

REPLICATE PATTERNS OF SPECIES RICHNESS, HISTORICAL BIOGEOGRAPHY, AND PHYLOGENY IN HOLARCTIC TREEFROGS

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Abstract.—In recent decades, the field of historical biogeography has become increasingly divorced from evolutionary biology, ecology, and studies of species richness. In this paper, we explore the evolutionary causes of patterns of biogeography and species richness in Northern Hemisphere treefrogs, combining phylogenetics, ancestral area reconstruction, molecular dating methods, and ecological niche modeling. We reconstructed phylogenetic relationships among 58 hylid taxa using data from two mitochondrial genes (12S, ND1) and two nuclear genes (POMC, *c-myc*). We find that parallel patterns of species richness have developed in Europe, Asia, and in two separate clades of North American hylids, with the highest richness at midtemperate latitudes (30–35°) on each continent. This pattern is surprising given that hylids overall show higher species richness in the New World tropics and given many standard ecological explanations for the latitudinal diversity gradient (e.g., energy, productivity, mid-domain effect). The replicate pattern in Holarctic hylids seems to reflect specialized tolerance for temperate climate regimes or possibly the effects of competition. The results also suggest that long-range dispersal between continental regions with similar climatic regimes may be easier than dispersal between geographically adjacent regions with different climatic regimes. Our results show the importance of ecology and evolution to large-scale biogeography and the importance of large-scale biogeography to understanding patterns of species richness.

Key words.—Amphibians, biogeography, community assembly, diversification, Hylidae, phylogeny, species richness.

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For the past several decades, the fields of historical biogeography and ecology have become increasingly divergent from each other, and both fields have generally neglected the evolutionary causes of large-scale patterns of biogeography and species richness (Wiens and Donoghue 2004). Historical biogeography has generally focused on using phylogenies to reconstruct the history of connections among specific biotas (e.g., Wiley 1988; Brooks and McLennan 1991; Page 1994; Morrone and Crisci 1995; Humphries and Parenti 1999; van Veller et al. 2002), without addressing more general questions (e.g., why there are more species in tropical regions) or considering ecology (e.g., the role of climatic tolerances or competition in preventing dispersal between regions). Conversely, ecologists who have considered large-scale patterns of species richness have typically sought environmental variables (e.g., temperature, precipitation, energy, productivity) that are correlated with species numbers (e.g., Francis and Currie 2003; Hawkins et al. 2003; Willig et al. 2003), without considering historical biogeography. This correlative approach is limited in some ways, because it does not directly address the processes that actually increase or decrease species richness in a region (i.e., in situ speciation, extinction, and dispersal of species into or out of the region). Instead, these processes (particularly dispersal and in situ speciation) may be best identified using historical biogeography (Wiens and Donoghue 2004). To begin to understand large-scale patterns of species richness, an integrative approach to biogeography is needed that considers phylogeny, ecological factors (e.g., environmental tolerances, competition), and evolutionary processes (e.g., speciation, adaptation to new climatic regimes) and how they interact to create patterns of species distributions (Ricklefs 2004; Wiens and Donoghue 2004).

We explore patterns of biogeography and species richness in the treefrogs (Hylidae) of the Northern Hemisphere by combining information from molecular phylogenetics, biogeography, and ecological niche modeling. Many clades of plants and animals are confined to temperate regions on two or more continents of the Northern Hemisphere (e.g., Li 1952; Sanmartín et al. 2001). Although some aspects of biogeography and diversity have been intensively studied in these clades, many other patterns have not. Several recent studies have addressed the biogeographic relationships of these temperate clades using phylogenetic methods (e.g., Sanmartín et al. 2001; Donoghue et al. 2001; Donoghue and Smith 2004). Another series of studies has explored differences in species richness of temperate floras in different continental regions, particularly the disparity in species richness between temperate Asia and North America (e.g., Qian and Ricklefs 1999, 2000; Guo and Ricklefs 2000; Xiang et al. 2004). Previous authors have also discussed temperate clades as exceptions to the general pattern of high tropical species richness, including ichneumonid wasps and conifers (Brown and Lomolino 1998).

Widely distributed temperate clades raise many other interesting questions in evolution, biogeography, and species richness that have not been thoroughly addressed. First, do similar latitudinal patterns of species richness develop on each continent? Many hypotheses have been proposed to explain the latitudinal gradient in species richness (e.g., Pianka 1966; Rosenzweig 1995; Willig et al. 2003). Several hypotheses predict that different lineages should develop parallel patterns of species richness on each continent, with the highest species richness at the lowest latitudes (i.e., regions with the highest energy and productivity; Francis and Currie

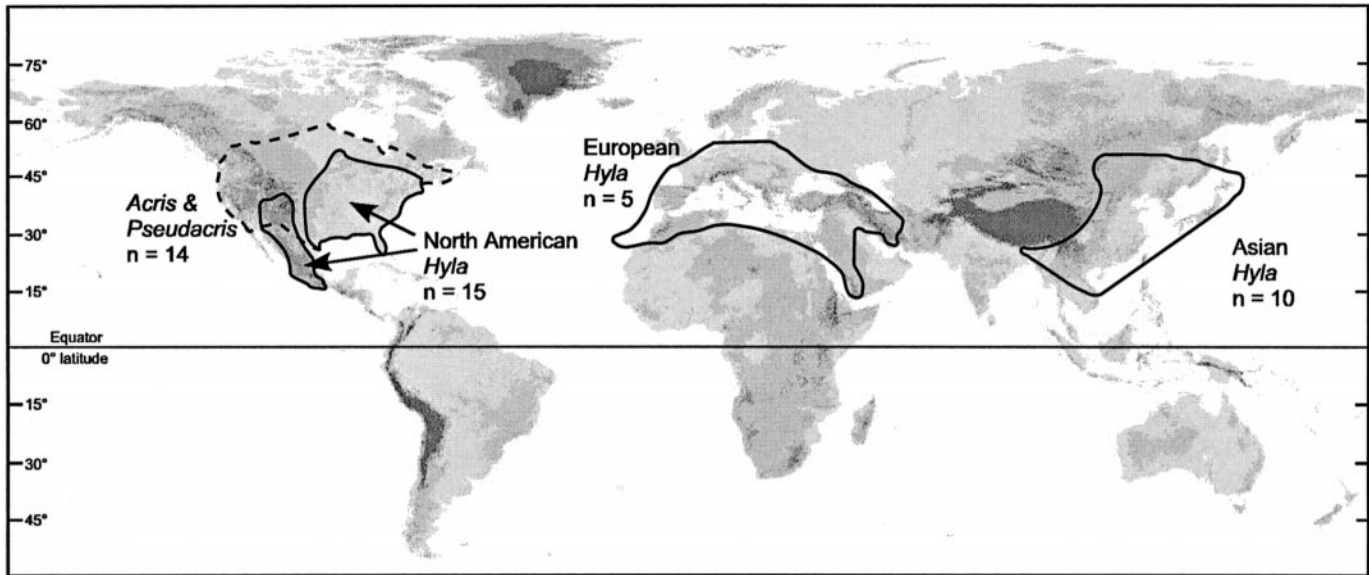


FIG. 1. Distribution and species richness of hylid frogs in Asia, Europe, and North America.

2003; Hawkins et al. 2003). Other hypotheses suggest that patterns of species richness develop stochastically within a continent (e.g., Colwell and Hurr 1994; Colwell and Lees 2000), with the highest species richness expected near the latitudinal midpoint of the landmass. Second, why are there monophyletic temperate clades on different continents? In other words, why do species in these clades not disperse freely between temperate and tropical areas? Ricklefs and Latham (1992) suggested that plant species in Europe and North America had similar geographic range sizes and latitudinal ranges because they inherited the relevant ecological traits from a common ancestor (i.e., niche conservatism). Alternately, if temperate clades are recently derived from tropical lineages, their reinvasion of tropical areas may be limited by competition with tropical species rather than intrinsic ecological or physiological traits (e.g., Darwin 1859; MacArthur 1972; Brown and Lomolino 1998).

