong environmental gradient (referred to as gradient speciation in Moritz et al. 2000), such as high and low elevation populations along a

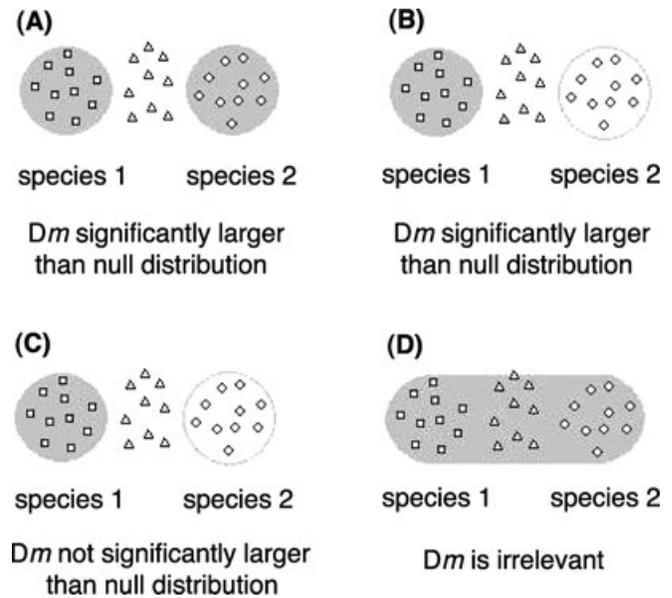


Figure 1. Hypothetical example illustrating four potential outcomes of niche modeling and D_m estimation, with their implications for speciation mechanisms. Significantly positive values of D_m indicate that the climatic distributions of sister species are more similar to each other than they are to the intervening absence localities. Squares represent the observed localities of species 1. Circles represent the observed localities of its sister species, species 2. Triangles represent locations where both species are known to be absent. The shading represents the spatial distribution of climatically suitable habitat for species 1 based on ecological niche modeling. In all four cases, we assume results based on species 1 are similar to those based on species 2 (but see Materials and Methods for discussion of potentially asymmetric outcomes). (A) Species 1 is predicted to occur in its sister species' geographic range, but not in the intervening gap area. D_m for the species is significantly larger than its null distribution. This pattern suggests that niche conservatism initiates the isolation between sister species and maintains their current allopatric distributions, given that results are similar for species 2. (B) Species 1 is not predicted to occur in either its sister species' geographic range or in the intervening gap region. D_m is significantly larger than its null distribution. This pattern (assuming similar results in species 2) suggests that niche conservatism initiates the geographic isolation between sister species and subsequent niche divergence prevents further gene flow between them. (C) Species 1 is not predicted to occur in either its sister species' geographic range or in the intervening gap, but D_m is not significantly larger than the null distribution. This pattern suggests that factors other than climatic niche conservatism may have initiated the geographic isolation of these species and that subsequent niche divergence prevents further gene flow. (D) Species 1 is predicted to occur both in its sister species' geographic range and in the intervening gap between their ranges. This pattern suggests that the current allopatric distributions of sister species are determined by factors other than climate, and so the climatic similarity of species (and similarity to gap locations) may not be relevant to speciation.

mountain slope. Adaptation to these different environments eventually leads to restricted gene flow and reproductive isolation between the populations that inhabit them (Coyne and Orr 2004). This model predicts that newly evolved sister species will be parapatrically distributed and occupy adjacent but distinct environments (Moritz et al. 2000).

Recent studies have begun to explore the relative importance of niche divergence and niche conservatism in speciation, particularly using GIS-based environmental datasets. However, these studies have found highly divergent results. For example, some studies have found evidence for speciation by niche conservatism in temperate montane regions (e.g., Kozak and Wiens 2006) and in the tropics (e.g., Peterson et al. 1999; Peterson and Nyári 2007). Others have found evidence for speciation by niche divergence along climatic gradients in the tropics (e.g., Graham et al. 2004; Kozak and Wiens 2007; Raxworthy et al. 2007), as well as a strong association between genetic distances and divergence in the ecological niche in temperate regions (e.g., Rissler and Apodaca 2007; but see Kozak and Wiens 2006; Stockman and Bond 2007).

Kozak and Wiens (2007) suggested that there might be a latitudinal pattern in the relative importance of niche divergence and niche conservatism in speciation, with niche conservatism being more common in temperate areas and niche divergence more prevalent in the tropics (also predicted by Moritz et al. 2000). By comparing climatic data from 16 temperate sister-species pairs and 14 tropical sister-species pairs in the salamander family Plethodontidae, they found that temperate sister species tend to occupy similar climatic niches (which are very different from the intervening “absence” locations) and that tropical sister species tend to occur at different elevations and in different climatic zones. Kozak and Wiens (2007) related this geographic pattern to Janzen’s (1967) hypothesis of “why mountain passes are higher in the tropics.” Janzen (1967) suggested that more limited temperature seasonality in the tropics selects for organism that are narrowly adapted to a given elevational band, potentially leading to more limited dispersal between different elevations in the tropics. If true, this pattern may promote gradient speciation along mountain slopes in the tropics (e.g., Moritz et al. 2000; Kozak and Wiens 2007), leading to a higher frequency of parapatric speciation and niche divergence in the tropics. Such differences in speciation mechanisms might also be important in explaining why there are more species in the tropics for so many groups of organisms. Indeed, tropical plethodontids have higher rates of diversification than temperate salamander clades (Wiens 2007), and the species richness of plethodontids in tropical Middle America is nearly twice that in temperate North America (even though plethodontids seem to have originated in the temperate zone and spread to the tropics relatively recently). If the results found in plethodontid salamanders apply widely to other groups

of organisms, these latitudinal differences in climatic zonation may be important for explaining both variation in mechanisms of speciation across studies and global-scale patterns of species richness.

In the present study, we test whether there is latitudinal variation in speciation mechanisms related to climate, using frogs as a model system. We focus on frogs because they are the sister group to salamanders and may show complementary patterns. We select 79 sister species pairs from across the world and across the phylogeny of frogs. For the first time, we compare the relative frequencies of allopatric, parapatric (or partially sympatric), and sympatric distributions of sister species in the tropics versus the temperate zone. We then test for latitudinal variation in elevational and climatic overlap between sister species. We also evaluate the relative importance of allopatric speciation via niche conservatism versus niche divergence in relation to latitude. In contrast to the results of Kozak and Wiens (2007) for salamanders, we find no tendency for tropical frog species to show greater divergence (or less overlap) in their elevational or climatic distributions than temperate species. Although our results are consistent with Janzen’s (1967) hypothesis that tropical species occupy narrower climatic regimes than temperate species, we show that the relationships between climate, latitude, and speciation are not straightforward, and may even be clade-specific.

Materials and Methods

Given that our methods are lengthy and somewhat complex, we provide a summary and overview in a flow chart (Fig. 2). Our description of methods follows the order in the numbered boxes in the flow chart, starting with the identification of sister species.

IDENTIFICATION OF SISTER SPECIES

We examined recently published phylogenetic analyses across all major frog clades, and identified 79 useable pairs of sister species (see Table S1). Although dozens of frog phylogenies have been published recently that collectively include hundreds of species, we only included sister-species pairs from phylogenetic studies in which all described species in their genera or species groups were included. This restriction greatly limited the number of species pairs, but was necessary to ensure (as much as possible) that included species pairs are actually sister species. However, we acknowledge the possibility that undescribed or extinct species might still interdigitate among these species in some cases (although relatively short branch lengths for many species suggest that they are relatively recent, and that there may have been too little time for undiscovered or extinct species to have arisen along these branches). To evaluate whether taxon sampling in a given study was sufficiently complete, we used the summary of taxonomy from Frost’s (2008) database. When a

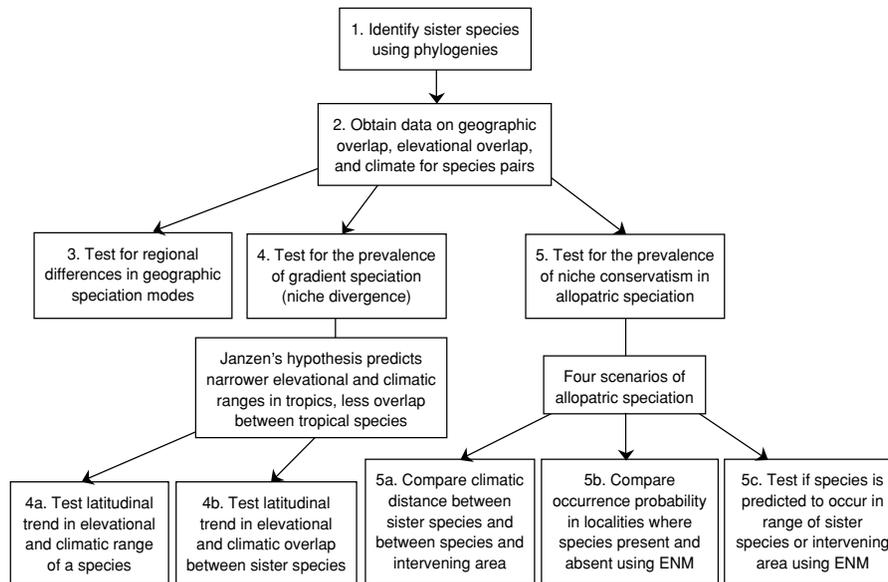


Figure 2. A flow chart summarizing the methods used in this study. ENM represents ecological niche modeling.

