LETTER

Phylogenetic origins of local-scale diversity patterns and the causes of Amazonian megadiversity

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Abstract

What explains the striking variation in local species richness across the globe and the remarkable diversity of rainforest sites in Amazonia? Here, we apply a novel phylogenetic approach to these questions, using treefrogs (Hylidae) as a model system. Hylids show dramatic variation in local richness globally and incredible local diversity in Amazonia. We find that variation in local richness is not explained primarily by climatic factors, rates of diversification (speciation and extinction) nor morphological variation. Instead, local richness patterns are explained predominantly by the timing of colonization of each region, and Amazonian megadiversity is linked to the long-term sympatry of multiple clades in that region. Our results also suggest intriguing interactions between clade diversification, trait evolution and the accumulation of local richness. Specifically, sympatry between clades seems to slow diversification and trait evolution, but prevents neither the accumulation of local richness over time nor the co-occurrence of similar species.

Keywords

Amazonia, amphibians, anurans, community ecology, diversification, phylogeny, species richness, tropical rainforest.

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INTRODUCTION

A major challenge in ecology is to explain why local species richness varies across the globe (e.g. Ricklefs 1987; Morin 1999) and why and how large numbers of species co-occur in some tropical rainforest sites (e.g. Hubbell 2001; Kraft *et al.* 2008). Species richness patterns at all scales are ultimately dependent on the processes that directly change the number of species in a given location: speciation, extinction (local or global) and dispersal (e.g. Ricklefs 1987). For living taxa, these processes of diversification and biogeographic dispersal can be revealed using time-calibrated molecular phylogenies. Phylogenies are now being used to address patterns of local community structure (e.g. Webb *et al.* 2002; Cavender-Bares *et al.* 2009) and large-scale regional patterns of richness (e.g. Ricklefs 2006; Wiens *et al.* 2006, 2009; Mittelbach *et al.* 2007). However, the application of phylogenetic approaches to understanding patterns of local richness remains largely unexplored (but for related studies see Brown *et al.* 2000; Stephens & Wiens 2003; Stevens 2006).

How might local-scale richness patterns within a group be related to the processes of speciation, extinction and dispersal? In general, one might expect local species richness to vary with local-scale ecological conditions (e.g. climate, pH, predation and disturbance), the size of the regional species pool or some combination of these two factors (e.g. Harrison & Cornell 2008). However, even a perfect relationship between local richness and local ecological conditions must still (ultimately) be related to the processes of speciation, extinction and dispersal. For example, variation in local species richness in a given group along an environmental gradient might occur if conditions at one end of the gradient promote diversification (i.e. higher rates of speciation or lower rates of extinction), yielding more species that can occur at these sites. Alternately, the group may have originated in ecological conditions at the species-rich end of the gradient and only

recently evolved traits that allowed some species to colonize the other end. Thus, communities at one end may have more species because the group occurred longer under those conditions, allowing more time for speciation to build up richness in those communities (e.g. the time-for-speciation effect; Stephens & Wiens 2003). A phylogenetic approach can allow these two, non-exclusive possibilities to be disentangled. Similarly, the size of the regional species pool must also be explained in terms of speciation, extinction and dispersal. Thus, some regions may have more species because they have been occupied longer (e.g. Stephens & Wiens 2003) or because ecological conditions in those regions promote more rapid diversification (e.g. Mittelbach *et al.* 2007).

Understanding the diversification and dispersal of species within clades is critical to understanding patterns of local richness, but local richness and species interactions may also influence these clade-level patterns of diversification (e.g. Harrison & Cornell 2008). Resolving these complex interrelationships may be critical to explaining patterns of local richness. For example, recent phylogenetic studies have hypothesized 'density-dependent' effects on diversification, where rates of species accumulation within a clade seem to slow down over time due to increasing species density (e.g. Phillimore & Price 2008; Rabosky & Lovette 2008) and ecological limits on how many species can co-exist (e.g. Rabosky 2009). Thus, given a broadly distributed group where some regions have multiple sympatric clades and other regions have only one, local richness might converge across regions regardless of the number of clades present, due to density dependence. Or alternately, local richness may be highest where multiple clades occur in sympatry over long periods of time. Such interactions between local richness and clade diversification remain poorly explored (but see Weir 2006).

Furthermore, species interactions may depend on overlap and divergence in traits related to resource utilization (Morin 1999).

Understanding the origins of local richness patterns may require considering how such traits evolve and facilitate sympatry, and how sympatry in turn influences the evolution of these traits (e.g. Schluter 2000; Davies *et al.* 2007; Carlson *et al.* 2009; Moen & Wiens 2009). These issues can be addressed with a phylogenetic approach.

Here, we test hypotheses about the interplay of local richness, diversification and trait evolution in treefrogs (Hylidae). Hylids offer an excellent model system for several reasons. Despite their high overall diversity (> 850 species; AmphibiaWeb; http://amphibia-web.org/), their phylogeny is relatively well understood, with most species assigned to seemingly monophyletic genera and a recent phylogeny includes all genera and > 350 species (Wiens *et al.* 2010a). Range maps are available for most species (e.g. http://www.iucnredlist.org/initiatives/amphibians) and their richness at many sites is well documented (e.g. Duellman 1978, 1988, 2005). Previous studies have addressed their regional diversity (Wiens *et al.* 2006; Algar *et al.* 2009) but not local richness. These studies suggested that hylid regional diversity is explained by time (Wiens *et al.* 2006) or climate (Algar *et al.* 2009), but neither directly analysed both factors. Previous research also showed that body size is the major axis of morphological variation in hylids (Moen *et al.* 2009), that body size strongly influences dietary resource-use (e.g. Duellman 2005), that diverse tropical communities have greater ranges of body sizes among species than temperate communities and that similar body-size ranges have evolved in tropical communities in different regions (Moen & Wiens 2009). Body size may be particularly important in that sympatric species tend to share broadly similar microhabitats (e.g. arboreal), life histories (e.g. aquatic larvae in ponds and streams) and diet (e.g. insects), whereas body-size differences among species seem to facilitate use of different dietary resources (e.g. Duellman 2005; Moen & Wiens 2009; Moen *et al.* 2009).

We combine a large-scale, time-calibrated hylid phylogeny (362 species) with data on local richness, climate and body size to address several general questions about the origins of local richness patterns and their relationship to diversification and trait evolution. (1) Are local richness patterns correlated with local climatic conditions, or with the timing of colonization of the regions in which these local assemblages are embedded? (2) Are there strong relationships between climatic variables and diversification rates, which might explain relationships between climate and richness? Importantly, this hypothesis subsumes numerous, more specific explanations for how ecological conditions in tropical rainforests might promote diversification and species co-existence (e.g. Mittelbach *et al.* 2007), leading to higher local species richness. Or alternately, do species-rich clades occur ancestrally in these (presently) high-richness climatic conditions? (3) Is there evidence that diversification and local richness of clades depend on the density of other clades, such that clades that are geographically isolated from other clades (e.g. on different continents) have higher rates of diversification and higher local richness (relative to clades that are sympatric with each other)? (4) Do sites with greater ranges of body sizes among species have higher richness (as predicted if greater body-size variation allows more species to co-exist)? (5) Do geographically isolated clades have higher rates of body-size evolution, as expected if extensive sympatry between clades limits trait evolution? We use hylids to address these broader questions and to address the causes of high local richness in Amazonia, where hylids (and many other groups) are particularly diverse.