We address these questions in hylid frogs of the Northern Hemisphere. Hylids consist of 42 genera and 861 species (AmphibiaWeb 2004). Most genera and species of hylids live in the New World tropics, although there is also a large radiation (three genera, 162 species) in the Australasian region (Duellman 2001; AmphibiaWeb 2004). There are also several species of hylids (genus *Hyla*) in Europe (five) Asia (10), and North America (10) and two endemic genera (*Acris*, two; *Pseudacris*, 12) in temperate North America (Global Amphibian Assessment, GAA hereafter; IUCN et al. 2004; Fig. 1). Previous studies have suggested that European and Asian *Hyla* are closely related to each other (traditionally recognized as the *H. arborea* group) and these species may in turn be closely related to the North American species (Duellman 2001; Wiens et al. 2005b). Thus, it should be possible to address diversification of treefrogs on these three continents by sampling a set of relatively closely related species. Furthermore, hylid frogs exhibit the typical latitudinal gradient in species diversity, at least in the New World. For example, there are six species of hylid frogs in Canada, 43

in Costa Rica, 137 in Ecuador, and 15 in Uruguay (GAA). Thus, it is possible to relate patterns of species richness in temperate regions to the larger-scale pattern of high tropical species richness.

MATERIALS AND METHODS

Taxon Selection and Sampling

A recent phylogenetic analysis based on nuclear and mitochondrial DNA sequences and morphology (Wiens et al. 2005b) confirmed that there is a Middle American clade of hyline frogs that contains all of the endemic hyline genera of Middle and North America, as well as the North American, European, and Asian species of *Hyla*. Taxon sampling for the present study focused on including: all available species of hylid frogs in Europe and Asia; all North American species of *Hyla*; representatives of the endemic North American genera *Acris* and *Pseudacris* (relationships within *Pseudacris* were addressed in detail by Moriarty and Cannatella 2004); and tropical representatives of the Middle American clade of hylids, including all currently recognized genera (*Anothea*, *Duellmanohyla*, *Plectrohyla*, *Pternohyla*, *Ptychohyla*, *Smilisca*, *Triprion*) and 12 of the 13 endemic Middle American species groups of *Hyla* (all but the *H. nubicola* group, for which samples were unavailable). We were not able to include all hylid species in the Northern Hemisphere, but our major conclusions should be robust to the absence of these unsampled species (see Results). We also included outgroups from several of the other major clades of hyline frogs, including the 30-chromosome clade of *Hyla*, the *Phrynohyas* clade (including *Osteocephalus*, *Osteopilus*, *Phrynohyas*, *Scinax*, and *Sphaenorhynchus* (Wiens et al. 2005b).

DNA Sequence Data

We obtained DNA sequence data from two mitochondrial genes and two nuclear genes (Table 1), all of which have

TABLE 1. Summary of sequence data characteristics from four sampled genes.

	12S	ND1	POMC	<i>c-myc</i>
Number of characters (excluding sites with ambiguous alignment)	838	1163	547	832
Parsimony-informative characters	269	545	168	146
Retention index	0.495	0.370	0.674	0.694
Consistency index (excluding uninformative characters)	0.269	0.193	0.347	0.426

proven to be useful for resolving relationships at many levels of divergence in hylid frogs (Wiens et al. 2005b). These included the mitochondrial ribosomal small subunit (12S; 1077 bp; also including the adjacent tRNA-Phe and tRNA-Val), the mitochondrial NADH dehydrogenase subunit 1 gene (ND1; 1218 bp; also including up to 372 bp of the adjacent 16S and tRNA genes), the nuclear proopiomelanocortin A gene (POMC; 547 bp), and portions of exons 2 and 3 of the nuclear proto-oncogene cellular myelocytomatosis (*c-myc*; 844 bp total). DNA was extracted from frozen and ethanol-preserved tissues using standard methods and was amplified using the polymerase chain reaction (PCR). Primer sequences are listed in Table 2. Both strands of each PCR product were purified and sequenced using an ABI 3100 automated sequencer. Sequences were edited using Sequence Navigator (ver. 1.0.1, Applied Biosystems, Foster City, CA).

Alignment of protein-coding sequences (ND1, POMC, and *c-myc*) was straightforward and was performed using Clustal X.1.81 (Thompson et al. 1994) with default parameters (gap

opening = 15; gap extension = 6.666; delay divergent sequences = 30%; transition:transversion = 50%), with adjustments by eye using Se-Al v1.0 (Rambaut 1996). Wiens et al. (2005b) provided an alignment for the 12S ribosomal gene based on an extensive analysis incorporating secondary structure. Because all major clades found in the present study were represented in that study, new 12S sequences were aligned primarily by fitting them to this alignment.

Voucher numbers and specimen localities are provided in Appendix 1 (available online only at <http://dx.doi.org/10.1554/05-284.1.s1>). Genbank accession numbers, including those for new sequences (DQ055730–DQ055843) and those from previous studies, are provided in Appendix 2 (available online only at <http://dx.doi.org/10.1554/05-284.1.s2>). A total of 58 taxa were included in the initial analysis. Thirty taxa were included based on four genes previously sequenced by Wiens et al. (2005b). For 19 other taxa, either one or two genes (12S and/or ND1) were sequenced by Wiens et al. (2005b) and up to three additional genes were added for this

TABLE 2. Primers used for amplification and sequencing of nuclear and mitochondrial genes in hylid frogs.

Primer	Sequence (5'–3')	Source
<i>12S</i>		
t-Phe-frog	ATAGCRCTGAARAYGCTRAGATG	Modified "MVZ 59" (Graybeal 1997)
t-Val-frog	TGTAAGCGARAGGCTTTKGTAAAGCT	Wiens et al. (2005b)
t-Phe2-frog	GCRCTGAARATGCTGAGATGARCCC	this study
t-Val2-frog	GTTGTAAGCGAGAGGCTT	this study
<i>ND1</i>		
16S-frog	TTACCCTRGGGATAACAGCGCAA	Wiens et al. (2005b)
tMet-frog	TTGGGGTATGGGCCAAAAGCT	Wiens et al. (2005b)
ND1 F1	AGCCATAATCATCTGAACC	this study
ND1 F2	GCMATAATYATYTGAACCC	this study
ND1 F6	ATTTATCTTGGCCCTATCCAGT	this study
ND1 R1	TCCTCCCTATCAAGGAGGTC	this study
ND1 R2	CATACTAAGTGTATAAGTTGGTC	this study
ND1 R3	GATTAGAGCTAGTGTGACTTC	this study
<i>c-myc</i> exon 2		
<i>c-myc</i> 1U	GAGGACATCTGGAARAARTT	Crawford (2003)
<i>c-myc</i> ex2d R	TCATTCAATGGGTAAGGGAAGACC	Wiens et al. (2005b)
<i>c-myc</i> F2	ACMGAGTTCCTGGGAGGGGACATGG	this study
<i>c-myc</i> exon 3		
<i>c-myc</i> ex3F	CCCACCAGTCCAGACCTCACCACAG	Wiens et al. (2005b)
<i>c-myc</i> ex3R	GTTCTCTTTGAGTTTAACTGTTC	Wiens et al. (2005b)
<i>c-myc</i> ex3F2	AYGTNCCYATYCAACAGCACAAC	Wiens et al. (2005b)
<i>c-myc</i> ex3R3	TCKCGNAKGAGYCKYCGCTCRTC	Wiens et al. (2005b)
<i>POMC</i>		
POMC-1	GAATGTATYAAAGMMTGCAAGATGGWCCT	Wiens et al. (2005b)
POMC-2	TAYTGRCCCTTYTTGTGGGCRTT	Wiens et al. (2005b)
POMC-5	GGARCACTTYCGATGGGGYAAACC	Wiens et al. (2005b)
POMC-5-r	GGTTTRCCCATCGRAAGTGYTCC	Wiens et al. (2005b)
POMC-6	TCTGCMGAGTCACCRGTGTTTC	this study
POMC-7	TGGCATT'TTGAAAAGAGTCAT	this study