phylogeny with adequate taxon sampling was available, we only used sister-species pairs if the stem uniting them had a likelihood or parsimony bootstrap value $>50\%$, or a Bayesian posterior probability >0.50 (but 88.6% of the 79 species pairs have a bootstrap value $>70\%$ or a posterior probability >0.90). In the few cases in which there were conflicts among different phylogenies from different datasets (e.g., different genes), we used the phylogeny based on the combined data, if possible. Otherwise, we used the relationships supported by the majority of phylogenies based on different datasets.

Among the phylogenetic analyses we used, phylogenies for the families Eleutherodactylidae (sensu Frost 2008), Hylidae (sensu Frost 2008), and Ranidae (sensu Wiens et al. 2009) are relatively complete. Over 60% (26.6% from Eleutherodactylidae, 30.4% from Hylidae, 5.1% from Ranidae) of the sister-species pairs we used are from these families. However, these are also among the three largest families of frogs (collectively including $\sim 42\%$ of all frog species) and they collectively span nearly the entire geographic range of all frog species combined (Amphibiaweb 2009).

SPECIES DISTRIBUTION AND SPECIATION MODES

We used the species distribution maps from the Global Amphibian Assessment (GAA hereafter; IUCN 2009). The GAA provides a recent estimate of geographic ranges for almost all amphibian species, based on minimum area polygons (IUCN 2009). We acknowledge that such maps are not necessarily without errors. However, any errors in these maps should only be relevant to our study if they influence the inferred geographic overlap of sister species. Further, most of our analyses are based on elevational distribution patterns and climatic data (from spe-

cific localities), and do not depend directly on these maps. For 14 species without distribution maps available from the GAA, we developed maps in ARCGIS version 8.3 (ESRI, Redlands, CA), based on maps and relevant descriptions from the original literature.

We first classified each species pair as being allopatric, parapatric (or partially sympatric), or sympatric, and then compared the frequency of each pattern in tropical versus temperate regions. We defined the tropics as the region between 23.5°S and 23.5°N latitude, the same angle as the tilt of the Earth's axis, and a standard geographic definition for the tropics. A sister-species pair was considered to occur in the tropics if its latitudinal midpoint (the average of the maximum and minimum latitudes of both sister species) falls within this latitudinal zone. Species pairs were considered allopatric when there was no geographic overlap between them, and a geographic gap or barrier (i.e., one species pair is separated by the Mississippi River) separated their localities. Pairs were considered sympatric if one species' range was entirely within the range of its sister species. Initially, we intended to have a category for parapatrically distributed species to represent this important geographic mode of speciation. However, we found no species pairs with distributions that were truly abutting, only ones that were allopatric, fully sympatric, or partially sympatric. We therefore created an intermediate, "partial sympatry" category for distributions that were partially overlapping.

Using these criteria described above, we classified all species pairs as either tropical or temperate and as either allopatric, partially sympatric, or sympatric. We then compared the frequency of each geographic pattern in each region using the 2×3 G-test with William's correction (Sokal and Rohlf 1995; Fig. 2; flow chart step 3).

ELEVATIONAL AND CLIMATIC DATA

To test the hypothesis that elevational overlap of sister species decreases at lower latitudes, we determined the elevational overlap for all 79 species pairs. We obtained the elevational ranges of most species from the summary provided for each species in the GAA (IUCN 2009). For species without elevational data in the GAA, we extracted data on elevational distribution from georeferenced specimen localities (see below for methods) and obtained the elevational range by subtracting the minimum elevation from the maximum elevation (Fig. 2; step 4a). The degree of elevational overlap between sister species was calculated using the following formula (Fig. 2; step 4b):

$$\text{Elevational overlap} = 0.5(O/R_A + O/R_B),$$

where R_A and R_B are the elevational ranges of species A and B, and O is the absolute elevational overlap of R_A and R_B . This formula is different from the one used in Kozak and Wiens (2007), where the degree of overlap was calculated by dividing the elevational overlap by the elevational range of the species with the smaller elevational range. For their formula, an index of 1.0 describes both sympatric sister species and allopatric sister species distributed over identical elevational ranges (none of the sister species included in Kozak and Wiens [2007] are sympatric). In our study, 12 of 79 pairs are sympatric sister species, in which one species has a narrower elevational range than the other whereas allopatric sister species usually share a similar elevational range. Given this difference, our index should be a better estimation of elevational overlap between sister species.

To quantify and compare the climatic distribution of sister species at different latitudes, we first obtained climatic data from each included species using GIS-based environmental layers from georeferenced specimen localities. Specimen localities were obtained from museum collections by searching the HerpNet (www.herpnet.org) database (which includes locality data for all species represented in dozens of the U.S. and foreign collections) and from the original literature. *Pseudacris* locality data were gathered from Lemmon et al. (2007), in which specimen localities were georeferenced by those authors. For species whose specimen localities were not georeferenced by the original collectors, we georeferenced the localities ourselves. Localities were only used that were within 1 km of a georeferenced landmark (e.g., 1 km from a village). All georeferenced localities were then checked against the species distribution maps (e.g., from IUCN 2009 or the original literature). Localities far away from the mapped ranges were eliminated as being potentially erroneous. Although we began with a large number of potential species pairs, we only included pairs if there were at least five useable georeferenced specimen localities for each species (to provide an adequate description of the climatic distribution). We also eliminated

species whose available georeferenced localities were highly localized compared to the overall geographic distribution of the species, such that the available climatic data might not match the species' overall climatic distribution. We eventually mapped a total of 2591 georeferenced specimen localities for 28 carefully selected sister-species pairs (see Tables S2 and S3 for a listing of species and their data).

For each locality, we extracted elevation and climatic variables from the WORLDCLIM database with 1 km² spatial resolution (Hijmans et al. 2005), using ARC GIS version 8.3. To quantify the temperature ranges of each species (Fig. 2; step 4a), we extracted the maximum temperature of the warmest month (Bio 5) and the minimum temperature of the coldest month (Bio 6) for each locality. The temperature range of the species is the difference between its maximum observed value of Bio 5 and minimum value of Bio 6. To quantify the temperature overlap between sister species, we first extracted the maximum and minimum temperature for each month across all of a species' localities. We then calculated the temperature range of a species for each month as the difference between the maximum and minimum temperatures. Finally, the degree of temperature overlap between sister species was calculated using the same formula in Kozak and Wiens (2007) as follows (Fig. 2; step 4b):

$$\text{Temperature overlap} = \sum_{i=1}^{12} 0.5(O_i/R_{Ai} + O_i/R_{Bi}),$$

where R_{Ai} and R_{Bi} are the temperature ranges of species A and B for month i , and O_i is the absolute overlap of R_{Ai} and R_{Bi} . We also calculated the degree of temperature overlap by the same formula as the one used for elevational overlap to account for the possibility that sister species differ somewhat in which months are coldest and warmest within their geographic ranges.

The overall climatic distribution of a species was characterized using 19 climatic variables derived from monthly temperature and precipitation values in the WORLDCLIM database (Hijmans et al. 2005). For each species, we extracted the maximum and minimum values of each variable across all of the species' localities, and calculated the species' range for each climatic variable as difference between the maximum and the minimum values (Fig. 2; step 4a). The overall climatic range of a species, therefore, is a vector of 19 derived variables (data for each species listed in Table S2). Similarly, we calculated the overlap of each climatic variable between sister species by the same formula as the one used for elevational overlap (Fig. 2; step 4b). The overall climatic overlap between two sister species is a vector of another 19 derived variables (see Table S3). To account for the potential redundancy among the 19 variables, a principal components analysis (PCA) was run for each matrix (19 variables with 28 sister-species pairs) of climatic ranges and climatic overlap (see the section below on

statistical analysis), using S-PLUS version 6 (Insightful Corporation 2001). The first several principal components (PCs) that cumulatively account for over 95% of the variation were used in the following statistical analyses (see below).