METHODS

Species richness of local sites

We obtained data on local species composition of 123 sites throughout the range of Hylidae, using literature and museum records (Tables S1–S3), emphasizing detailed studies of amphibian faunas of local sites (e.g. Duellman 1978, 2005). Sites were typically several km^2 in size and represented a single biome (e.g. lowland tropical rainforest) but multiple habitats (e.g. forest, pond and stream). These sites are standard for studies of tropical amphibians (e.g. Duellman 1978, 1988, 2001, 2005), and our goal was to compare richness among sites. We consider this the most appropriate scale for 'local' richness for hylids, but we acknowledge that smaller scales could be used (in theory) and may be more appropriate for other organisms (e.g. plants).

For most analyses, we used a well-sampled locality to represent each biogeographic region (Fig. 1; Table 1) to reduce potential problems of imbalanced numbers of sites among regions, spatial autocorrelation, and inadequately surveyed sites. For each region, we used the site with the maximum local richness (although other sites within each region had similar richness). We considered 12 major biogeographic regions, corresponding to six large-scale regions where hylids occur (North America, Middle America, the West Indies, Europe, Asia and Australia), and six ecogeographic regions within South America [Amazon Basin, Andes mountains, Atlantic rainforest, Chocó, Guyana highlands and Cerrado (and adjacent grasslands, including Chaco, Pampas and Monte)]. More details on site selection and justification for regions used are provided in Appendix S1A. We confirmed the lack of spatial autocorrelation in richness among the 12 focal sites with maximum richness (Appendix S1B). We also performed alternate analyses using sites with the mean local richness in each region (Appendix S1L; Table S13) and analyses that subdivided these regions (Appendix S1M; Table S14), and both gave generally similar results.

Among these 12 focal sites, nine had richness data for other anurans. Hylids make up 20–72% of anuran species in these nine communities (mean = 46%). There is a strong relationship between hylid local richness and overall anuran richness ($r^2 = 0.581$; $P = 0.017$), suggesting that hylids offer a reasonable proxy for anuran richness.

Climatic data for local sites

We tested the hypothesis that local richness is related to climatic variables (but recognizing that climate alone does not directly change richness). We extracted climatic data from each georeferenced locality (Table S2) using ArcView GIS 3.3 and the WorldClim database with 1 km^2 resolution (Hijmans *et al.* 2005). Analyses focused on annual precipitation (Bio1) and annual mean temperature (Bio12) as obvious and intuitive descriptors of climate (Table S2).

We also conducted principal components analysis (PCA) with all 19 climatic variables from the WorldClim database (Table S10), and utilized PC1 (Appendix S1C). Preliminary analyses using AET (annual evapotranspiration) and NPP (net primary productivity) showed no significant relationships with local richness, so these variables were not used. Here and throughout, 'climate' indicates current climate, but past climates were presumably similar for these regions and timescales (e.g. currently tropical regions were also tropical).
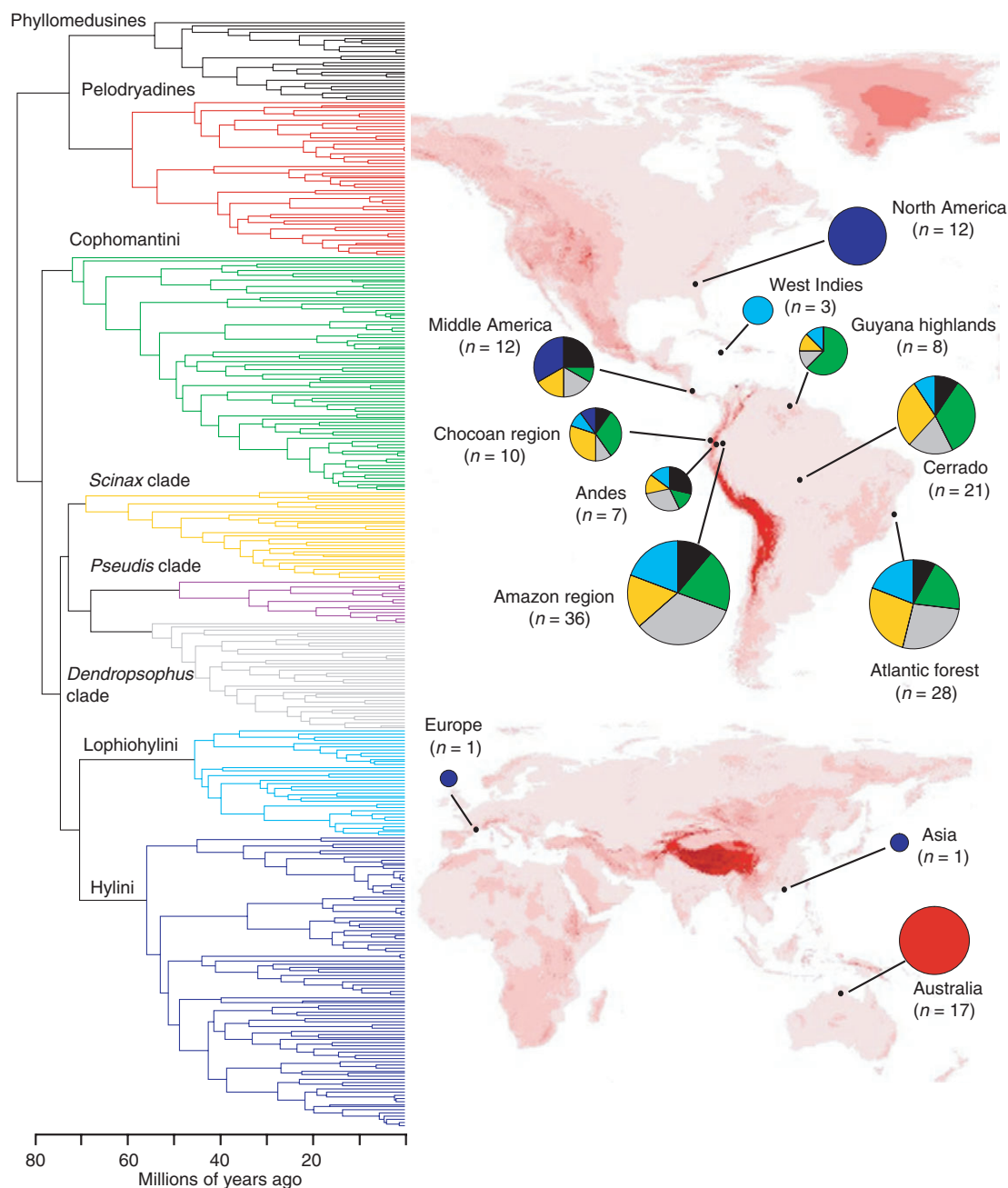
Figure 1 Time-calibrated phylogeny of hylid frogs (major clades coloured), with a map (darker red showing higher elevations) indicating location, local diversity (*n* = species, also indicated by relative size of circles) and clade composition of select communities. Each site represents the community with the highest richness in each of 12 major biogeographic regions (Table 1; see Tables S1–S3 for information on all sites). The eight major clades of hylids used in many analyses are also shown. Pelodryadinae and Phyllomedusinae are subfamilies. Hylini, Cophomantini and Lophiohylini are tribes within the subfamily Hylinae. The *Dendropsophus* clade (*Dendropsophus* and *Xenohyla*), *Pseudis* clade (*Lysapsus, Podonectes, Pseudis* and *Scarthyla*) and *Scinax* clade (*Scinax* and *Sphaenorhynchus*) are clades within Hylinae.