study. Nine taxa were previously unsampled for any gene and were sequenced for up to four genes; five of these taxa failed to amplify for one or two genes (Appendix 2 available online only) but were nevertheless included in the combined analysis. For some taxa we lacked tissues and included them (in a separate analysis) based only on 12S sequences downloaded from GenBank (*Acris gryllus*, *Hyla chrysocelis*, *H. eximia*, *H. suweonensis*, *Pseudacris brachyphona*, *P. brimleyi*, *P. clarkii*, *P. feriarum*, *P. illinoensis*, *P. kalmi*, *P. maculata*, *P. ornata*, *P. streckeri*, *P. triseriata*). Furthermore, *H. simplex* successfully amplified only for a single locus (*c-myc* exon 2) and was included in this latter analysis only. Simulations (Wiens 2003) and analyses of empirical datasets (e.g., Driskell et al. 2004; Phillipe et al. 2004; Wiens et al. 2005b) suggest that highly incomplete taxa can be accurately placed in phylogenetic analyses regardless of the number or proportion of missing data cells they bear, if the overall number of characters is sufficiently large (i.e., data from one gene or part of one gene may be adequate to accurately place a taxon on the tree, regardless of how many other genes lack data).

Phylogenetic Methods

Data were analyzed using parsimony and Bayesian methods. Each of the four genes was initially analyzed alone (using methods described below), to look for areas of incongruence that are strongly supported by two or more datasets, by comparing bootstrap values and Bayesian posterior probabilities (Wiens 1998). Strong support was arbitrarily defined as parsimony bootstrap values $\geq 70\%$ (Hillis and Bull 1993; but see their extensive caveats) and Bayesian posterior probabilities (Pp) $\geq 95\%$ (for justification see Wilcox et al. 2002; Alfaro et al. 2003; Erixon et al. 2003; Huelsenbeck and Rannala 2004). Combined nuclear (POMC and *c-myc*) and combined mitochondrial (ND1 and 12S) datasets were also analyzed and compared. Areas of strongly supported incongruence might indicate deviations between gene phylogeny and species phylogeny, conditions where combined analysis might be expected to perform poorly (Wiens 1998). However, no strongly supported incongruence was found, and all datasets were therefore combined to increase the overall sample size of characters.

Parsimony analyses were implemented in PAUP* 4.0b10 (Swofford 2002), using a heuristic search with tree-bisection-reconnection (TBR) branch swapping and 1000 random taxon-addition-sequence replicates per search. Support for clades was evaluated using nonparametric bootstrapping (Felsenstein 1985). Bootstrap analyses used 500 pseudoreplicates each with TBR branch swapping and 10 random taxon-addition-sequence replicates per bootstrap pseudoreplicate. All characters were equally weighted.

Bayesian analyses were implemented using MrBayes version 3.0b4 (Huelsenbeck and Ronquist 2001). A previous analysis of these genes for hylid frogs (Wiens et al. 2005b) used a mixed strategy to find the best combination of models and partitioning strategies, in which hierarchical likelihood-ratio tests (implemented in MrModeltest, ver. 2.0; Nylander 2004) were used to choose reasonable models for separate genes, and comparison of Bayes factors (Nylander et al. 2004) was used to select the best partitioning strategy (Brandley et

al. 2005; Wiens et al. 2005a). These analyses suggest that the best overall modeling and partitioning strategy for the combined molecular data uses the GTR + I + Γ model (general time reversible [Rodriguez et al. 1990] with a proportion of sites invariable [Gu et al. 1995] and rates at other sites varying according to a gamma distribution [Yang 1994]), with separate partitions for each gene and additional partitions within each gene including: 12S stems; 12S loops; ND1 structural regions; ND1 loops; and first, second, and third codon positions for ND1, POMC, and *c-myc*. We did not perform maximum likelihood analyses of these data because current implementations of this approach (e.g., in PAUP*) do not allow for different partitions to have different models, and our comparisons using Bayes factors suggest that a single, shared model of evolution for all genes fits the combined data very poorly.

Each separate and combined dataset was analyzed using two replicate searches with 5.0×10^6 generations each, sampling every 1000 generations. Plots of log-likelihoods over time were examined for stationarity, and trees generated prior to achieving stationarity were discarded as burn-in. To further test for stationarity, we summarized the harmonic mean of the log-likelihoods of the putative postburn-in trees from each analysis using the *sump* command in MrBayes to evaluate whether the separate analyses converged on similar mean log-likelihoods. We also confirmed that the topologies and clade posterior probabilities for each replicate analysis were similar, as an additional check for stationarity. All analyses appeared to reach stationarity before 70,000 generations. Each analysis used four chains and default priors (i.e., Dirichlet for substitution rates and state frequencies; uniform for the Γ shape parameter and proportion of invariable sites; all topologies equally likely a priori; branch lengths unconstrained:exponential). The phylogeny was estimated from the majority-rule consensus of the pooled postburn-in trees from the two analyses.

Most phylogenetic results were similar between parsimony and Bayesian analyses. However, we generally prefer Bayesian analysis over parsimony, because the parsimony analyses assumed a model of evolution (equal rates of change among states and between characters) that is significantly rejected for these data.

We did not perform commonly used statistical tests of alternate phylogenies (e.g., Templeton 1983; Huelsenbeck et al. 1996; Goldman et al. 2000), because these tests do not allow for combined analyses with partitioned models (at least as typically implemented). Thus, they require either use of inadequate models (either for calculating likelihoods or simulating data), piecemeal analysis of the data, or analysis based on parsimony alone. Instead, we interpreted the statistical support for alternate phylogenies based on the posterior probabilities from the Bayesian analyses (i.e., monophyly of a clade is rejected when the alternate topology has Pp ≥ 0.95).

Patterns of Species Richness

Species richness of hylid lineages in Asia, Europe, and North America was calculated from distribution maps available from the GAA. The GAA provides a recent, conservative estimate of geographic ranges for almost all amphibian spe-

cies, and each species account is reviewed by several experts (IUCN et al. 2004). Richness was measured as the number of species occurring in each 5° band of latitude (starting at the equator and working both north and south) within each continental region (North America, Europe, and Asia; Fig. 1). In Mexico we counted only the number of species in the two North American clades under consideration (*Acris-Pseudacris* and *Hyla* originating from North America; see Results). We also used the species-level taxonomy in the GAA. Although there is uncertainty about the status of some hylid taxa, the number of temperate hylid species recognized varies only slightly between recent summaries, and alternate taxonomies should have little impact on our analyses (e.g., GAA recognizes 12 *Pseudacris* and 10 Asian *Hyla* species, whereas Frost [2004] and AmphibiaWeb [2004] both recognize 14 *Pseudacris* and nine Asian *Hyla*). We used the map of *P. triseriata* from Stebbins (1985) because the GAA inexplicably shows the northern distribution of this species ending at the U.S. border.

To test the hypothesis that empirical patterns of species richness differed from those that would be predicted by random distribution of species ranges within continental regions (i.e., the mid-domain effect; Colwell and Hurtt 1994), the program Mid-Domain Null (McCain 2004) was used to shuffle the ranges of species from each region within the latitudinal boundaries of the continental landmass that they live on. This analysis used default settings, empirical range limits, and sampling of species without replacement. Latitudinal boundaries were rounded to the nearest 5° to approximate the method used to measure actual species richness. Ten thousand replicates were used to generate 95% prediction curves for each assemblage. In cases where a given assemblage occurs in a continent that is connected by land to another continent (e.g., North and South America), analyses were repeated using the northern and southern edges of both the continent the assemblage occurs in and the continent the assemblage is connected to. Species were shuffled randomly between the following latitudinal boundaries in each region: (1) North America: between 70°N (Cape Bathurst, Canada) and 5°N (border between Panama and Columbia), and again between 70°N and 55°S (Cape Horn, Argentina); (2) Asia: between 80°N (Cape Chelyuskin, Russia) and 0° (Singapore); (3) Europe: between 70°N (North Cape, Norway) and 10°N (southwestern corner of Yemen), and again between 70°N and 35°S (Cape Agulhas, South Africa).