We also tested the hypothesis that niche conservatism causes populations to become isolated as they track suitable climatic conditions over time (Fig. 2; step 5a). We calculated separately (1) the climatic distance (see below for methods) between allopatric sister species, and (2) the climatic distance between each species of the sister-species pair and locations in the gap between their geographic ranges (i.e., “absence localities” where neither species occurs). Among the 28 species pairs with adequate climatic data, there were nine allopatric species pairs (i.e., no geographic overlap between sister species’ geographic ranges, and a geographic gap separating their ranges). To obtain climatic data for absence locations, we first drew a maximum convex polygon in the most narrow part of the gap between the geographical ranges of the two sister species (i.e., the place where their ranges most closely approach each other). We then randomly generated pseudoabsence locations within this polygon. The number of pseudoabsence locations was set to be equal to the average of the number of sampling localities for both species of the sister-species pair. Data for all 19 climatic variables were then extracted from each absence location.

To quantify the climatic distances, we first performed a PCA on the correlation matrix of the 19 climatic variables extracted from the localities of each species in a sister-species pair and their corresponding absence locations. Due to the limited number of localities for some species, we selected the first several PCs by the Kaiser criterion (Kaiser 1960). Using this criterion, we included fewer PCs than using an arbitrary threshold of the PCs that explain 95% of the variance, but still included PCs that account for at least 80% of the variance for all the allopatric sister-species pairs. For a given sister-species pair (species A and species B, for example), we calculated Mahalanobis distances using the selected PCs, first between species A and species B, then between species A and absence locations, and then between species B and absence locations. To test whether the distance between species A and B is significantly smaller than that between species A and absence locations (the same procedure is applied to species B), we pooled and reshuffled the localities of species B and the absence locations 1000 times using PopTools version 3.0.6 (www.cse.csiro.au/poptools). In each replicate, we recalculated the Mahalanobis distances between species A and B, and between species A and absence locations. The difference between these two Mahalanobis distances (D_m hereafter) for species A is then calculated using the following formula:

$$D_m = (\text{distance between species A and absence locations} \\ - \text{distance between species A and B}) / \text{distance between} \\ \text{species A and B.}$$

If fewer than 5% of the 1000 randomizations had a difference larger than the observed one, we considered the result to be significant support for the potential importance of niche conservatism in explaining their allopatric distributions (i.e., the climatic distributions of sister species are more similar to each other than they are to the intervening absence localities). Similarly, if more than 95% of the 1000 randomizations had a difference larger than the observed one, the climatic distributions of sister species are more different from each other than they are from the absence localities. This could be the result of niche divergence between sister species or possibly spatial autocorrelation (i.e., a species has more similar climatic distribution to the absence localities than to its sister species because it occurs geographically closer to the absence localities).

Warren et al. (2008) proposed a promising statistical test to address whether species climatic niches are conserved over evolutionary time scales. However, we did not use their test in the present study, for two main reasons. First, the statistics used in their test are based on probabilities of occurrence estimated from niche modeling, instead of directly analyzing climatic data or presence/absence data. Because the accuracy of niche modeling depends on many factors (see discussion in Warren et al. (2008)), this may introduce unnecessary biases to the final results as opposed to directly using climatic data. Second, their test does not include the absence locations (between the ranges of allopatric sister species) in the test of niche conservatism. Theoretically, we could extend the test by performing niche modeling based on absence localities and comparing the similarity indices used in Warren et al. (2008) between sister species and between species and their absence locations. However, basing niche modeling on absence localities could be problematic, especially considering that the absence localities in our study are randomly sampled within somewhat arbitrarily defined gap areas between the ranges of sister species.

We consider a significantly large D_m as potential evidence for niche conservatism in allopatric speciation. However, this test alone cannot distinguish between the model of allopatric speciation via niche conservatism and niche conservatism followed by major niche divergence, because it does not address whether the geographic range of one species is climatically suitable for its sister species, or whether the intervening absence locations are suitable. To distinguish these two models, we estimated the spatial patterns of predicted climatic suitability of the nine pairs of allopatric sister species using ecological niche modeling, as implemented in Maxent version 3.2 (Phillips et al. 2006). The combination of the D_m test with ecological niche modeling allows us to potentially distinguish among several possible speciation scenarios involving niche conservatism and/or niche divergence (Fig. 1).

Under the scenario of speciation via niche conservatism, we expect each species niche model to predict its occurrence in a

substantial portion of its sister species' geographic range, but not in the intervening gap area that separates where they are found today (Fig. 1A). In addition, the D_m for each species is expected to be significantly larger than its null distribution. In contrast, under the scenario of speciation via niche divergence, we expect that niche modeling will show that each species' geographic range is climatically unsuitable for its sister species, as is the intervening gap between their ranges. In addition, when D_m is significantly larger than its null distribution for both species, the model of speciation via niche divergence may have involved an initial isolation by niche conservatism (Fig. 1B). However, when D_m is not significantly larger than its null distribution, the model of speciation may involve an initial isolation by either niche conservatism or by other factors not included in this study (Fig. 1C). Although it is hard to rule out the role of niche conservatism in initiating allopatry, under this model, niche divergence maintains the allopatric distribution between sister species.

Theoretically, if species' niches are fully conserved during the speciation process, we expect a species to predict 100% of its sister species' localities as suitable. In contrast, when species' niches are completely divergent after an initial isolation, we expect a species to predict 0% of its sister species' localities as suitable. However, many factors may cause the results to deviate from these expectations somewhat (e.g., differences in sampling or species range sizes).

To test if sister species distributions were significantly more suitable than the intervening absence locations (Fig. 2; step 5b), we mapped the georeferenced localities of each species and their absence localities onto the Maxent predictive map of its sister species, and extracted the occurrence probabilities for each locality. For each species, we conducted a one-sided Wilcoxon rank-sum test between the distribution of occurrence probabilities for its sister species and the distribution of occurrence probabilities for the absence localities (most distributions significantly differ from normal distribution as indicated by the Kolmogorov–Smirnov test). Under the model of niche conservatism, we expect a species to predict its sister species' presence localities with significantly greater probability than the absence localities. Under the model of niche divergence, we expect probabilities for the sister species to be no greater than for the absence localities. This general approach follows that of Kozak and Wiens (2006). However, these differences in probabilities do not directly address if a species should be able to survive in a substantial portion of its sister species' range. To address this (Fig. 2; step 5c), we calculated for each species the proportion of its sister species' localities and the absence localities that were predicted as climatically suitable (see below for details). We considered a species to fail to predict the geographic range of its sister species, or the gap area that separates their ranges, when <30% of its sister species' localities or the absence localities were predicted as suitable. Although this

threshold is arbitrary, we found that use of alternate thresholds gave similar overall results.

In general, we expect the Wilcoxon test and the proportion of predicted localities to be concordant. However, to be conservative, we only considered a species pair as supporting niche conservatism if, for both species: (1) D_m values were significantly large, (2) the Wilcoxon test was significant, (3) >30% of the sister species' localities were predicted as suitable, and (4) <30% of the absence localities were predicted as suitable.

It is important to note that the three scenarios above assume that the current allopatric distributions of sister species are maintained by climatic factors, so that species should not be predicted to occur in the “gap” area between their geographic ranges using ecological niche modeling. When a species' predicted distribution includes >30% of the absence localities, we assumed that factors other than climate are presumably involved in determining the current allopatric distributions of sister species. Under this scenario, the climatic similarity of species (and similarity to gap locations) may not be relevant to speciation (Fig. 1D).

Sometimes, two sister species may have asymmetric results, potentially indicating different speciation scenarios for each species. For example, if allopatric speciation is driven by niche divergence, but the current distribution of one species is limited by factors other than climate, we might expect this species to support the scenario that involves factors other than climate, whereas its sister species supports the scenario of niche divergence. To account for these asymmetric results, we assigned each sister species separately into one of the four possible speciation scenarios, instead of treating a sister species pair as a unit.