Phylogeny and divergence times

Testing hypotheses about dispersal and diversification required a time-calibrated phylogeny. The phylogeny was based on a combined, partitioned likelihood analysis of 362 hylid taxa (and 33 outgroup species) with data for up to 11 genes each (Wiens *et al.* 2010a). Bayesian divergence-time estimation with BEAST version 1.4.7 (Drummond & Rambaut 2007) utilized these data and 10 fossil calibration points (details in Appendix S1D).

Time, biogeography and regional richness

We tested the hypothesis that species richness is higher at localities embedded in regions that have been occupied for longer periods of time. The timing of colonization of each region was estimated using likelihood-based biogeographic reconstruction with LAGRANGE (Ree & Smith 2008; details in Appendix S1E; Appendix S2).

We used two main approaches for assessing how long hylids have been present in each region (each with advantages and disadvantages;

Table 1 Basic information on the 12 local sites representing the 12 major regions (see Fig. 1), including local hylid richness, annual mean temperature, annual precipitation, PC1 (from a principal components analysis of 19 climatic variables across all 123 sites), the regional species richness of each major region, the estimated timing of the first colonization of hylids in each region (in millions of years before present), the timing of colonization of each major clade in each region (summed across clades, in millions of years) and estimated mean diversification rates and mean rates of body-size evolution for the genera and species present

Local site	Region	Local richness	Temperature (°C)	Precipitation (mm)	PC1
Ecuador: Santa Cecilia	Amazon	36	25.4	3670	5.360
Brazil: Estacion VeraCruz	Atlantic rainforest	28	24.0	1540	1.723
Venezuela: Gran Sabana	Guyana highlands	8	18.4	2162	1.183
Ecuador: Palenque	Chocó	10	23.8	3463	4.398
Brazil: Dardanelos Dam	Cerrado	21	24.4	2256	1.312
Ecuador: Rio Salado	Andes	7	19.8	2742	2.740
China: Cha Chang	Asia	1	19.8	1674	−0.458
Australia: Nitmiluk National Park	Australia	17	26.7	1417	0.545
France: Provence-Alpes-Cote-d'Azur	Europe	1	14.3	900	−0.302
Costa Rica: La Selva	Middle America	12	26.1	4147	5.537
USA: Savannah River Ecology Lab	North America	12	16.3	1290	−2.182
Jamaica: Quick Step	West Indies	3	23.0	1742	1.895

Local site	Regional richness	Time first colonization	Summed clade ages	Mean diversification rate	Mean size rate
Ecuador: Santa Cecilia	142	77.605	333.601	0.0660	0.0021
Brazil: Estacion VeraCruz	166	38.438	134.873	0.0567	0.0015
Venezuela: Gran Sabana	23	35.905	58.922	0.0686	0.0009
Ecuador: Palenque	31	20.498	76.692	0.0621	0.0016
Brazil: Dardanelos Dam	127	37.974	160.882	0.0691	0.0017
Ecuador: Rio Salado	82	52.203	103.811	0.0629	0.0023
China: Cha Chang	10	26.575	26.575	0.0715	0.0031
Australia: Nitmiluk National Park	189	58.410	58.410	0.0641	0.0044
France: Provence-Alpes-Cote-d'Azur	8	22.113	22.113	0.0715	0.0031
Costa Rica: La Selva	159	55.220	163.157	0.0525	0.0021
USA: Savannah River Ecology Lab	29	44.397	44.397	0.0552	0.0032
Jamaica: Quick Step	142	31.017	49.983	0.0402	0.0082

Appendix S1F). First, we estimated the timing of the first colonization of hylids in each region, based on the age of the oldest clade unambiguously reconstructed as occurring in that region, using ancestral area reconstructions on our time-calibrated phylogeny (Appendix S2). This approach assumes that the first colonization of each region is the most important in driving richness patterns, even if there were multiple colonizations by different clades.

Second, to account for multiple colonizations of a single region by different clades, we also considered the timing of the oldest colonization of each major clade in each region, summed across clades. We tallied the oldest unambiguous occurrence of each clade in each region (Appendix S2), for eight clades (Fig. 1) that together include all known hylid species. Note that averaging colonization times across the clades that are present removes all information on the potential contribution of multiple colonization events to richness; this is why summing clade ages is necessary.

A potential third approach is to estimate the total amount of time that hylids have been present in each region, based on the summed lengths of the terminal and internal branches reconstructed as unambiguously occurring in that region. However, this index is potentially influenced by the number of species present in each region and the number included in the phylogeny and was therefore not used. Nevertheless, it gives similar results to those from summed clade ages (Appendix S1F).

All approaches are potentially influenced by the ambiguity of ancestral biogeographic reconstructions. For example, some regions appear to have been colonized relatively recently, but this may reflect

ambiguity in biogeographic reconstructions associated with frequent dispersals between adjacent regions (e.g. Atlantic forest, Cerrado).

We expect the timing of colonization of each region to influence local richness primarily by allowing the buildup of a larger regional species pool in regions colonized longer (and/or multiple times). We therefore tested for a relationship between (1) regional richness and maximum local richness in each region and (2) regional richness and our two measures for the time hylids that have been present in each region. We estimated regional richness using species range maps from the Global Amphibian Assessment (http://www.iucnredlist.org/initiatives/amphibians).

Climate and diversification rates

A relationship between climate and local richness may be explained by either higher diversification rates in more favourable climatic conditions or by greater time (see next section). To test the relationship between climate and diversification, we obtained climatic data for most species in the phylogeny ($n = 337$; Table S4). We obtained locality data from literature and museum records and obtained climatic data for each locality using the WorldClim dataset (Hijmans *et al.* 2005). We summarized annual mean temperature and annual mean precipitation for each species (averaged across localities) and obtained averages of species values for each genus (Table S5). We also conducted a PCA on species means for all 19 climatic variables and obtained mean values for genera for PC1. PC1 for species was similar to that for sites (compare Tables S10 and S11). We then tested the relationship between each

of these three climatic variables and the estimated diversification rate (see below) for each genus, using phylogenetic generalized least squares (PGLS; Martins & Hansen 1997).