Estimating Dates of Diversification and Dispersal

We used penalized likelihood (Sanderson 2002) to estimate absolute dates for major events in the radiation of Northern Hemisphere hylids by combining information on molecular branch lengths and ages of fossil taxa. Methods generally followed Chippindale et al. (2004). Dates were estimated using the topology from the Bayesian analysis of the combined data. However, branch lengths were based on the combined nuclear data alone, which are more slowly evolving and less likely to be influenced by saturation. Branch lengths were estimated using maximum likelihood with PAUP* version 4.0b10 using the GTR + I + Γ model. We excluded all outgroup taxa outside of the Middle American clade for this

analysis, because we lacked fossil calibration points for hylid lineages outside of this clade.

We constrained the minimum ages of several clades based on evidence from the fossil record. For a fossil assigned to a period of time in the literature, we used the latest date for that period, given that we were estimating the minimum ages of clades. Five dates were used.

Most recent common ancestor (MRCA) of Acris-Pseudacris clade, at least 15 million years ago.—Holman (2003) suggested that the extinct fossil taxon *Acris barbouri* is likely the sister group to extant *Acris* species and is at least 15–19 million years old (Miocene Hemingfordian North American Land Mammal Age; NALMA hereafter). Thus, the split between *Acris* and *Pseudacris* was at least 15 million years ago. Various *Pseudacris* fossils are known from the middle Miocene Barstovian of North America (~12–15 million years ago; Holman 2003), but given that these fossils cannot be assigned confidently to clades within *Pseudacris* and given that *Acris* fossils already show that the *Acris-Pseudacris* clade is at least 15 million years old, we did not use this information.

MRCA of European Hyla, at least 10 million years ago.—Carroll (1988) and Holman (1998) noted the presence of *Hyla* in the Lower Miocene (~10–23 million years ago) of Europe. We assume that these *Hyla* are closely related to the *Hyla* presently extant in Europe, and that the common ancestor of extant European *Hyla* species is at least 10 million years old.

MRCA of H. squirella–H. cinerea clade.—*Hyla goini* is a fossil species from Miocene Hemingfordian NALMA (15–19 million years ago) that is thought to be closely related to, if not actually conspecific with, the extant species *H. squirella* (Holman 2003). Thus, we assume that the split between *H. squirella* and its sister species (*H. cinerea*) occurred at least 15 million years ago.

MRCA of North American Hyla, at least 33 million years ago.—*Hyla swanstoni* is a fossil taxon similar to the extant *H. gratiosa*, occurring in the late Eocene Chadronian NALMA (33–35 million years ago; Holman 2003). We assume the common ancestor of extant North America species (including *H. gratiosa*) is at least 33 million years old.

Hyla gratiosa–H. versicolor clade, at least 15 million years ago.—*Hyla miocenica* is thought to be closely related to *H. chrysocelis* and *H. versicolor* and occurs in the early Miocene Barstovian (14–16 million years ago; Holman 2003). *Hyla miofloridana* (Miocene, Hemingfordian NALMA; 15–19 million years ago) is similar to *H. gratiosa* (Holman 2003). Among the species included in the penalized likelihood analysis, *H. avivoca*, *H. gratiosa*, and *H. versicolor* form a clade of uncertain relationships, and we assume that the MRCA of this clade is at least 15 million years old.

We used r8s (ver. 1.6 for Unix; Sanderson 2003) to implement penalized likelihood, which requires a date for the root of the tree. We used two dates for the MRCA of the Middle American clade. The first estimate (42 million years ago) is based on the minimum age of the Middle American clade from a similar penalized likelihood analysis that included many additional outgroup taxa and additional geological calibration points for these (nonhylid) outgroup taxa (J. J. Wiens, unpubl. data). The second, older estimate (60 million years ago) is based on the approximate overall age

of hylid frogs in the fossil record (Duellman and Trueb 1986; Zug et al. 2001), which may be an appropriate upper bound for the age of the Middle American clade. The r8s analysis was implemented using the TN algorithm (truncated Newton method with bound constraints). Smoothing parameters were chosen by cross-validated assessment, using values from 10 to 10^4 in increments of 0.5. Analyses using both root ages for the Middle American clade (42 and 60 million years ago) choose 100 as the optimal smoothing value. Because fossil calibration points can only provide minimum estimates of clade age, our estimates of clade age must be considered only minimal estimates as well. Overall, we attempted to infer the general time frame over which major events occurred, and none of the conclusions of this paper hinge upon precise estimates of absolute dates.

Reconstructing Biogeographic Patterns

Major biogeographic changes were reconstructed on the combined-data Bayesian tree using parsimony (implemented in MacClade, version 4.0; Maddison and Maddison 2000). Each species was assigned to one of the following regions, which were coded as character states: 0 (South America, including Trinidad and Tobago); 1 (Middle America; Mexico to Panama); 2 (North America; United States and Canada); 3 (West Indies); 4 (Europe, North Africa, and the Middle East); and 5 (Asia). Most species were endemic to one of these areas, with relatively few species occurring in more than one area. Parsimony was used because the complex ordering of states would have been problematic using available likelihood methods.

We performed three analyses, generally following methods described in Stephens and Wiens (2003): (1) areas as unordered character states: species found in more than one area are coded as polymorphic (sensu Wiens 1999); (2) areas with partial ordering to reflect generalized differences in geographic proximity of areas: (a) South America to North America ordered to prefer passing through Middle America; (b) Middle America to Europe and Asia passes through North America; (c) one step between North America and Europe, North America and Asia, and Asia and Europe; species found in more than one area were coded as polymorphic; and (3) with partial ordering and with polymorphic species (those found in more than one area) coded using the scaled method (sensu Wiens 1999), with a character state for each combination of regions (most were Middle America + South America, but some were North America + Middle America); transitions between polymorphic states are given half a step (e.g., South America + Middle America to South America is 0.5 step, South America + Middle America to North America is 1.5). Although results were similar using all three methods, we preferred the third method because it incorporates the most realistic assumptions. Ancestral area reconstruction can also be performed using Ronquist's (1997) DIVA method, but this method tends to infer ancestors with very large geographic ranges. Conversely, our approach will tend to infer ancestors that are confined to a single geographic area.

Ecological Niche Modeling

We hypothesized that the southward dispersal of temperate hylid lineages into tropical regions may be constrained by

(among other things) limited tolerances to climatic conditions and/or competition with ecologically similar species. To address the potential role of climate, we performed ecological niche modeling to test how well the spatial distribution of climatic variables predicted the range limits of select hylid species. We constructed niche models for six *Hyla* species that live adjacent to lowland tropical regions, specifically those in southeastern Asia (i.e., *H. annectans*) and the montane Middle American species of North American origin (i.e., *H. arenicolor*, *H. euphorbiacea*, *H. eximia*, *H. plicata*, *H. wrightorum*; see Results). However, we also obtained locality and climatic data for all 58 species included in the phylogenetic analysis to reconstruct climatic niche evolution on the phylogeny (see below).

We obtained georeferenced localities for each of the 58 species included in our phylogenetic analysis from museum collection databases (California Academy of Sciences, Museum of Vertebrate Zoology at University of California at Berkeley, Field Museum of Natural History, Museum of Comparative Zoology at Harvard University, and the National Museum of Natural History) and literature sources (Taylor 1962; Ohler et al. 2000). The localities were checked for overall consistency with range maps from the GAA. We also used the CONABIO database for species in Mexico (C. Alvarez, pers. comm.), but only localities that were consistent with those in Duellman's (2001) monograph were included. An average of 19.2 localities per species were examined, ranging from one to 95 (the species with $n = 1$ is only known from a single locality). A list of these localities is available from the authors.