We estimated a species' climatic niche envelope based on its georeferenced localities, using the default modeling parameters in Maxent and environmental layers with 1 km² grid resolution for seven climatic variables selected from the WORLDCLIM database. These variables are: Bio2, mean diurnal temperature range; Bio5, maximum temperature of the warmest month; Bio6, minimum temperature of the coldest month; Bio9, mean temperature of the driest quarter; Bio15, precipitation seasonality; Bio17, precipitation of the driest quarter; and Bio18, precipitation of the warmest quarter. Each variable was selected to represent a group of highly correlated variables in which the selected variable is most likely to set the range limits of anuran species (e.g., we selected maximum temperature rather than mean temperature). Groups of variables were identified by Pearson-product correlation analysis of the 19 bioclimatic variables in SPSS version 11.5 (SPSS, Chicago, IL), and variables were grouped if their $r \geq 0.70$. To decide the background area used in niche modeling, we first divided the nine sister-species pairs into four large-scale geographic regions: northern South America, Mexico-Central America, North America, and Western Australia. We then drew a rough polygon for each region that encompassed all of the geographic ranges of

the species pairs occurring in that region. This polygon was then used as the background area for each species pair in its corresponding region and the background data (data that represent the range of environmental conditions in the modeled region) were then drawn randomly from the background area. These analyses confirm that the niche models for all 18 species used have AUC values larger than 0.75 (where the AUC is the probability that a tested locality has higher presence probability than a randomly chosen site in the background area; Phillips et al. 2006). Thus, the niche models for all the species we tested are considered useful (Elith 2000). We presented results using logistic values (the default output value in Maxent version 3.2; it gives an estimate between 0 and 1 of the probability of presence) and generated binary prediction maps of presence–absence for each species using an arbitrary threshold of 0.3 (grid value is absence if its logistic value < 0.3). We chose this threshold because for most species, it appears to include adequate grids that cover the actual geographic range of the species under estimation, but yields few predicted presence locations outside the range. However, this threshold also captures the qualitatively similar pattern of climatic suitability outside the range as those produced using higher threshold values (e.g., a species that is predicted to occur in its sister species's geographic range when using a threshold higher than 0.3 is still predicted to occur in that area when using the threshold of 0.3).

STATISTICAL ANALYSIS

To test for regional differences in species' elevational ranges and temperature ranges (as predicted by Janzen's hypothesis), we performed linear regressions, in which elevational range and temperature range were regressed separately on the latitudinal midpoint of each species' distribution (Fig. 2; step 4a). However, because limited sampling of a species' localities might lead to underestimation of its temperature range, we also performed a multiple regression analysis with the number of georeferenced localities per species and species' latitudinal midpoint as the independent variables. Unless otherwise noted, all analyses were carried out using S-PLUS, version 6 (Insightful Corporation 2001).

To test for regional differences in elevational overlap and temperature overlap between sister species (Fig. 2; step 4b), we regressed the degree of elevational overlap and temperature overlap separately on the sister-species pair's latitudinal midpoint (see above). However, sister species with a larger area of overlap in their spatial distribution may tend to have larger elevational and temperature overlap as well. In addition, uneven sampling effort along the latitudinal gradient (i.e., less sampling in the tropics) could also introduce a bias in the estimation of elevational and temperature overlap. To account for the effects of area overlap and sample size, we performed a multiple regression analysis in which the area of geographic range overlap between sister species, the number of localities sampled per sister-species pair, and the sister-

species pair's latitudinal midpoint were the independent variables. The area of geographic range overlap between sister species was calculated using IDRISI version 14.0 (Eastman 2003) with 30-sec resolution.

Similarly, to test for regional differences in overall climatic ranges (Fig. 2; step 4a) and climatic overlap between sister species (Fig. 2; step 4b), we regressed the matrix of climatic ranges on the latitudinal midpoint of each species' distribution, and the matrix of climatic overlap on the latitudinal midpoint of both sister species's distribution using multivariate regression by the general linear method (GLM). A PCA was run for each matrix prior to the regressions and the selected PCs (see above) were used to generate new matrices in the following regressions. However, lower rank PCs that explain less variation but have a strong relationship with the independent variable might bias the overall relationship between the set of dependent variables and the independent variables. To examine the robustness of our results to this potential bias, we did several additional regressions using fewer PCs (if seven PCs were originally included, we ran additional regressions based on PC1, then PC1 and PC2, etc.) and compared the results. We also did multivariate multiple regressions that included the area of overlap between sister species and the number of localities sampled per sister-species pair in the set of independent variables.

All the variables used in the above regressions were first tested for normality by the Kolmogorov–Smirnov test. When data significantly departed from normality, the Robust MM regression method (Yohai and Zamar 1998) was used for the univariate regressions. This method is robust to violation of the assumption of normality, but is more efficient than traditional nonparametric regressions (Insightful Corporation 2001). For multivariate analyses, variables were normal-score transformed prior to the analyses.

Results

The frequency of allopatric: partially sympatric: sympatric distribution modes is 42: 25: 12 across a total of 79 sister-species pairs of anurans. The frequency for temperate frogs is 16: 8: 3 of 27 sister-species pairs. The frequency for tropical frogs is 26: 17: 9 of 52 sister-species pairs. The 2×3 G-test indicates no significant differences between the tropical and temperate regions ($G_{adj} = 0.34$, $P = 0.84$, $df = 2$). In the category of partial sympatry, 7 of 25 sister species have geographic overlap <20% of the area of the sister species with smaller distribution, indicating that few species have ranges that approach expectations for parapatric species.

As predicted by Janzen's (1967) hypothesis, the extents of species' temperature ranges are positively related to latitude ($F_{1,53} = 10.36$, $P < 0.0001$; Fig. 3B). This relationship remains significant even after controlling for sample size, which

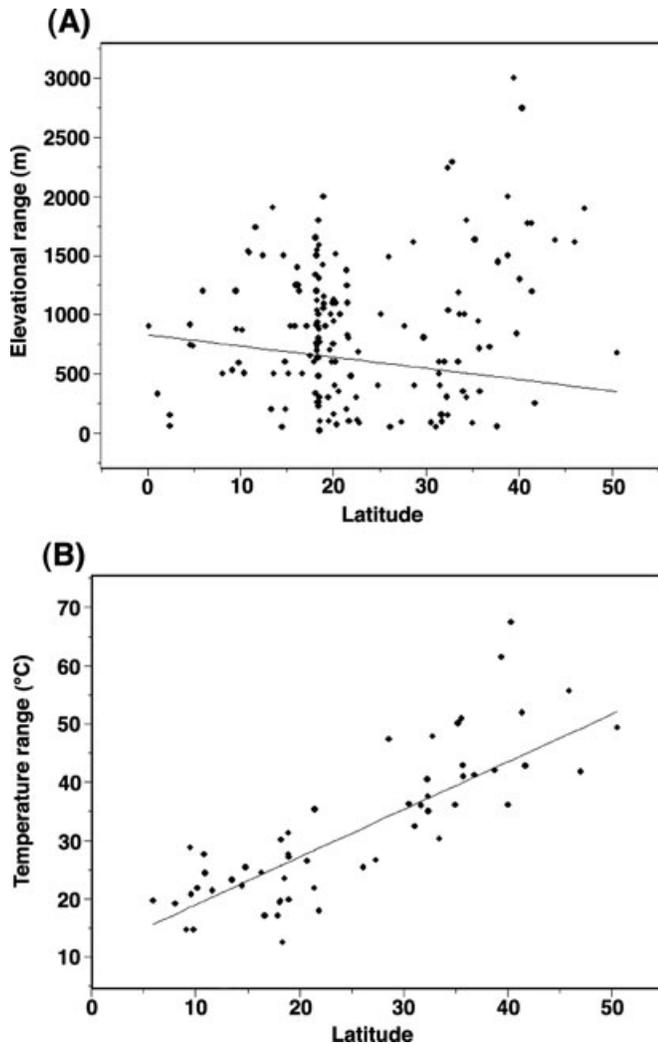


Figure 3. Regressions of species' elevational range (A) and temperature range (B) on the latitudinal midpoint of the species range, using the Robust MM method. (A) There is no relationship between species' elevational range and latitude ($F_{1,156} = -1.24$, $P = 0.22$). (B) Species' temperature ranges are positively related to latitude ($F_{1,53} = 10.36$, $P < 0.0001$).

has a significant influence on the relationship between temperature ranges and latitude ($F_{1,53} = 3.88$, $P = 0.0003$). In contrast, there is no significant relationship between species' elevational ranges and latitude ($F_{1,156} = -1.24$, $P = 0.22$; Fig. 3A). These results are consistent with those of Kozak and Wiens (2007).