All PGLS analyses and ancestral reconstructions were conducted in R version 2.9.2, with the packages Ape (Paradis *et al.* 2004) and GEIGER (Harmon *et al.* 2008). All analyses used the same time-calibrated phylogeny (Fig. 1).

We estimated the diversification rate for each genus given the total number of described species (Amphibiaweb; http://amphibia-web.org/), estimated stem-group age (from our chronogram) and the method-of-moments estimator of rates (Magallón & Sanderson 2001; see Appendix S1G).

Climatic reconstructions

To address whether time might help explain climate-richness relationships instead of diversification rates (i.e. more time for speciation in climatic zones inhabited longer), we estimated ancestral values for temperature, precipitation and PC1 for the eight major clades (Table 2). We first tested whether each variable better fit an Ornstein-Uhlenbeck (OU) or Brownian motion model, using comparison of AIC values across all 337 species. All three variables best fit the OU model (ΔAIC = 953, 194, 89 respectively). In addition, likelihood-ratio tests significantly rejected a 'white noise' model of no phylogenetic signal (Pagel's (1999) lambda of 0 is rejected for all variables; $P < 0.0001$). These two results strongly support phylogenetic niche conservatism (Kozak & Wiens 2010; Wiens *et al.* 2010b). We then reconstructed values for each climatic variable under the OU model using PGLS (exponential model, with estimated alpha values of 0.06, 0.12 and 0.04), utilizing COMPARE 4.6b (Martins 2004). We also used these reconstructions to test if the estimated time in each climatic zone (for PC1) is related to its mean local richness, given the

ages of clades reconstructed with a given climatic distribution (see Appendix S1H, Table S12).

Diversification rates and local richness in allopatric and sympatric clades

We tested the hypothesis that clades that do not co-occur with other (hylid) clades will have higher rates of diversification. Two major clades are either partially (Hylini) or entirely allopatric (Pelodryadinae) with respect to other hylid clades. The six other clades occur sympatrically with each other in many South American communities (Table S3). We estimated diversification rates for clades as described for genera (Appendix S1I). We then compared rates in allopatric and sympatric clades using PGLS, which allows testing the effect of categorical independent variables on continuous dependent variables (Martins & Hansen 1997). Diversification rates for these eight clades were also strongly related to clade richness ($r^2 = 0.794$; $P = 0.003$) but not clade age ($r^2 = 0.112$; $P = 0.4181$), strongly suggesting that these rates are important for explaining richness patterns.

As a complementary test utilizing the entire phylogeny, we evaluated whether sympatry influenced diversification rates using the BiSSE approach (Maddison *et al.* 2007) in Mesquite version 2.52 (Maddison & Maddison 2007), comparing the likelihood of models with equal and unequal rates of diversification in allopatric and sympatric lineages on our phylogeny of 362 species (Appendix S1I).

We also tested whether sympatric clades show more strongly decreasing diversification over time within clades (as expected for density-dependent diversification), as indicated by more negative values of the gamma statistic (Pybus & Harvey 2000), following Phillimore & Price (2008). To account for incomplete sampling, we used simulations to test whether the difference between the observed gamma and simulated critical value for each clade was more negative

Table 2 The eight major clades of hylids, including their estimated ancestral values for annual mean temperature, annual precipitation and PC1 (from a principal components analysis of 19 climatic variables for 337 species), overall species richness, estimated stem ages, estimated diversification rates, gamma values (observed − expected, with more negative values indicating support for a slowdown in diversification over time within a clade, after accounting for incomplete taxon sampling; Table S9) and maximum local richness for each clade across all sites

Major clades	Ancestral temp. (°C)	Ancestral precip. (mm)	Ancestral PC1	Species richness
Hylini*	17.25	2014.91	−3.09	181
Cophomantini	20.84	2049.62	0.44	160
Lophiohylini	23.45	2081.72	1.48	69
Dendropsophus clade	23.53	2061.97	1.66	96
Scinax clade	21.48	2050.57	0.26	110
Pseudis clade	22.39	2041.52	0.53	13
Phyllomedusinae	23.08	2062.97	1.68	61
Pelodryadinae*	22.19	2045.20	0.13	189

Major clades	Stem age	Div. rate	Gamma (observed − simulated)	Maximum local richness	Body-size rate
Hylini*	69.55	0.066	0.563	12	0.00352
Cophomantini	77.60	0.058	0.117	12	0.00222
Lophiohylini	69.55	0.052	−0.317	7	0.00608
Dendropsophus clade	67.16	0.059	−0.942	12	0.00125
Scinax clade	72.07	0.057	−0.502	8	0.00099
Pseudis clade	67.16	0.030	0.258	2	0.00296
Phyllomedusinae	71.91	0.049	0.028	6	0.00227
Pelodryadinae*	71.91	0.065	0.767	17	0.00444

Largely allopatric clades are asterisked. Note that the estimated rates of body-size evolution for each clade are shown for illustrative purposes only (the actual test involves the entire tree). The high rate in Lophiohylini is due in part to a high rate in the largely allopatric genus *Osteopilus* in the West Indies (Moen & Wiens 2009).

in the six sympatric clades (using the Mann–Whitney U-test; see Appendix S1I).

We also tested whether clades that are primarily allopatric have higher maximum local richness than sympatric clades. We determined the maximum local richness of the eight clades (Table 2) from our survey of local sites (Table S3), and tested for differences with the Mann–Whitney U-test. For all three analyses, we used the one-tailed test, given the prediction that allopatric clades should have higher diversification rates, less slowing of diversification over time and higher local richness (see introduction). However, we acknowledge that sample sizes in each category are unavoidably unequal and that this could influence the results.

In theory, similarities between allopatric clades could be unrelated to their geographic isolation *per se*. However, our estimates for diversification rate, gamma and local richness for these clades are uncorrelated with their reconstructed climatic variables (Table 2; $P > 0.10$). It is unclear what would explain similarities between these allopatric clades that occur in Australia (Pelodryadinae) and the Northern Hemisphere (Hylini).

Body-size variation and local richness

We next tested if more species-rich communities have greater variation in body sizes. We used the maximum snout-vent length (SVL) reported in males of each species as a measure of body size (Table S7; see Appendix S1J). Data on SVL are widely available and SVL is strongly correlated with multivariate measures of size ($r = 0.991$), based on morphometric analyses including all hylid genera (Moen *et al.* 2009). Body sizes in males and females of hylid species are typically similar but not identical (e.g. Duellman 2001, 2005) and males are more frequently collected. We obtained data on maximum male SVL for almost all described species in the 123 included sites (Table S3). We initially used linear regression to test if maximum and minimum SVL (among species) and ranges of SVLs (Table S8) are greater in more species-rich communities (excluding communities with < 3 species to reduce sampling effects). We then performed simulations to explicitly address the effects of sampling on this relationship (see Appendix S1J). These simulations involved randomly resampling species to create new communities, analysing the size-diversity relationship across these communities and comparing the observed and simulated relationships.

Rates of body-size evolution and species diversification

We tested the hypothesis that allopatric clades have higher rates of body-size evolution. We also tested if more diverse communities contain genera with higher rates of body-size evolution and whether rates of species diversification and morphological evolution are correlated (e.g. Harmon *et al.* 2003; Adams *et al.* 2009).