Data for 19 climatic variables, each describing temperature or precipitation, were obtained for each locality from the WORLDCLIM dataset, version 1.3 (Hijmans et al. 2004) with 2.5" spatial resolution. Many of the variables are potentially redundant, and the most strongly correlated variables were identified using Spearman's rank correlation (using SPSS for windows, SPSS Inc., Chicago, IL) on a dataset consisting of all unique localities for all 58 species. A single variable was chosen to represent each group of strongly correlated variables in subsequent analyses; variables that were strongly correlated were arbitrarily considered to be those with correlation coefficients ≥ 0.750 . Within a group of strongly correlated variables we chose those that seemed most likely to limit the geographic range of a species (e.g., extreme cold or hot temperatures rather than annual mean temperatures). The following six variables were selected for use in ecological niche modeling: Bio2, mean diurnal temperature range (mean of monthly [maximum—minimum temperature]); Bio4, temperature seasonality (standard deviation of monthly mean temperatures $\times 100$); Bio5, maximum temperature of warmest month; Bio6, minimum temperature of coldest month; Bio16, precipitation of the wettest quarter; Bio17, precipitation of driest quarter.

Ecological niche modeling focused on only six species (*H. annectans*, *H. arenicolor*, *H. eximia*, *H. euphorbiacea*, *H. plicata*), one of which (*H. eximia*) was not one of the 58 included in the primary phylogenetic analysis. Niche models were based on an average of 82.8 localities per species (range = 10–334); these localities are available from the authors. Niche modeling was implemented using the BIOCLIMav (Beta1.1)

extension (A. Moussalli, Cooperative Research Centre for Tropical Rainforest Ecology and Management, Brisbane, Australia) on ArcView GIS 3.3 (Environmental Systems Research Institute, Redlands, CA, 1992). For each of the six species a niche model was calculated for each variable, and for all six variables combined. Both upper and lower profile constraints were used for each variable. We used BIOCLIM to determine the range of environmental conditions in which a species is able to persist (the ecological niche envelope) and then projected the distribution of these conditions back onto a map to visualize the geographic extent of those conditions. If the climatic niche envelope closely matches the geographic distribution of a species, this result suggests that climatic tolerances for those variables may set the range limits of that species (although this finding does not rule out other factors, such as competition). Conversely, if the climatic niche envelope extends well beyond the geographic range, then other factors besides climate presumably are limiting the dispersal of that species. By repeating this procedure on each of the climatic variables separately, it is also possible to infer which of the individual variables may be important in limiting the spread of the relevant species into lowland tropical regions.

To objectively quantify and compare the degree to which climate niche models overpredicted into lowland tropical regions, we first obtained a series of absence localities adjacent to the range of each species in the areas of interest (i.e., lowland tropical habitats). We then quantified the number of absence localities that were within the predicted niche envelope of a given species; variables with niche envelopes that overpredicted into many absence localities were considered unlikely to determine the range limits of that species. For species in Middle America, these absence localities represented localities where other hylid species have been collected in the lowland tropics but not the species of interest (i.e., those derived from temperate North America). We generally used localities that were within 100 km of the edge of the range of the species of interest, and only used localities that were more than 20 km distant from each other. However, for *H. wrightorum*, we had no localities within 100 km, but used those within 200 km instead. The absence localities were obtained using the same sources used to obtain presence localities and were based mainly on the species *Smilisca baudinii* (for most species), *Pachymedusa dacnicolor* (Pacific coast only), and *Pternohyla fodiens* (for *H. wrightorum* only).

In Asia, no hylids exist south of the Holarctic lineages. Therefore, we used localities for rhacophorid treefrogs as absence localities. Because relatively few rhacophorid localities were available in the desired geographic area, we included localities in lowland Cambodia, Laos, Myanmar, Thailand, and Vietnam that were up to 1400 km south of the range of *H. annectans* (the southernmost Asian hylid included) and only used localities that were at least 50 km distant from each other.

Elevation and Latitude

If climate is important in limiting the dispersal of temperate lineages into tropical regions, we might also expect that temperate lineages will live at higher elevations at lower latitudes

on each continent. We tested the relationship between latitude and elevation with standard regression using individual localities as datapoints and pooling localities across species (126 for the *Acris-Pseudacris* clade; 279 localities for *Hyla* in North America, 96 for Europe, and 46 for Asia). A separate analysis was performed for each continent, and the two major hylid clades in North America (*Acris-Pseudacris* and *Hyla*) were treated separately. It should be noted that this analysis is potentially biased by the nonindependence of species and conspecific populations; however, an approach using phylogenetically independent contrasts would lose information on intraspecific shifts in elevation (given that we lack extensive intraspecific phylogeographic data). The results should be interpreted with appropriate caution.

Patterns of Clade Overlap and Diversity

Competition from ecologically similar species may be important in setting the range limits of species and clades (e.g., Darwin 1859; Connell 1961; MacArthur 1972; Case and Taper 2000). Although our data cannot resolve this issue, we can test some predictions of this hypothesis using distributional data. We were specifically interested in explaining why some hylid species fail to invade tropical lowland rainforests in Asia and Middle America.

All Asian hylid species belong to the *H. arborea* group, and competition with other hylids therefore cannot prevent temperate hylids from invading tropical Asia. However, there is a large radiation of ecologically and morphologically similar frogs in Asia (rhacophorid treefrogs; Duellman and Trueb 1986; Zug et al. 2001) that are particularly diverse in tropical areas and that might play a role in limiting the spread of hylids into tropical regions in Asia. If this were the case, we might expect a negative relationship between numbers of hylid and rhacophorid species in Asia at larger geographic scales. As a crude test of this hypothesis (lacking sufficient data on local-scale diversity of these families in Asia), we tallied the number of rhacophorid and hylid species occurring in each 5° square of latitude/longitude in Asia (using GAA distribution maps, limited to the areas where *Hyla* occur) and performed a linear regression analysis with Statview (ver. 5, SAS Institute, Cary, NC). If the diversification of rhacophorids in tropical lowland southeast Asia has prevented the invasion of hylids into these regions, we would expect to see a negative relationship between the number of hylid species and the number of rhacophorid species at the regional scale (although other explanations for a negative relationship are possible).

Competition might also have prevented species of the North American *Hyla* lineage in montane Middle America from invading tropical lowland habitats (see Results). We obtained data on local hylid species richness and species composition at 23 relatively well-studied sites in Mexico from Duellman (2001; mostly from table 73) and analyzed the relationship between the number of tropical Middle American species and the number of species derived from North America. If competition prevents the invasion of tropical lowlands by the North American lineages, then one expected signature of this pattern would be a negative rela-

tionship between the number of species from North American and Middle American lineages in local communities.

We acknowledge that these large-scale patterns of geographic overlap and diversity cannot provide rigorous tests of the effects of competition. However, given that we are specifically interested in the effects of competition on large-scale patterns of diversity and distribution, testing for the predicted effects of competition at this scale is useful for our purposes. There is some evidence for competition between hylid species based on experiments with tadpoles (e.g., Wilbur 1982; Morin 1983), but these studies are not necessarily relevant to explaining large-scale biogeographic patterns.

Reconstructing Niche Evolution and Testing Niche Conservatism

To visualize evolutionary change and stasis in the important climatic niche characteristics of hylid lineages, we mapped the most generally limiting variable identified from the ecological niche modeling onto the phylogeny. Minimum temperature of the coldest month (Bio6) generally provided the best fit to geographic range limits of hylid lineages in tropical Asia and Middle America (see Table 3). Ancestral values of coldest winter temperatures (Bio6) were reconstructed using the linear generalized least-squares method of Martins and Hansen (1997) implemented with COMPARE version 4.6 (Martins 2004). For extant species the value of Bio6 was obtained by averaging this variable across all georeferenced localities for a species (mean of 19.2 localities per species, with a range from one to 95). For this analysis, we obtained locality data for all 58 species included in the phylogenetic analysis (although the number of localities was limited for the distant outgroup taxa). Ancestral values of Bio6 were reconstructed on the Bayesian tree with branch lengths estimated from the combined nuclear genes using maximum likelihood.