However, contrary to the evolutionary predictions derived from Janzen's hypothesis (and the results of Kozak and Wiens 2007), we found no evidence that the extent of elevational overlap ($F_{1,77} = 0.94$, $P = 0.35$; Fig. 4A) or temperature overlap ($F_{1,24} = -0.36$, $P = 0.72$; Fig. 4C) between sister species were higher as latitude increased. Temperature overlap also has no significant relationship with latitude when calculated by the same formula as the one used for elevational overlap ($F_{1,26} = -0.91$, $P = 0.37$). Neither sample size ($F_{1,13} = 0.00$, $P = 1.00$) nor area

of overlap ($F_{1,13} = 0.00$, $P = 1.00$) has a significant influence on the relationship between temperature overlap and latitude. Similarly, area of overlap has no influence on the relationship between elevational overlap and latitude ($F_{1,33} = 1.26$, $P = 0.22$). Sister species produced by allopatric speciation via niche conservatism may have high niche overlap, which might obscure any latitudinal pattern in gradient speciation. However, a regression analysis that excludes allopatric sister species also indicated no linear relationship between either elevational overlap and latitude ($F_{1,33} = 0.75$, $P = 0.46$; Fig. 4B) or between temperature overlap and latitude ($F_{1,13} = -0.00$, $P = 1.00$; Fig. 4D). These different results of the two studies cannot be explained by the use of different indices of elevational overlap. Our index estimates a lower degree of overlap between sympatric sister species than the index used in Kozak and Wiens (2007), and there are more sympatric sister species in the tropics than in the temperate zone. Thus, the use of our index makes it even easier to detect a positive relationship between elevational overlap and latitude, as found in Kozak and Wiens (2007).

The overall climatic range of a species increases significantly as one goes from the tropics to the poles (regression coefficient for PC1 = 0.050, which explains 49.5% climatic variance among species; $F_{7,47} = 18.59$, $P < 0.0001$). Results of additional regressions indicate that this significant result is not caused by lower rank PCs that explain less variation but have strong relationships with the independent variable (all $P < 0.0001$; Table 1). In contrast, the degree of overall climatic overlap between sister species does not show a significant tendency to increase as latitude increases ($F_{10,15} = 2.48$, $P = 0.055$). Instead, additional regressions with fewer PCs show a significant trend in the opposite direction (i.e., climatic overlap is higher in tropical species). In particular, the regression coefficient for PC1 equals -0.026 ($P = 0.0076$), which explains 39.1% climatic variance among species (Table 1). Regressions that exclude allopatric sister species indicated no linear relationship between climatic overlap and latitude ($F_{7,7} = 1.31$, $P = 0.366$; Table 1).

Considering the results of both niche modeling (Table 2; see also Fig. S1) and D_m estimation (Table 2), we tentatively assigned 18 species in the nine allopatric species pairs (with adequate climatic data) into one of four possible speciation scenarios (Fig. 1). The results show little unambiguous evidence for speciation through niche conservatism, in either tropical or temperate species pairs. Only one species, *Engystomops pustulosus*, has results consistent with scenario B, a scenario that involves both niche conservatism and niche divergence. Results from 10 species support scenario C, which involves significant niche divergence with no evidence for niche conservatism. The remaining seven species (of 18 total) support scenario D, indicating that factors other than climate are involved in determining the current allopatric distributions of sister species.

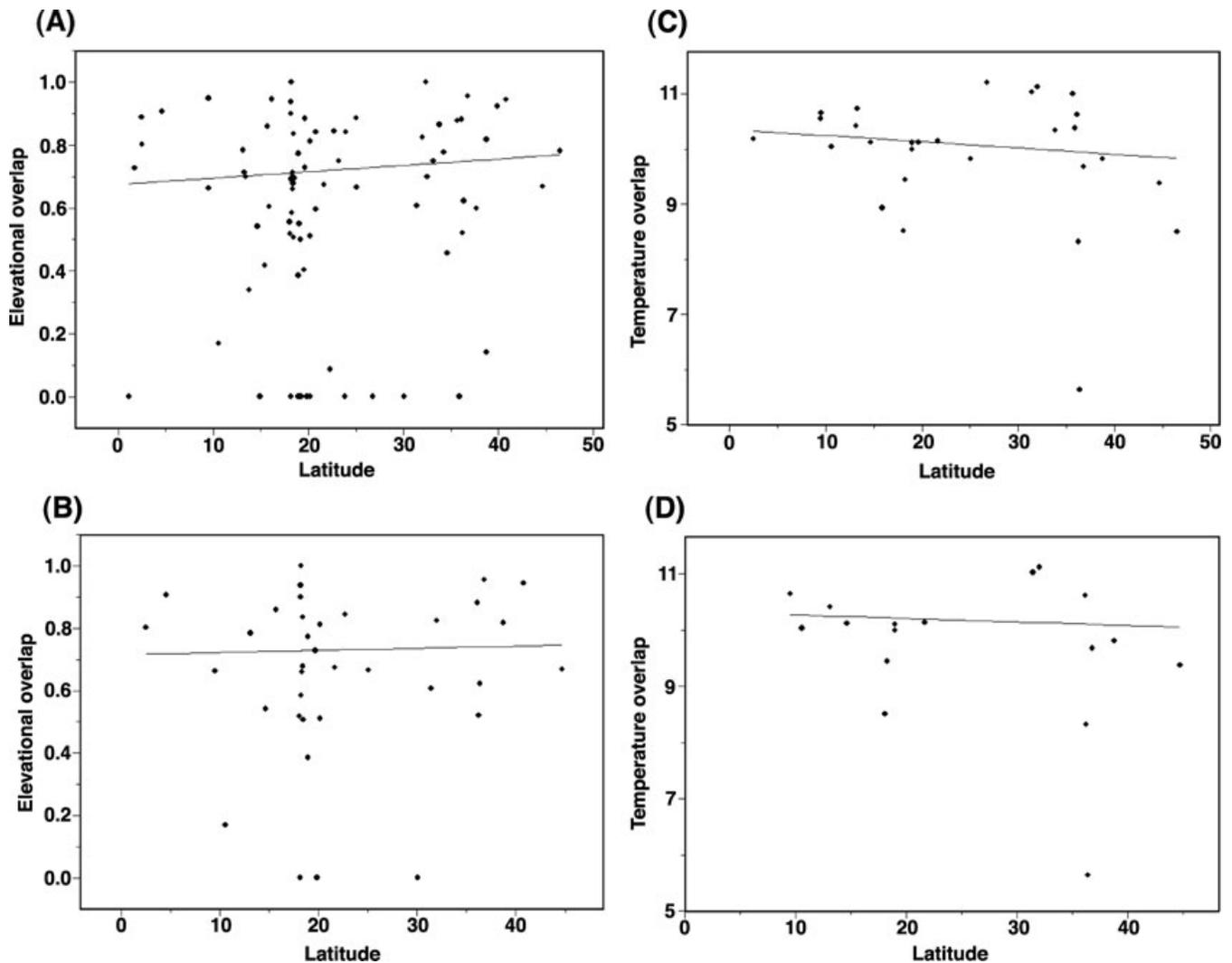


Figure 4. Regressions of sister species' elevational overlap (A, B) and temperature overlap (C, D) on the latitudinal midpoint of the species pair, using the Robust MM method. A and C are regressions including all the sister species ($n = 79$ and $n = 28$, respectively). B and D are regressions that exclude allopatric sister species ($n = 35$ and $n = 17$, respectively). There is no significant relationship between elevational overlap and latitude (A: $F_{1,77} = 0.94$, $P = 0.35$; B: $F_{1,33} = 0.75$, $P = 0.46$), nor between temperature overlap and latitude (C: $F_{1,24} = -0.36$, $P = 0.72$; D: $F_{1,13} = 0.00$, $P = 1.00$).

To test for potential regional differences in the extent of niche divergence during allopatric speciation, we also regressed the climatic overlap between these allopatric sister species on the latitudinal midpoint of their distributions using GLM. The results show that allopatric sister species in lower latitudes do not have a significantly higher degrees of climatic overlap ($F_{5,2} = 2.30$, $P = 0.330$; Table 1), although such a trend is nearly significant when only using PC1, which explains 35.3% of the variance among species pairs (regression coefficient = -0.154 , $P = 0.066$).

Discussion

In this study, we test for latitudinal differences in the relative importance of niche conservatism and niche divergence in spe-

ciation, using frogs as a model system. In general, our results support Janzen's (1967) hypothesis that tropical species have narrower climatic ranges. However, our results do not support the hypothesis that these narrower climatic ranges lead to a greater tendency for speciation through elevational and climatic divergence in the tropics, in contrast to recent results from salamanders (Kozak and Wiens 2007). Instead, some of our results suggest that sister species may be more climatically similar to each other in the tropics (Table 1). To our knowledge, these two amphibian studies represent the first attempts to systematically test for latitudinal differences in speciation mechanisms related to climatic distributions (i.e., niche conservatism vs. divergence), although they come to quite different conclusions. We also present possibly the first test for latitudinal differences in geographic modes of speciation

Table 1. Regression coefficients (β) and P -values for the multivariate regressions of sister species' climatic overlap on the latitudinal midpoint of the pair, using different numbers of principal components (PCs) for climatic data. PCs are used in the order of the amount of variance that each explains. In each row, for example, when the number of PCs is two, the P -value is the statistical significance for the multivariate regression using PC1 and PC2, and β is the independent regression coefficient for PC2.