We obtained data on maximum male body size (SVL) from the literature for most hylid species ($n = 343$) in the phylogeny (Table S7). We tested whether rates of body-size evolution are higher in primarily allopatric clades (i.e. Hylini, Pelodryadinae) using maximum likelihood (with Brownie; O'Meara *et al.* 2006) and the time-calibrated phylogeny. Statistical significance was determined using a chi-squared test and confirmed with parametric bootstrapping, based on rate estimates across the tree (not the rate estimates in Table 2).

We also used Brownie to estimate rates of body-size evolution for 22 genera (Table S5) that contain at least three species in our phylogeny (estimates from 1 to 2 species are problematic). We then tested whether communities with greater species richness contain higher average rates of body-size evolution (based on generic-level estimates), using the locality with maximum richness in each region (Table 1). We used PGLS to test whether rates of body-size evolution are related to rates of species diversification among these 22 genera (Tables S5 and S6).

Finally, we tested if average rates of diversification are higher in more diverse communities (e.g. if sites with high richness are dominated by rapidly diversifying clades and if local ecological conditions promote higher diversification rates). We estimated a weighted average diversification rate for each of the 12 localities with maximum richness, by adding the diversification rates for each species of each genus (estimated as described above) and dividing by the total number of species in each community. We then tested the hypothesis that average rates of diversification will be higher in more diverse communities using linear regression (see Appendix S1K for details and caveats).

RESULTS

Across 123 sites, local richness of hylids varies from 1 to 36 species (Fig. 1), with the highest richness in the Amazonian and Atlantic rainforests and the lowest in Europe and Asia, and at high latitudes and elevations.

Dividing the range of hylids into 12 regions and utilizing the most diverse site in each region (Fig. 1; Table 1), we find that local richness is related to annual mean temperature ($r^2 = 0.345$; $P = 0.04$) but not annual precipitation ($r^2 = 0.103$; $P = 0.30$) or PC1 ($r^2 = 0.160$; $P = 0.20$). When all communities are considered (Table S2), there are significant relationships between these climatic variables and local richness (temperature: $r^2 = 0.239$; $P < 0.001$; precipitation: $r^2 = 0.241$; $P < 0.001$; PC1: $r^2 = 0.299$; $P < 0.001$). However, these climate-diversity relationships must still be explained by the processes of speciation, extinction and dispersal. Phylogenetic analyses (using PGLS) show no relationship between diversification rates of clades (genera) and temperature ($r^2 = 0.059$; $P = 0.097$), precipitation ($r^2 = 0.012$; $P = 0.454$), or PC1 ($r^2 = 0.003$; $P = 0.720$), suggesting that the observed relationship between climate and diversity is not primarily due to effects of climate on diversification.

Instead, biogeographic reconstructions on a time-calibrated phylogeny reveal that the maximum local richness in each region is related to the length of time that hylids have been present in each region (first colonization: $r^2 = 0.454$; $P = 0.0162$; summed clade ages: $r^2 = 0.705$; $P = 0.0006$: Fig. 2; Table 1). This relationship may occur because more time in a region leads to higher regional richness (first colonization: $r^2 = 0.490$; $P = 0.011$; summed clade ages: $r^2 = 0.369$; $P = 0.036$) and higher regional richness is related to higher local richness ($r^2 = 0.564$; $P = 0.005$). Preliminary path analyses (data not shown) suggest that both time and regional richness may directly influence local richness. Surprisingly, the highest local richness in tropical sites in Australia, Middle America and the Chocó (South America) is similar to that in temperate North America (Fig. 1; Table 1). These four regions were all colonized recently relative to Amazonia, strongly supporting the idea that time is more important than climate alone in determining local richness. Analyses using mean local richness also generally showed significant relationships between local richness and time but not between local richness and climatic variables (Appendix S1L).
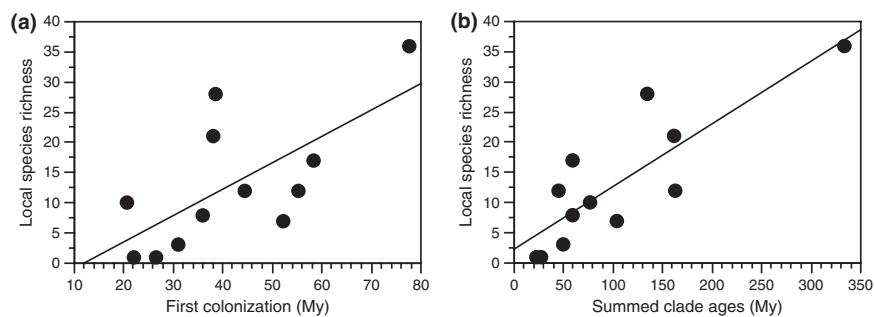
Figure 2 Relationship between local species richness of 12 focal sites (Fig. 1; representing 12 biogeographic regions) and time, where time is based on either (a) the amount of time that each region has been occupied by hylids overall (in millions of years before present, My), based on the timing of the first colonization of hylids in each region ($r^2 = 0.454$; $P = 0.0162$) or (b) the amount of time that each region has been occupied by each major hylid clade, summed across clades to account for multiple colonizations of a region ($r^2 = 0.705$; $P < 0.001$).

Reconstructions of these climatic variables (Table 2) suggest that most clades originated in relatively warm, wet environments (annual mean temperature > 20 °C for all but Hylini; annual precipitation > 2000 mm). There is also a significant relationship between the estimated time in each climatic zone (using PC1) and its mean local richness ($r^2 = 0.338$; $P = 0.029$). Overall, these analyses suggest that higher local richness in warm, wet climates is related to greater time, rather than more rapid diversification.

Diversification rates are marginally higher (Table 2) in allopatric clades ($P = 0.04$ for Mann–Whitney test, but phylogenetic tests show $P = 0.11$ for PGLS and $P > 0.05$ for BiSSE). Although these results alone are somewhat equivocal, the allopatric clades also show less slowing of diversification over time within clades ($P = 0.05$; Tables 2 and S9) and their maximum local richness is higher compared with sympatric clades ($P = 0.04$; Table 2). Taken together, these three results suggest that sympatric clades may constrain each other's diversification and local richness. However, allopatric clades alone reach less than 50% of the highest local richness found where multiple clades occur together (e.g. 12–17 vs. 36; Fig. 1; Table 2).

More species-rich communities have a larger range of maximum male body sizes among species ($r^2 = 0.431$; $P < 0.001$; for simulations $P = 0.0152$), implying that morphological diversity might facilitate co-existence and high local richness. Relationships between richness and maximum and minimum size are also significant (both $P < 0.001$). However, dividing species into standard body-size classes illustrates that the only size class that is absent in temperate communities (very large) has few species, even in the most species-rich tropical communities (Fig. 3). Thus, the presence of this size class cannot explain high local richness. Instead, the most diverse communities have many species with similar body sizes. For example, the most species-rich community included (Santa Cecilia, Ecuador; 36 species) has seven species of *Dendropsophus* with very similar maximum male body sizes, ranging only from *c.* 20 to 25 mm (Duellman 1978).