To test explicitly for phylogenetic conservatism in this climatic variable, we used a measure of phylogenetic correlation (λ) introduced by Pagel (1999); λ generally varies between zero and one, with zero indicating no phylogenetic signal and one indicating strong phylogenetic signal (Freckleton et al. 2002). Again, we focused on Bio6. We estimated the log-likelihood of the data when λ is at its estimated maximum likelihood value and when λ is set to zero, assuming the null hypothesis that the data are not phylogenetically conserved. We then used the likelihood ratio test statistic ($-2\log_e[H_0/H_1]$) to assess the difference between these models, where H_0 represents the null model ($\lambda = 0$) and H_1 the alternative model (λ estimated). Analyses were performed with the program Continuous, version 1.0d13 (M. Pagel, University of Reading, Reading, U.K.), using the combined Bayesian tree with branch lengths estimated from the combined nuclear data.

RESULTS

Phylogeny and Biogeography

Combined analysis of the four genes included 3380 aligned nucleotide positions, of which 1991 were variable and 1128 were parsimony informative (Table 1). Separate analyses of

the nuclear and mitochondrial genes using parsimony and Bayesian methods resulted in similar topologies. The Bayesian phylogeny is generally strongly supported, and many clades are recovered by separate analyses of the combined nuclear genes and combined mitochondrial genes (Fig. 2).

Mapping geographic location onto this phylogeny shows two separate invasions of North America from Middle America (Fig. 3): the *Acris-Pseudacris* clade and North American species of *Hyla*. Most of the sampled species of the *arborea* group from Europe and Asia each form a monophyletic group, which together make up the sister group to North American *Hyla*. Remarkably, the North American *Hyla* do not form a monophyletic group. Instead, they gave rise to a second invasion of Asia from the New World. Specifically, *H. japonica* is nested among the North American species. Furthermore, this group of North American species also launched a secondary invasion of montane Middle America (i.e., the clade including *H. arenicolor*, *H. euphorbiacea*, *H. plicata*, and *H. wrightorum*). The nonmonophyly of North American *Hyla* is surprising but is supported by separate analyses of the mitochondrial and nuclear genes (Fig. 2).

An additional Bayesian analysis (not shown) was performed to confirm that additional species (for which only fragmentary data were available) are placed in the clades established in the larger dataset. This analysis confirms that: (1) the Asian *H. simplex* is in the clade of Asian species with *H. chinensis*; (2) the Mexican *H. eximia* is in the clade of montane Middle American species including *H. arenicolor*, *H. wrightorum*, *H. plicata*, and *H. euphorbiacea* (corresponding to the *H. eximia* species group of Duellman 2001); (3) the North American *H. chrysocelis* is in the clade of North American *Hyla* with *H. avivoca*, *H. gratiosa*, and *H. versicolor*; (4) the Korean *H. suweonensis* is in the clade of North American *Hyla* clade, which also includes *H. japonica* (although placement of this species is very weakly supported); (5) *Acris gryllus* is the sister species of *A. crepitans*; and (6) all species of *Pseudacris* have been correctly assigned to that genus.

Given that we supported the monophyly of the *H. eximia* group for the five species included and showed that this group is nested within a clade of temperate North American (and Asian) species, we considered there to be seven species of *Hyla* in montane Middle America derived from temperate North America (i.e., we assume the *H. eximia* species group is monophyletic and that *H. bocourti* and *H. walkeri* are correctly assigned to this group, even though they were not included in our phylogenetic analyses). We also assume that the two unsampled species of European treefrogs (*H. intermedia*, *H. sarda*) belong to the same clade as the European species we sampled; in fact these species have been considered conspecific with *H. arborea* by some previous authors (Frost 2004). Similarly, we assume that the unsampled species of Asian *Hyla* (*H. hallowelli*, *H. immaculata*, *H. sanchiangensis*, *H. tsinlingensis*, *H. zhaopingensis*) belong to one of the two Asian clades. Immunological distance data (Riehl et al. 1995) suggested that *H. hallowelli* belongs to the clade with *H. chinensis* rather than the clade with *H. japonica*.

Patterns of Species Richness

The hylid faunas of Asia, Europe, and North America show very similar latitudinal pattern of species richness (Fig. 3),

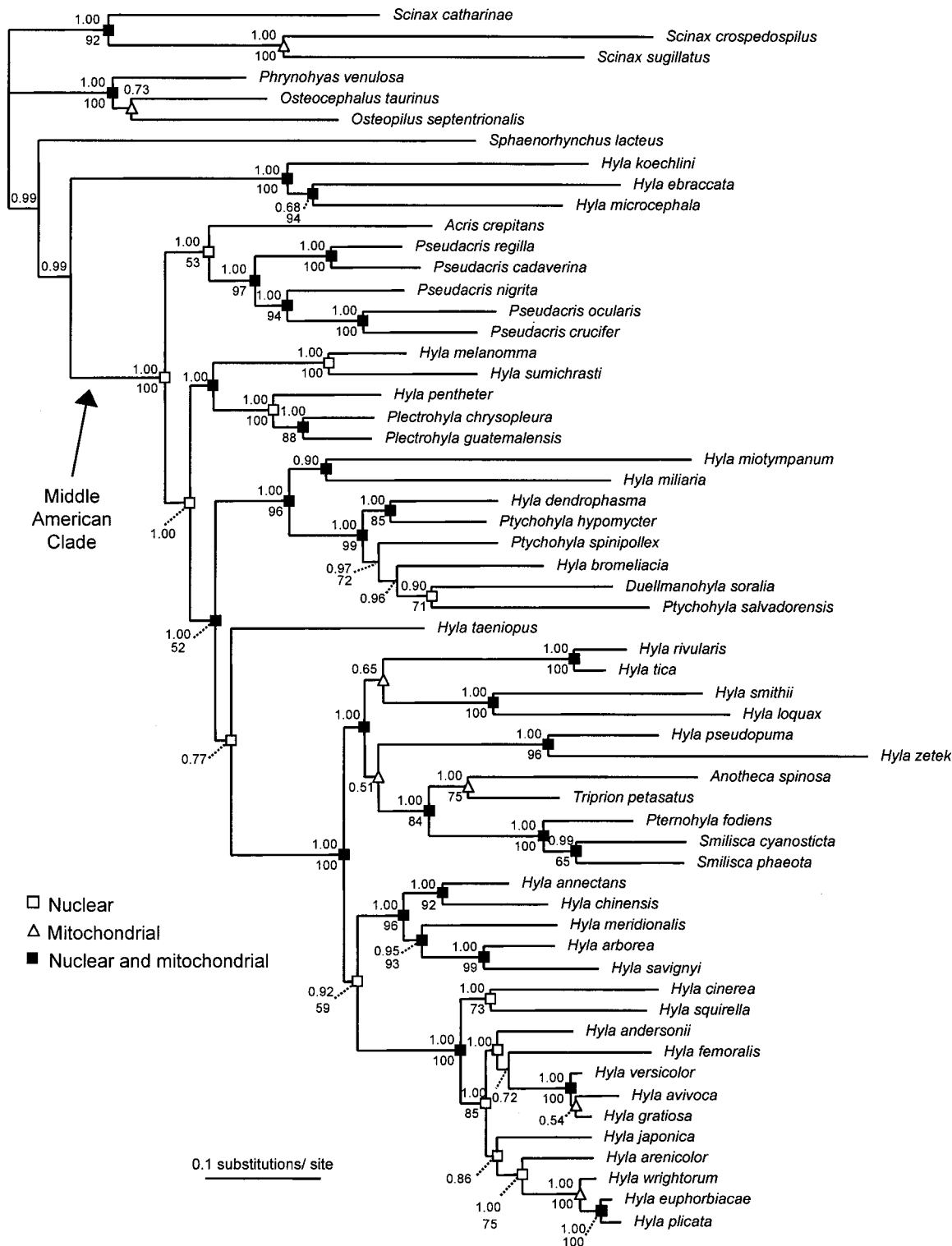


FIG. 2. Phylogeny based on combined, partitioned Bayesian analysis of two nuclear and two mitochondrial genes. Numbers above branches are Bayesian posterior probabilities (Pp) and numbers below branches are bootstrap support values for clades that were also found in the parsimony analysis. Open squares indicate nodes supported in separate Bayesian analysis of the combined nuclear data, open triangles mark nodes supported in analysis of combined mitochondrial data, and filled squares indicated nodes supported by separate analyses of both nuclear and mitochondrial data. Clades without symbols are unique to the combined analysis. Support values less than Pp 0.50 or bootstrap 50% are not shown.