Number of PCs	Climatic range		Climatic overlap (all species pairs)		Climatic overlap (no allopatric pairs)		Climatic overlap (allopatric pairs)	
	β	P	β	P	β	P	β	P
1	0.050	<0.0001	-0.026	0.0076	-0.013	0.3617	-0.154	0.0665
2	-0.148	<0.0001	0.016	0.0195	-0.006	0.5594	0.018	0.2869
3	0.022	<0.0001	0.001	0.0516	0.007	0.5721	-0.036	0.3700
4	-0.014	<0.0001	0.000	0.1083	-0.008	0.5771	-0.012	0.5432
5	0.000	<0.0001	-0.001	0.1929	0.005	0.1403	-0.040	0.3303
6	-0.001	<0.0001	0.008	0.0068	0.001	0.2376	-	-
7	-0.010	<0.0001	-0.002	0.0146	0.000	0.3661	-	-
8	-	-	-0.002	0.0162	-	-	-	-
9	-	-	0.001	0.0296	-	-	-	-
10	-	-	-0.001	0.0550	-	-	-	-

(or at least, geographic distribution of sister species). Our results suggest that there is no significant difference in the frequency of different speciation modes between tropical and temperate regions.

Our major results are as follows. First, as predicted by Janzen's hypothesis, we found that anuran species inhabit significantly wider climatic regimes as one goes from the tropics to the pole, based on data from 158 species. However, the elevational

Table 2. Results of niche modeling and D_m (P -value) estimation for each species in the nine allopatric sister-species pairs. Positive D_m with P -value ≤ 0.05 indicates that sister species occupy climatic niches that are significantly more similar to each other than they are to the absence localities that separate them. Negative D_m with P -value ≥ 0.95 indicates that sister species occupy climatic niches that are significantly less similar to each other than they are to the intervening absence localities. The P -value in the Wilcoxon rank-sum test represents the probability that a species predicts its sister species' localities with greater probability than the absence localities (significantly greater when P -value ≥ 0.95). The proportion of suitable sister species localities and absence localities are estimated based on niche modeling for a given species using seven climatic variables (see Fig. S1). Categories of speciation scenarios correspond to letters in Figure 1. Species are classified into scenarios based on the combination of niche modeling and D_m values.

Sister species	D_m (P -value)	P -value in Wilcoxon rank-sum test	Proportion of suitable sister species localities	Proportion of suitable absence localities	Scenario
<i>Engystomops petersi</i>	-0.101 (0.703)	0.016	0.38	0.65	D
<i>E. pustulosus</i>	0.781 (0.013)	0.688	0.23	0.05	B
<i>Phyllobates vittatus</i>	-0.569 (0.982)	0.999	0.20	0	C
<i>P. lugubris</i>	0.028 (0.535)	0.170	0	0	C
<i>Agalychnis annae</i>	0.134 (0.254)	0.092	0.08	0.14	C
<i>A. moreletii</i>	-0.572 (1.000)	0.999	0.81	0.36	D
<i>Tlalocohyla picta</i>	-0.254 (0.879)	<0.001	0	0	C
<i>T. smithii</i>	-0.425 (0.907)	0.001	0	0.22	C
<i>Rana tarahumarae</i>	-0.256 (0.977)	0.141	0.17	0.38	D
<i>R. pustulosa</i>	-0.541 (0.994)	0.004	0.25	0.88	D
<i>Arenophryne rotunda</i>	-0.415 (0.912)	0.003	0.20	1.00	D
<i>A. xiphorhyncha</i>	-0.644 (1.000)	0.036	0.75	1.00	D
<i>Bufo californicus</i>	-0.269 (1.000)	<0.001	0	0	C
<i>B. microscaphus</i>	-0.230 (0.996)	<0.001	0	0	C
<i>Pseudacris brimleyi</i>	-0.074 (0.538)	0.002	0.16	0.60	D
<i>P. brachyphona</i>	-0.313 (0.995)	0.004	0.13	0.25	C
<i>Ascaphus montanus</i>	-0.422 (1.000)	0.003	0.08	0.11	C
<i>A. truei</i>	0.068 (0.606)	0.960	0	0	C

ranges of species do not show the same pattern of increasing width with latitude, a surprising result also found by Kozak and Wiens (2007; but see McCain [2009] for more extensive analyses of this particular question). Second, there is no evidence that anuran sister species occurring in lower latitude exhibit less overlap in their elevational and climatic ranges (based on data from 79 and 28 species pairs, respectively). In fact, some analyses even show significant evidence for the opposite trend (i.e., tropical species pairs tend to be climatically more similar to each other than temperate pairs). Taken together, these two results suggest that species in lower latitudes, although having narrower climatic regimes, do not show greater propensity for divergent speciation along environmental gradients. Finally, our analyses of nine allopatric pairs suggest that climatic niche conservatism may not be commonly important in allopatric speciation for anurans. We find no species pairs that unambiguously support this scenario. Instead, niche divergence and factors other than climate seem to determine the current distributions of allopatric sister species. But we find no evidence that tropical allopatric species pairs show greater niche divergence than temperate pairs. However, these latter results on niche conservatism and divergence are based on only nine allopatric sister-species pairs, and so should be taken with appropriate caution. In the sections that follow, we discuss the major assumptions of our analyses and the implications of our results for studies of parapatric speciation and patterns of species richness.

MAJOR ASSUMPTIONS

Our analyses rest on several assumptions. However it is important to note that our methods are similar to those of Kozak and Wiens (2007), and so we make very similar assumptions. Thus, even if the geographic and elevational patterns that we analyze do not fully address the causes of speciation, the biogeographic patterns found in frogs are still very different from those found in salamanders. One notable difference between our methods and those of Kozak and Wiens (2007) is that we only included species pairs in which both species were represented by at least five localities (for climatic data). However, Kozak and Wiens (2007) concluded that the different patterns in tropical and temperate species pairs were not explained by different sample sizes in tropical and temperate species pairs.

First, we assume that the 79 species sampled are representative of overall patterns in frogs, and not some unusual subset of taxa. Our sampling of species pairs (see Table S1) includes taxa in both the New World and Old World, the primitive and advanced frogs, and in the two major clades of advanced anurans (Hylloidea, Ranoidea). Our phylogenetic and geographic sampling for frogs is more diverse than that of Kozak and Wiens (2007) for salamanders, although most tropical salamanders occur in only one clade (Bolitoglossinae) in one geographic region (Middle America).

Second, we assume that the current geographic distributions of sister species reflect their original geographic modes of speciation. However, many factors can drive significant post-speciational range shifts that increase or decrease species' geographic overlap (e.g., Futuyma 1998; Barraclough and Vogler 2000; Losos and Glor 2003). Most importantly, the sister species in the "partial sympatry" category in our study could be the result of secondary contact between allopatric species or changes in distributions after parapatric and sympatric speciation. However, even though there may be some shifts in distribution after speciation, our results from analyses of geographic overlap, elevational overlap, and climatic distribution are all concordant. These analyses all suggest that there is no greater propensity for parapatric speciation along environmental and elevational gradients in the tropics.

Similarly, it is possible that post-speciational climatic changes may influence our analyses of niche conservatism and divergence. For example, environmental changes after speciation might cause the geographic area separating the ranges of two allopatric species to become less hospitable over time, leading to inflated values of *Dm* and overestimated support for niche conservatism. However, our analyses do not rely on *Dm* values alone as a test of niche conservatism. Perhaps more importantly, our results showed little evidence for allopatric speciation through niche conservatism in the nine allopatric species pairs that we examined, suggesting that such an inflation was not important in our study.

Third, we assume that there is some relationship between the climatic distribution of species (based on environmental data from known localities) and their climatic tolerances. For example, when sister species occur in very different climatic regimes, we assume that one species could not tolerate the climatic conditions experienced by the other, such that the climatic distributions may serve to isolate them from gene flow. Similarly, if the absence localities separating members of an allopatric species pair are very different from the presence localities, we assume that these differences may serve as a barrier to dispersal and gene flow between species. In partial support of this assumption, Kozak and Wiens (2007) showed a general relationship between the climatic distribution of selected salamander species and their body temperatures in the field. However, we acknowledge that this does not directly address physiological tolerances per se. Thus, the climatic distributions that we analyze here could overestimate the actual climatic tolerance range of a species, especially given that some amphibians have effective thermoregulatory behaviors and can hibernate or migrate to avoid extreme temperatures (Zug et al. 2001).