Phylogenetic analyses (using Brownie) show higher rates of body-size evolution in the allopatric clades ($P = 0.02$, using chi-square and bootstrapping tests; $rate_{allopatric} = 0.003853$, $rate_{sympatric} = 0.002667$ in $ln(mm)^2$ per million years). This result suggests that sympatry between clades limits rates of body-size evolution. In South America, different clades tend to occupy different portions of a broad range of body sizes among species (Moen & Wiens 2009), whereas in other regions (Australia, Middle America, Caribbean islands), this range is occupied through *in situ* evolutionary change within single clades.

Intriguingly, although this pattern suggests that competitive interactions constrain rates of trait evolution, competitive interactions do not seem to prevent the co-occurrence of species with similar values for this same trait in local communities (Fig. 3; Table S3).

We find no relationship between average rates of body-size evolution in a locality and local richness ($r^2 = 0.129$; $P = 0.252$; Table 1) or between mean diversification rates and local richness ($r^2 = 0.002$; $P = 0.899$; Table 1). Thus, sites with exceptionally high richness and diverse body sizes are made up of species from clades (genera) with unexceptional rates of species diversification and body-size evolution. We also find no relationship between rates of body-size evolution and rates of species diversification among genera ($r^2 = 0.007$; $P = 0.710$ using PGLS), further weakening the hypothesis that variation in this trait drives richness.

DISCUSSION

In this study, we demonstrate the importance of a phylogenetic perspective in understanding patterns of local richness. Our results shed light on this general topic and on the origin of high local richness in Amazonian rainforests. Phylogenetic analyses reveal that the timing of colonization of each major region where hylids occur is critical for explaining their local-scale richness within these regions. Thus, the megadiversity of Amazonian treefrog communities is explained by the accumulation of species in multiple sympatric clades in this region since the Cretaceous (time-for-speciation effect) and not rapid diversification linked to climatic variables or body-size evolution. This explanation might not be apparent from simply looking at correlations between climate and richness or from analysing communities or clades in Amazonia without comparisons to other regions. Thus, our results suggest the potential benefits of using broad-scale phylogenetic analyses to help understand patterns of local richness. Our results also suggest intriguing interactions between clade diversification, trait evolution and the accumulation of species at the local scale.

Some recent reviews have downplayed the importance of time in explaining patterns of species richness (e.g. Mittelbach *et al.* 2007; Rabosky 2009). However, time is crucial for explaining local richness patterns in treefrogs and may underlie many other regional and local-scale richness patterns in other organisms, including those related to latitude (e.g. Stephens & Wiens 2003; Stevens 2006; Wiens *et al.* 2009), elevation (e.g. Wiens *et al.* 2007; Li *et al.* 2009; Kozak & Wiens 2010) and predation regimes (Brown *et al.* 2000).
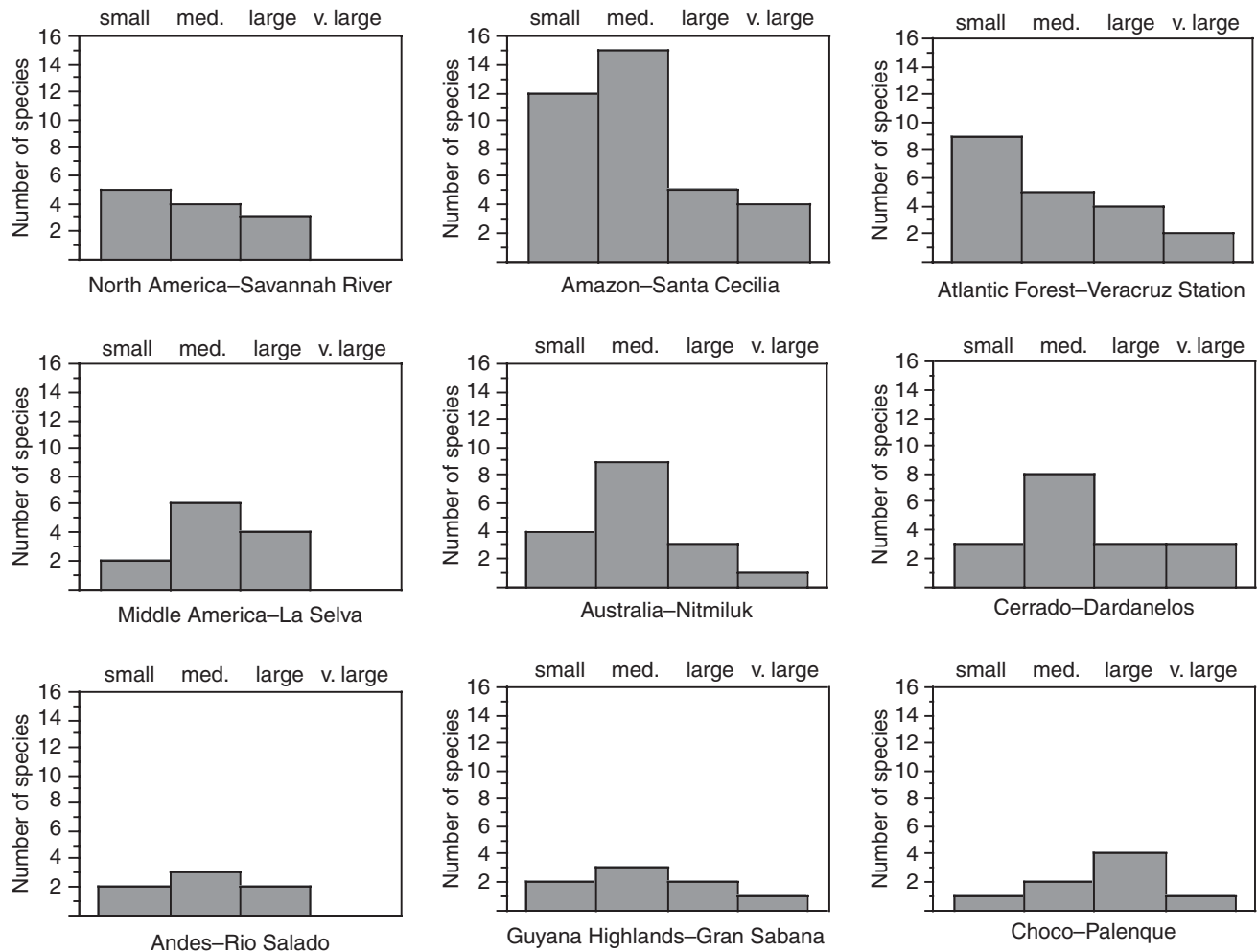
Figure 3 The number of species in each body-size class in 12 local communities of hylid frogs (Fig. 1), where each species is represented by the maximum male body size (snout-vent length). Body-size classes are small (15–29 mm), medium (30–49 mm), large (50–79 mm) and very large (80–132 mm). These classes follow standard usage in treefrogs and are merely used for illustrative purposes.