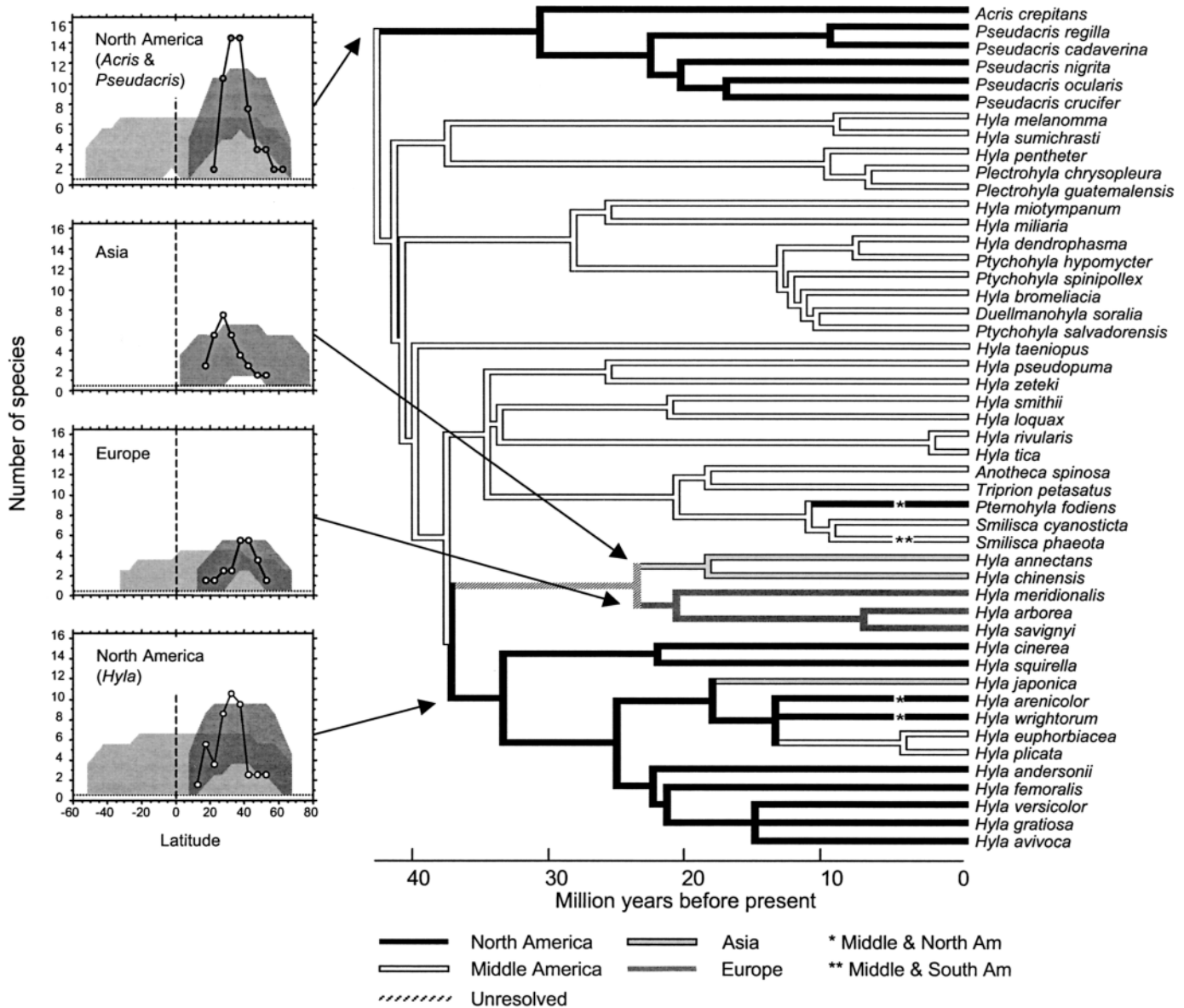


FIG. 3. Species richness, biogeography, and divergence time estimates for Northern Hemisphere hylids. Graphs at left show the number of hylid species occurring in each 5° band of latitude for Europe, Asia, and the two clades in North America. Gray shaded portions of this graph indicate the expected distribution of species richness (95% confidence interval) if ranges are randomly distributed within the boundaries of a continental region; dark gray represents a narrow geographic definition for the region and lighter gray a broader definition. The tree topology is from the combined Bayesian analysis (Fig. 2) with branch lengths proportional to penalized likelihood estimates of clade ages (based on a root age of 42 million years; results are generally similar for an alternate root age of 60 million years). Branch shadings indicate generalized geographic ranges of species and clades (reconstructed using parsimony).

with the highest species richness at midtemperate latitudes (25–30° in Asia, 35–45° in Europe, 30–35° in North America). In North America, this same pattern occurs in both the *Acris-Pseudacris* clade (30–40°) and within *Hyla* (30–35°). The peak of species richness at these latitudes is significantly higher than expected for Asian *Hyla* and the two North American hylid lineages (Fig. 3), given a null model in which species ranges are randomly distributed among latitudes within continental regions (i.e., the mid-domain effect). For European *Hyla* the peak is at the edge of the 95% prediction curve (Fig. 3), but clearly above the curve for randomizations

that include Europe and Africa. Furthermore, the latitudinal distribution is significantly narrower than predicted for the *Acris-Pseudacris* clade and North American *Hyla*. The similarity in their geographic patterns of species richness is also surprising given the differences in the overall numbers of hylid species between regions and clades (Fig. 3).

Dates of Diversification and Dispersal

Penalized likelihood analyses generally gave similar results regardless of whether the Middle American clade was

TABLE 3. Summary of results of ecological niche modeling. Climatic niche models (based on presence localities) that erroneously predicted the species as occurring in the most absence localities were considered to have the poorest fit. Conversely, climatic variables that correctly predicted the absence localities were considered to be potentially important in limiting dispersal of Holarctic hylid lineages into lowland tropical regions. Bio2, mean diurnal temperature range; Bio4, temperature seasonality; Bio5, maximum temperature of warmest month; Bio6, minimum temperature of coldest month; Bio16, precipitation of wettest quarter; Bio17, precipitation of driest quarter.

Species	No. of presence localities	No. of absence localities	Proportion of absence localities predicted (%)	
			Combined model	Individual climatic variables (best fitting to worst fitting)
<i>Hyla annectans</i>	14	35	23	Bio6 (43), Bio4 (51), Bio16 (51), Bio5 (94), Bio2 (97), Bio17 (97)
<i>H. arenicolor</i>	95	71	49	Bio6 (73), Bio17 (87), Bio2 (89), Bio16 (89), Bio5 (93), Bio4 (96)
<i>H. euphorbiacea</i>	14	19	5	Bio6 (10), Bio4 (26), Bio5 (32), Bio2 (47), Bio16 (47), Bio17 (47)
<i>H. eximia</i>	334	80	36	Bio6 (44), Bio17 (73), Bio5 (91), Bio16 (91), Bio4 (93), Bio2 (95)
<i>H. plicata</i>	30	43	5	Bio5 (7), Bio6 (12), Bio4 (42), Bio17 (58), Bio2 (74), Bio16 (81)
<i>H. wrightorum</i>	10	11	0	Bio5 (0), Bio6 (0), Bio17 (18), Bio4 (64), Bio16 (73), Bio2 (82)

considered to originate 42 million years ago (Fig. 3) or 60 million years ago, particularly for younger nodes. The two North American clades diversified over a similar time frame: North American *Hyla* arose ~33 million years ago (using 42 or 60 million years ago as the root age) and the *Acris-Pseudacris* clade arose 30–43 million years ago (using 42 vs. 60 million years ago). The split between European and Asian *Hyla* occurred 23–28 million years ago. The European hylid fauna diversified 21–25 million years ago, whereas the Asian sister clade diversified at least 18–22 million years ago. The second invasion of Asia occurred no less than 18.1–18.9 million years ago, based on the split between *H. japonica* and the related North American species, and the invasion of montane Middle America from North America occurred 13.5–14.3 million years ago. Although our sampling of species is not comprehensive, results for the included species suggest that all speciation events in this group are several million years old (with many extant species > 10 million years old) and were not associated with Pleistocene climatic changes.