Further, species distributions may also be influenced by biotic interactions, rather than by climate alone (Lomolino et al. 2006). For example, Heyer (1967) showed that herpetofaunal distributions correlated with particular vegetation zones that create necessary microhabitats. Competition between closely related

species may also set limits to species ranges, potentially resulting in parapatric distributions between sister species (e.g., Twomey et al. 2008). Consequently, the observed climatic-regime width could underestimate the climatic tolerance range of a species. Although it would be useful to have more data on the roles of physiological tolerances and biotic interactions on species distributions in frogs, our results do not suggest that there are obvious latitudinal differences in their geographic and climatic patterns of speciation to be explained. Finally, our own results suggest that climatic factors may not be universally important in setting geographic range limits in frogs (at least for the nine pairs we studied in detail).

We also acknowledge that the factors limiting geographic distributions are only one of many aspects of speciation (Coyne and Orr 2004). Other intrinsic isolating mechanisms may be important, especially for initiating parapatric or sympatric speciation (Turelli et al. 2001) and maintaining the reproductive isolation of allopatric lineages that have become partially sympatric. For example, reproductive isolation based on calls has been recorded for allopatric, parapatric, and sympatric sister species of frogs (e.g., Littlejohn 1965; Fouquette 1975; Loftus-Hills and Littlejohn 1992). There is now growing evidence that the evolution of male calls and female preferences are important drivers of speciation in frogs. For example, Hoskin et al. (2005) showed that premating isolation caused by natural selection against hybridization drove both rapid parapatric speciation and rapid allopatric speciation in treefrogs (Hylidae: *Litoria*) in Australia. Similarly, Boul et al. (2007) showed that divergent sexual selection on female preferences and male calls drove premating isolation and speciation in Amazonian frogs (*Physalaemus/Engystomops*).

PARAPTRIC SPECIATION ALONG ENVIRONMENTAL GRADIENTS

Our results from anurans do not support the hypothesis that parapatric speciation along environmental gradients is more frequent in the tropics (e.g., Moritz et al. 2000; Kozak and Wiens 2007). This hypothesis is derived partly from Janzen's (1967) observation that tropical species have narrower climatic regimes due to limited temperature seasonality, which may lead to narrower elevational ranges and greater opportunities for parapatric speciation along mountain slopes. Our results partially support Janzen's hypothesis by showing that species in lower latitudes occupy narrower climatic regimes (Fig. 1). However, we found no tendency for greater elevational or climatic divergence between species pairs in the tropics (and some evidence for the opposite trend; Table 1), as would be predicted under a model of parapatric speciation along environmental gradients.

The prediction of a higher frequency of parapatric speciation and niche divergence in the tropics is based on two assumptions following from Janzen's (1967) hypothesis. First, more limited

temperature seasonality in the tropics should select for organism that are tightly adapted to a narrow elevational band. Second, tropical species should also evolve limited thermal acclimation, leading to limited dispersal between different elevations (Ghalambor et al. 2006). In fact, we find that tropical species do not have significantly narrower elevational ranges (see also Kozak and Wiens 2007; but see McCain 2009), and tropical sister species do not show less elevational and climatic overlap along elevational gradients. Thus, one of the reasons why we do not support the prediction of more gradient speciation in the tropic is because the elevational distribution patterns we observed do not support this prediction from Janzen's hypothesis. In fact, our finding that species in lower latitudes occupy narrower climatic regimes simply supports the assumption of less climatic seasonality in the tropics, rather than supporting any direct effects of limited seasonality on the relationship between elevation, climate, latitude, and speciation.

Overall, our results suggest that parapatric speciation along environmental gradients may be uncommon or absent among the 79 frog species pairs that we sampled, regardless of whether they are tropical or temperate. Perhaps the best evidence for parapatric speciation is the finding that sister species have abutting distributions with no obvious geographic barriers in between. However, we found no species pairs with such a distribution pattern, only species distributions that are allopatric, sympatric, or partially sympatric. In theory, some species might have speciated parapatrically and then become partially sympatric, however only 7 of 25 species pairs in the "partial sympatry" group have overlap <20%, an arbitrary threshold that has been used as evidence for parapatric speciation in previous studies (e.g., Lynch 1989). Moreover, if sister species speciated parapatrically along environmental gradients that limited their dispersal, then we would not expect any overlap in their geographic ranges after speciation (assuming that the environmental gradient that caused them to speciate is still present).

SPECIATION PATTERNS AND PATTERNS OF SPECIES RICHNESS

Latitudinal differences in speciation mechanisms might potentially contribute to the latitudinal diversity gradient, if different speciation mechanisms influence the rate of speciation. Anurans and plethodontid salamanders both seem to be ancestrally temperate, and have dispersed into the tropics subsequently, and both have high species richness in the tropics (Wiens 2007). Wiens (2007) attributed this pattern to differential diversification rates along the latitudinal gradient. Indeed, he found that diversification rates in both amphibian groups increased significantly with decreasing latitude (but in frogs, this only pertains to the largest time scales, not within diverse, primarily tropical families; Wiens et al. 2006, 2009). Higher diversification rates may be caused by

either higher speciation rates or lower extinction rates, and distinguishing the relative contribution of these two factors is relatively difficult (e.g., Ricklefs 2007). In fact, higher diversification rates in tropical amphibian clades may be due to higher extinction rates in the temperate zone rather than faster tropical speciation rates (Wiens 2007).

Many factors have been proposed that might promote faster speciation in tropical faunas (e.g., Willig et al. 2003; Mittelbach et al. 2007). Although climatic stratification in the tropics does not seem to drive widespread speciation along climatic gradients in tropical frogs (based on our results), climatic stratification could also make allopatric speciation via niche conservatism more effective (Ghalambor et al. 2006). However, our findings also show that climatic niche conservatism may not be widely important in allopatric speciation in anurans. Instead, niche divergence and factors other than climate may be more important in determining the current distributions of allopatric sister species in frogs (at least for the nine species pairs we studied in detail). Most importantly, our results do not support the idea that speciation mechanisms are fundamentally different between tropical and temperate regions. Thus, our results provide little basis for predicting that speciation rates should be higher in tropical frogs, a finding echoed in analyses of diversification rates across latitudes in species-rich frog clades (e.g., Wiens et al. 2006, 2009) and across amphibians (Wiens 2007). Overall, our study shows different latitudinal trends in speciation mechanisms in frogs and in salamanders, suggesting that the relationships between climatic zonation, speciation mechanisms, and species richness are not straightforward.