We find that climate alone is insufficient to explain local richness in hylids, and the process by which climate does influence richness may also be related to time (contra Algar *et al*. 2009). The coldest and driest sites clearly have few hylid species, but there is dramatic variation in richness between sites with similar climate in different regions (e.g. low diversity in tropical rainforests in the Chocoan region, Middle America and Australia). Furthermore, although climate predicts some variation in richness, these climate-richness relationships must still be explained through the processes of speciation, extinction and dispersal. We find that rates of diversification (speciation minus extinction) among hylid genera are unrelated to their climatic distributions. Instead, phylogenetic reconstructions of these climatic variables (and regional-scale biogeographic analyses) suggest that the observed relationships between climatic variables and local richness may be related to greater time for speciation in mesic tropical environments and limitations on dispersal related to niche conservatism.

Our results also shed new light on the origins of Amazonian megadiversity. Although many hypotheses have been proposed to explain high Amazonian biodiversity (review in Hoorn & Wesselingh 2010), most involve different speciation mechanisms within the region and not comparisons between regions. One recent study (Santos *et al*.

2009) proposed that Amazonian amphibian diversity is explained primarily by late Miocene dispersal from the Andes, based on phylogenetic patterns in dendrobatid frogs. However, dendrobatids have low local richness in the Amazon (*c*. 3–7 species per site; Duellman 1988) and high regional richness in the Andes (Santos *et al*. 2009). Conversely, hylids have high local richness in the Amazon (where they seemingly originated in the Cretaceous; Fig. 1) and lower local and regional richness in the Andes (where they colonized more recently; Table 1). Thus, dendrobatids may not be as representative as hylids for understanding the causes of local-scale diversity of Amazonian amphibians (e.g. hylids include 43% of anuran species in some megadiverse Amazonian sites; Duellman 1978). We suggest that richness patterns in both dendrobatids and hylids can be explained by the same general principle (time-for-speciation effect), which predicts that a given group will be more species-rich in the region or habitat where it has been present and speciating for longer periods of time. However, open questions remain, such as why hylids originated in Amazonia, and what causes variation in their richness across the Amazon Basin.

Our results from treefrogs also offer an intriguing perspective on how local richness might interact with species diversification and

morphological trait evolution. First, we find that geographically isolated clades in Australia and Middle to North America have marginally higher net diversification rates (but not significant in some analyses), less slowing of within-clade diversification over time and significantly higher local species richness, relative to the six sympatric clades in South America (Fig. 1; Table 1). Although our results suggest that geographic overlap (density) of clades may slow within-clade accumulation of species over time, this does not mean that species richness cannot continue to accumulate (i.e. slowing is not stopping). Indeed, if there were strict limits on local richness (e.g. Rabosky 2009), there should be no relationship between time and local richness. Instead, we find the highest local richness where multiple clades have occurred in sympatry for long periods of time. Importantly, our results suggest that evidence for slowing diversification due to higher density of species and clades is not necessarily evidence that local richness will stop increasing over time.

We also find that geographically isolated clades have significantly higher rates of body-size evolution, suggesting that sympatry between clades limits body-size diversification. These allopatric clades have evolved broad ranges of body sizes, whereas sympatric clades in South America tend to occupy more limited portions of a similar range (e.g. Moen & Wiens 2009). Although these results suggest that species interactions may reduce rates of body-size evolution, we nevertheless find that species-rich communities contain many morphologically similar species occurring in sympatry (e.g. small *Dendropsophus*). Of course, species can diverge in other traits in sympatry besides body size, and these species are not necessarily ecologically identical (although many are similar in general diet, life-history mode and microhabitat; Duellman 1978, 2005). Thus, it is not necessarily surprising that species with similar body size co-occur, but it is more surprising given that large-scale sympatry of clades seems to slow rates of body-size evolution. Overall, our results suggest the intriguing possibility that competition might influence large-scale macroevolutionary patterns of trait evolution and species diversification even when its effects are not apparent at the local scale.

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REFERENCES

Adams, D.C., Berns, C.M., Kozak, K.H. & Wiens, J.J. (2009). Are rates of species diversification correlated with rates of morphological evolution? *Proc. R. Soc. Lond. B*, 276, 2729–2738.

Algar, A.C., Kerr, J.T. & Currie, D.J. (2009). Evolutionary constraints on regional faunas: whom, but not how many. *Ecol. Lett.*, 12, 57–65.

Brown, J.M., McPeek, M.A. & May, M.L. (2000). A phylogenetic perspective on habitat shifts and diversity in the North American *Enallagma* damselflies. *Syst. Biol.*, 49, 697–712.

Carlson, R.C., Wainwright, P.C. & Near, T.J. (2009). Relationship between species co-occurrence and rate of morphological change in *Percina* darters (Percidae: Etheostomatinae). *Evolution*, 63, 767–778.

Cavender-Bares, J., Kozak, K.H., Fine, P.V.A. & Kembel, S.W. (2009). The merging of community ecology and phylogenetic biology. *Ecol. Lett.*, 12, 693–715.

Davies, T.J., Meiri, S., Barraclough, T.G. & Gittleman, J.L. (2007). Species co-existence and character divergence across carnivores. *Ecol. Lett.*, 10, 146–152.

Drummond, A.J. & Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.*, 7, 214.

Duellman, W.E. (1978). The biology of an equatorial herpetofauna in Amazonian Ecuador. *Misc. Publ. Mus. Nat. Hist. Univ. Kansas*, 65, 1–352.

Duellman, W.E. (1988). Patterns of species diversity in anuran amphibians in the American tropics. *Ann. Mo. Bot. Gard.*, 75, 79–84.

Duellman, W.E. (2001). *Hylid Frogs of Middle America*, 2nd edn. Society for the Study of Amphibians and Reptiles, Lawrence, Kansas.

Duellman, W.E. (2005). *Cusco Amazónico: The Lives of Amphibians and Reptiles in an Amazonian Rainforest*. Comstock Publishing Associates, Ithaca, New York.

Harmon, L.J., Schulte, J.A. II, Larson, A. & Losos, J.B. (2003). Tempo and mode of evolutionary radiation in iguanian lizards. *Science*, 301, 961–964.

Harmon, L.J., Weir, J., Brock, C., Glor, R.E. & Challenger, W. (2008). GEIGER: investigating evolutionary radiations. *Bioinformatics*, 24, 961–964.

Harrison, S. & Cornell, H.V. (2008). Toward a better understanding of regional causes of local species richness. *Ecol. Lett.*, 11, 969–979.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005). The WorldClim Interpolated Global Terrestrial Climate Surfaces. Version 1.3. Available at: http://www.worldclim.org.

Hoorn, C. & Wesselingh, F. (2010). *Amazonia: Landscape and Species Evolution. A Look into the Past*. Wiley-Blackwell, West Sussex, UK.

Hubbell, S. (2001). *The Unified Neutral Theory of Biodiversity and Biogeography*. Princeton University Press, Princeton, NJ.

Kozak, K.H. & Wiens, J.J. (2010). Niche conservatism drives elevational diversity patterns in Appalachian salamanders. *Am. Nat.*, 176, 40–54.

Kraft, N.J.B., Valencia, R. & Ackerly, D.D. (2008). Functional traits and niche-based tree community assembly in an Amazonian forest. *Science*, 322, 580–582.

Li, J., He, Q., Hua, X., Zho, J., Xu, H., Chen, J. *et al.* (2009). Climate and history explain the species richness peak at mid-elevation for *Schizothorax* fishes (Cypriniformes: Cyprinidae) distributed in the Tibetan Plateau and its adjacent regions. *Glob. Ecol. Biogeogr.*, 18, 264–272.

Maddison, W.P. & Maddison, D.R. (2007). *Mesquite: A Modular System for Evolutionary Analysis*. Version 2.5. Available at: http://mesquiteproject.org.

Maddison, W.P., Midford, P.E. & Otto, S.P. (2007). Estimating a binary character's effect on speciation and extinction. *Syst. Biol.*, 56, 701–710.

Magallón, S. & Sanderson, M.J. (2001). Absolute diversification rates in angiosperm clades. *Evolution*, 55, 1762–1780.

Martins, E.P. (2004). *COMPARE, ver. 4.6: Computer Programs for the Statistical Analysis of Comparative Data*. Distributed by the author. Department of Biology, Indiana University, Bloomington. http://compare.bio.indiana.edu/.

Martins, E.P. & Hansen, T.F. (1997). Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *Am. Nat.*, 149, 646–667.

Mittelbach, G.G., Schemske, D.W., Cornell, H.V., Allen, A.P., Brown, J.M., Bush, M.B. *et al.* (2007). Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. *Ecol. Lett.*, 10, 315–331.

Moen, D.S. & Wiens, J.J. (2009). Phylogenetic evidence for competitively-driven divergence: body-size evolution in Caribbean treefrogs (Hylidae: *Osteopilus*). *Evolution*, 63, 195–214.

Moen, D.S., Smith, S.A. & Wiens, J.J. (2009). Community assembly through evolutionary diversification and dispersal in Middle American treefrogs. *Evolution*, 63, 3228–3247.

Morin, P.J. (1999). *Community Ecology*. Wiley-Blackwell, Malden, Massachusetts.

O'Meara, B.C., Ané, C., Sanderson, M.J. & Wainwright, P.C. (2006). Testing for different rates of continuous trait evolution using likelihood. *Evolution*, 60, 922–933.

Pagel, M. (1999). Inferring the historical patterns of biological evolution. *Nature*, 401, 877–884.

Paradis, E., Claude, J. & Strimmer, K. (2004). APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, 20, 289–90.

Phillimore, A.B. & Price, T.D. (2008). Density dependent cladogenesis in birds. *PLoS Biol.*, 6, e71.

Pybus, O.G. & Harvey, P.H. (2000). Testing macro-evolutionary models using incomplete molecular phylogenies. *Proc. R. Soc. Lond. B*, 267, 2267–2271.

Rabosky, D.L. (2009). Ecological limits and diversification rate: alternative paradigms to explain the variation in species richness among clades and regions. *Ecol. Lett.*, 12, 735–743.

Rabosky, D.L. & Lovette, I.J. (2008). Density dependent diversification in North American wood-warblers. *Proc. R. Soc. Lond. B*, 275, 2363–2371.

Ree, R.H. & Smith, S.A. (2008). Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Syst. Biol.*, 57, 4–14.

Ricklefs, R.E. (1987). Community diversity: relative roles of local and regional processes. *Science*, 235, 167–171.

Ricklefs, R.E. (2006). Global variation in the diversification rate of passerine birds. *Ecology*, 87, 2468–2478.

Santos, J.C., Coloma, L.A., Summers, K., Caldwell, J.P., Ree, R.H. & Cannatella, D.C. (2009). Amazonian amphibian diversity is primarily derived from Late Miocene Andean lineages. *PLoS Biol.*, 7, e56.

Schluter, D. (2000). *The Ecology of Adaptive Radiations*. Oxford University Press, Oxford.

Stephens, P.R. & Wiens, J.J. (2003). Explaining species richness from continents to communities: the time-for-speciation effect in emydid turtles. *Am. Nat.*, 161, 112–128.

Stevens, R.D. (2006). Historical processes enhance patterns of diversity along latitudinal gradients. *Proc. R. Soc. Lond. B*, 273, 2283–2289.

Webb, C.O., Ackerly, D.D., McPeek, M.A. & Donoghue, M.J. (2002). Phylogenies and community ecology. *Ann. Rev. Ecol. Evol. Syst.*, 33, 475–505.

Weir, J.T. (2006). Divergent timing and patterns of species accumulation in lowland and highland Neotropical birds. *Evolution*, 60, 842–855.

Wiens, J.J., Graham, C.H., Moen, D.S., Smith, S.A. & Reeder, T.W. (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., Parra-Olea, G., Garcia-Paris, M. & Wake, D.B. (2007). Phylogenetic history underlies elevational patterns of biodiversity in tropical salamanders. *Proc. R. Soc. Lond. B*, 274, 919–928.

Wiens, J.J., Sukumaran, J., Pyron, R.A. & Brown, R.M. (2009). Evolutionary and biogeographic origins of high tropical diversity in Old World frogs (Ranidae). *Evolution*, 63, 1217–1231.

Wiens, J.J., Kuczynski, C.A., Hua, X. & Moen, D.S. (2010a). An expanded phylogeny of treefrogs (Hylidae) based on nuclear and mitochondrial sequence data. *Mol. Phylogenet. Evol.*, 55, 871–882.

Wiens, J.J., Ackerly, D.D., Allen, A.P., Anacker, B.L., Buckley, L.B., Cornell, H.V. *et al.* (2010b). Niche conservatism as an emerging principle in ecology and conservation biology. *Ecol. Lett.*, 13, 1310–1324.

SUPPORTING INFORMATION

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

Appendix S1 Expanded Materials and Methods.
Appendix S2 Biogeographic reconstructions.

Table S1 Hylid species richness at 123 local sites, including region and source.
Table S2 Latitude, longitude and climate at 123 local sites.
Table S3 Species composition and body sizes at 123 local sites.
Table S4 Climatic data for 337 hylid species.
Table S5 Climatic data and rates of body-size evolution for hylid genera.
Table S6 Clades, ages, richness and diversification rates for hylid genera.
Table S7 Male body sizes for hylid species.
Table S8 Minimum, maximum and ranges of body sizes for species in 123 sites.
Table S9 Estimates of the gamma statistic for major clades of hylids.
Table S10 Eigenvectors from PCA of 19 climatic variables for PC1 for 123 local sites.
Table S11 Eigenvectors from PCA of 19 climatic variables for PC1 for 337 species.
Table S12 Estimates of local richness and time for each climatic zone.
Table S13 Data on 12 local sites representing mean local richness in each region.
Table S14 Data on 19 local sites representing the maximum local richness in each region, where some regions have been subdivided to evaluate the robustness of the results to different delimitations of regions.

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