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LITERATURE CITED

- AmphibiaWeb: Information on amphibian biology and conservation. [web application]. 2009. Berkeley, California: AmphibiaWeb. Available at <http://amphibiaweb.org/>. Accessed: June 4, 2009.
- Barracough, T. G., and A. P. Vogler. 2000. Detecting the geographical pattern of speciation from species-level phylogenies. *Am. Nat.* 155:419–434.
- Boul, K. E., W. C. Funk, C. R. Darst, D. C. Cannatella, and M. J. Ryan. 2007. Sexual selection drives speciation in an Amazonian frog. *Proc. R. Soc. Lond. B* 274:399–406.
- Coyne, J. A., and H. A. Orr. 2004. *Speciation*. Sinauer Associates, Sunderland, MA.
- Eastman, R. 2003. *Idrisi Kilimanjaro manual and tutorial*. Clark Labs, Clark Univ., Worcester, MA.
- Elith, J. 2000. Quantitative methods for modeling species habitat: comparative performance and an application to Australian plants. Pp. 39–58 in S. Ferson and M. Burgman, eds. *Quantitative methods for conservation biology*. Springer, New York.
- Fouquette, M. J. 1975. Speciation in chorus frogs. I. Reproductive character displacement in the *Pseudacris nigrata* complex. *Syst. Zool.* 24:16–23.
- Frost, D. R. 2008. *Amphibian Species of the World: an online reference*. American Museum of Natural History, New York, USA. Available at <http://research.amnh.org/herpetology/amphibia>. Accessed: September, 2008.
- Futuyma, D. J. 1998. *Evolutionary biology*. 3rd ed. Sinauer Associates, Sunderland, MA.
- Ghalambor, C. K., R. B. Huey, P. R. Martin, J. J. Tewksbury, and G. Wang. 2006. Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integr. Comp. Biol.* 46:5–17.
- Graham, C. H., S. R. Ron, J. C. Santos, C. J. Schneider, and C. Moritz. 2004. Integrating phylogenetics and environmental niche models to explore speciation mechanisms in dendrobatid frogs. *Evolution* 58:1781–1793.
- Heyer, R. W. 1967. A herpetofaunal study of an ecological transect through the Cordillera de Tilarán, Costa Rica. *Copeia* 1967:259–271.
- Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* 25:1965–1978.
- Hoskin, C. J., M. Higgie, K. R. McDonald, and C. Moritz. 2005. Reinforcement drives rapid allopatric speciation. *Nature* 437:1353–1356.
- Hutchinson, G. E. 1957. *A Treatise on Limnology*. Vol. 1. John Wiley & Sons, New York. 1015pp.
- Insightful Corporation. 2001. *S-Plus 6 for Windows: Guide to Statistics*. Vol. 1. Insightful Corporation, Seattle, WA.
- IUCN. 2009. *IUCN Red List of threatened species*. Version 2009.1. Available at <http://www.iucnredlist.org>. Accessed: June 2008.
- Janzen, D. H. 1967. Why mountain passes are higher in the tropics. *Am. Nat.* 101:233–249.
- Kaiser, H. F. 1960. The application of electronic computers to factor analysis. *Educ. Psychol. Meas.* 20:141–151.
- Kozak, K. H., and J. J. Wiens. 2006. Does niche conservatism promote speciation? A case study in North American salamanders. *Evolution* 60:2604–2621.
- . 2007. Climatic zonation drives latitudinal variation in speciation mechanisms. *Proc. R. Soc. Lond. B* 274:2995–3003.
- Lemmon, E. M., A. R. Lemmon, J. T. Collins, J. A. Lee-Yaw, and D. C. Cannatella. 2007. Phylogeny-based delimitation of species boundaries and contact zones in the trilling chorus frogs (*Pseudacris*). *Mol. Phylogenet. Evol.* 44:1068–1082.
- Littlejohn, M. J. 1965. Premating isolation in the *Hyla ewingi* complex (Anura, Hylidae). *Evolution* 19:234–243.
- Loftus-Hills, J. J., and M. J. Littlejohn. 1992. Reinforcement and reproductive character displacement in *Gastrophryne carolinensis* and *G. olivacea* (Anura, Microhylidae)—a reexamination. *Evolution* 46:896–906.
- Lomolino, M. V., B. R. Riddle, and J. H. Brown. 2006. *Biogeography*, 3rd ed. Sinauer Associates, Sunderland, MA.
- Losos, J. B., and R. E. Glor. 2003. Phylogenetic comparative methods and the geography of speciation. *Trends Ecol. Evol.* 18:220–227.
- Lynch, J. D. 1989. The gauge of speciation: on the frequencies of modes of speciation. Pp. 527–553 in D. Otte and J. A. Endler, eds. *Speciation and its consequences*. Sinauer Associates, Sunderland, MA.
- McCain, C. M. 2009. Vertebrate range sizes indicate that mountains may be 'higher' in the tropics. *Ecol. Lett.* 12:550–560.
- Mittelbach, G. G., D. W. Schemske, H. V. Cornell, A. P. Allen, J. M. Brown, M. B. Bush, S. P. Harrison, A. H. Hurlbert, N. Knowlton, H. A. Lessios et al. 2007. Evolution and the latitudinal diversity gradient: speciation, extinction, and biogeography. *Ecol. Lett.* 10:315–331.
- Moritz, C., J. L. Patton, C. J. Schneider, and T. B. Smith. 2000. Diversification of rainforest faunas: an integrated molecular approach. *Annu. Rev. Ecol. Syst.* 31:533–563.

- Nosil, P., T. H. Vines, and D. J. Funk. 2005. Perspective: reproductive isolation caused by natural selection against immigrants using divergent environments. *Evolution* 59:705–719.
- Ogden, R., and R. S. Thorpe. 2002. Molecular evidence for ecological speciation in tropical habitats. *Proc. Natl. Acad. Sci. USA* 99:13612–13615.
- Peterson, A. T., and A. S. Nyári. 2007. Ecological niche conservatism and Pleistocene refugia in the thrush-like mourner, *Schiffornis* sp., in the Neotropics. *Evolution* 62:173–183.
- Peterson, A. T., J. Soberón, and V. Sánchez-Cordero. 1999. Conservatism of ecological niches in evolutionary time. *Science* 285:1265–1267.
- Phillimore, A. B., C. D. Orme, G. H. Thomas, T. M. Blackburn, P. M. Bennett, K. J. Gaston, and I. P. F. Owens. 2008. Sympatric speciation in birds is rare: insights from range data and simulations. *Am. Nat.* 171:646–657.
- Phillips, S. J., R. P. Anderson, and R. E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. *Ecol. Model.* 190:231–259.
- Raxworthy, C. J., C. M. Ingram, N. Rabibisoa, and R. G. Pearson. 2007. Applications of ecological niche modeling for species delimitation: a review and empirical evaluation using day geckos (*Phelsuma*) from Madagascar. *Syst. Biol.* 56:907–923.
- Ricklefs, R. E. 2007. Estimating diversification rates from phylogenetic information. *Trends Ecol. Evol.* 22:601–610.
- Rissler, L. J., and J. J. Apodaca. 2007. Adding more ecology into species delimitation: ecological niche models and phylogeography help define cryptic species in the black salamander (*Aneides flavipunctatus*). *Syst. Biol.* 56:924–942.
- Rundle, H. D., and P. Nosil. 2005. Ecological speciation. *Ecol. Lett.* 8: 336–352.
- Schluter, D. 2001. Ecology and the origin of species. *Trends Ecol. Evol.* 16:372–380.
- . 2009. Evidence for ecological speciation and its alternative. *Science* 323:737–741.
- Sokal, R. R., and F. J. Rohlf. 1995. *Biometry: the principles and practice of statistics in biological research*. 3rd edn. W. H. Freeman and Co., New York.
- Stockman, A. K., and J. E. Bond. 2007. Delimiting cohesion species: extreme population structuring and the role of ecological interchangeability. *Mol. Ecol.* 16:3374–3392.
- Turelli, M., N. H. Barton, and J. A. Coyne. 2001. Theory and speciation. *Trends Ecol. Evol.* 16:330–343.
- Twomey, E., V. Morales, and K. Summers. 2008. Evaluating condition-specific and asymmetric competition in a species-distribution context. *Oikos* 117:1175–1184.
- Warren, D. L., R. E. Glor, and M. Turelli. 2008. Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. *Evolution* 62:2868–2883.
- Wiens, J. J. 2004a. Speciation and ecology revisited: phylogenetic niche conservatism and the origin of species. *Evolution* 58:193–197.
- . 2004b. What is speciation and how should we study it? *Am. Nat.* 163:914–923.
- . 2007. Global patterns of diversification and species richness in amphibians. *Am. Nat.* 170:S86–S106.
- Wiens, J. J., and C. H. Graham. 2005. Niche conservatism: integrating evolution, ecology, and conservation biology. *Annu. Rev. Ecol. Syst.* 36:519–539.
- Wiens, J. J., C. H. Graham, D. S. Moen, S. A. Smith, and T. W. Reeder. 2006. Evolutionary and ecological causes of the latitudinal diversity gradient in hylid frogs: treefrog trees unearth the roots of high tropical diversity. *Am. Nat.* 168:579–596.
- Wiens, J. J., J. Sukumaran, R. A. Pyron, and R. M. Brown. 2009. Evolutionary and biogeographic origins of high tropical diversity in Old World frogs (Ranidae). *Evolution* 63:1217–1231.
- Willig, M. R., D. M. Kaufman, and R. D. Stevens. 2003. Latitudinal gradients of biodiversity: pattern, process, scale and synthesis. *Annu. Rev. Ecol. Syst.* 34:273–309.
- Yohai, V. J., and R. H. Zamar. 1998. Optimal locally robust M-estimates of regression. *J. Statist. Plann. Inference* 64:309–323.
- Zug, G. R., L. J. Vitt, and J. P. Caldwell. 2001. *Herpetology: an introductory biology of amphibians and reptiles*. 2nd ed. Academic Press, New York.

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Supporting Information

The following supporting information is available for this article:

Figure S1. Predicted geographic distributions for each species of the nine selected allopatric sister species pairs, based on ecological niche modeling.

Table S1. The 79 species included in analyses of geographic mode and elevational distribution, along with their distribution patterns, latitudinal midpoints, maximum and minimum elevations, and elevational overlaps, and the literature sources that suggest they are sister species.

Table S2. The 28 sister-species pairs used in climatic analyses, including the ranges of values of each species for each of the 19 variables (maximum – minimum), sample size (georeferenced localities per species), temperature ranges, and additional literature resources for localities (all others are from museum localities from HerpNet).

Table S3. The 28 sister-species pairs used in the climatic analyses, included along with the latitudinal midpoint of the species pair (the average of the latitudinal midpoints of the two species), temperature overlap, geographic area overlap, and overlap in values for each of the 19 climatic variables.

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

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

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