Ecological Niche Modeling

Ecological niche modeling supports the hypothesis that specialized thermal tolerances (or preferences) may limit the spread of many temperate hylids into the tropics (Table 3). Visual inspection shows that niche envelopes predicted using the combined variables closely match the geographic distributions of each species at their southern and lower elevational range limits. Niche envelopes based on the combined climatic variables included less than 25% of the relevant absence localities for all species except *H. eximia* (36%) and *H. arenicolor* (49%). The distribution of coldest winter temperatures (Bio6) overpredicts into fewer absence localities in the lowland tropics than any other variable for four of the six species, is tied with hottest summer temperatures (Bio5) in one species, and is almost as good a predictor as Bio5 in another species (7% vs. 12%). Surprisingly, Bio5 is not one of the two best predictors in four other species. In general, temperature seasonality (Bio4), rainfall based variables (Bio16, Bio17), and diurnal temperature variation (Bio2) did not accurately predict species range limits. Overall, the distribution of coldest winter temperatures (Bio6) seems to most closely match the range limits of these six hylid species.

Elevation and Latitude

Regression analyses of elevation and latitude shows some support for the hypothesis that the geographic ranges of temperate hylid species and lineages tend to shift upward in elevation at lower latitudes on each continent (Asia: $r^2 = 0.296$, $P < 0.0001$; Europe: $r^2 = 0.234$, $P < 0.0001$; North American *Hyla*: $r^2 = 0.488$, $P < 0.0001$; *Acris-Pseudacris*: $r^2 = 0.021$, $P = 0.1055$). This relationship is strongest in North American *Hyla* clade, which contains a lineage of montane Middle American species, and weakest in the North American *Acris-Pseudacris* clade, which does not extend into tropical Middle America.

Patterns of Clade Overlap and Diversity

There is no association between the number of sympatric hylid species and the number of coexisting rhacophorids at the scale of 5° latitude/longitude ($r^2 = 0.13$, $P = 0.303$). We did not find the negative relationship between hylid and rhacophorid diversity expected if rhacophorids have excluded hylids from invading tropical lowland southeast Asia.

Among local-scale hylid communities in Mexico, there is a significant negative relationship between the number of species of Middle American lineages and those representing lineages recently derived from temperate North America ($r^2 = 0.435$, $P = 0.0006$). This is the pattern expected if Middle American lineages have prevented these North American lineages from invading tropical lowland habitats (although other explanations are possible).

Niche Evolution and Conservatism

Given that the distribution of coldest winter temperatures seems to be the most generally important variable in predicting range limits of Holarctic hylid lineages (Table 3), we reconstructed ancestral values of Bio6 on the Bayesian phylogeny (Fig. 4). These analyses suggest that there have been three major invasions of temperate climatic regimes from tropical regions: (1) the *Acris-Pseudacris* clade; (2) the clade of European and (most) Asian *Hyla*; and (3) the clade of North American, Asian, and high montane Middle American *Hyla* (although it seems likely that the latter two clades might represent a single invasion of temperate regions). The results

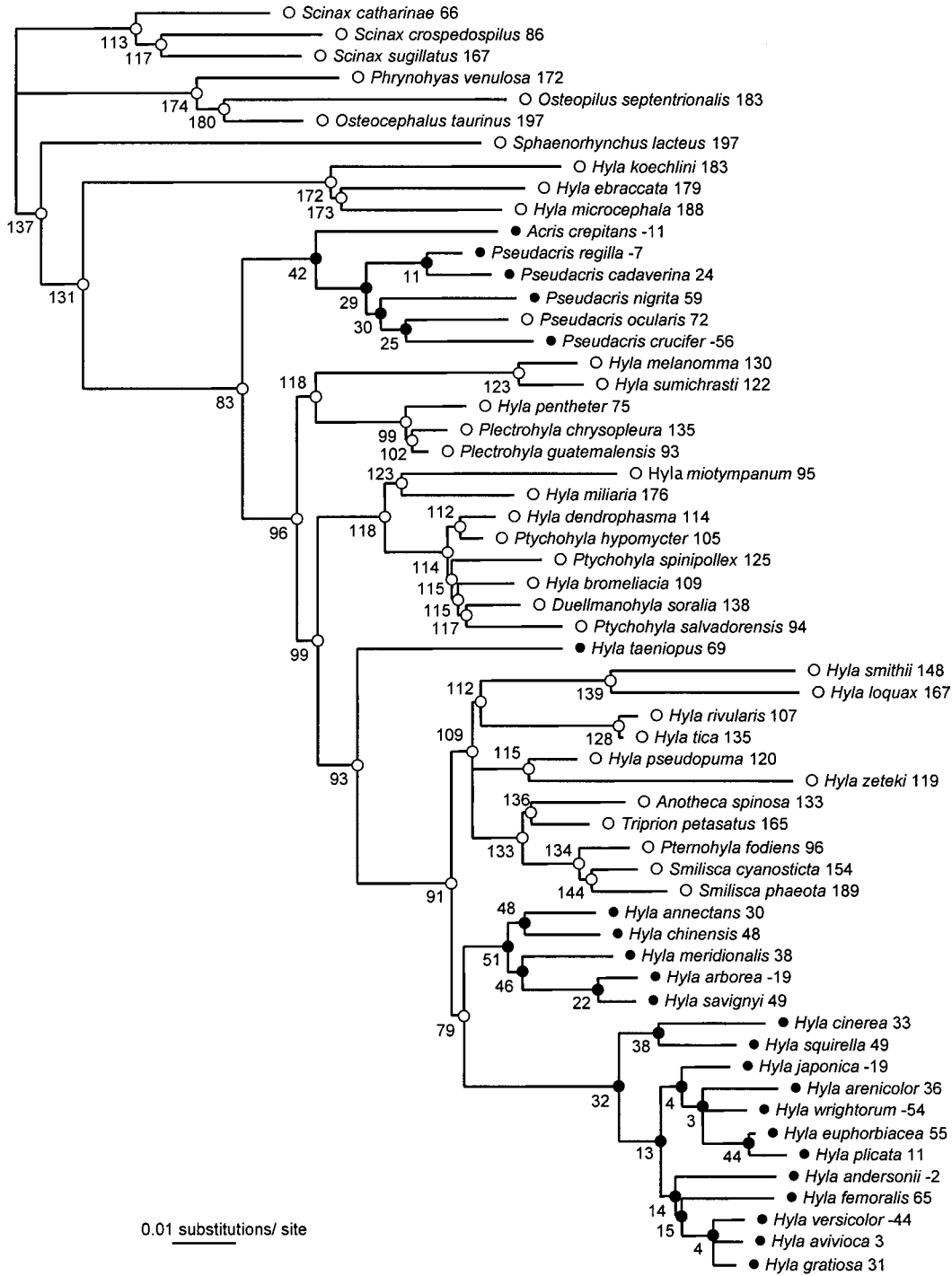


FIG. 4. Evolution and conservatism in a climatic niche trait. Ancestral values of minimum temperature of coldest month (Bio6) reconstructed from average values of extant populations using the linear generalized least-squares method of Martins and Hansen (1997). For illustrative purposes, we use closed circles to indicate species or nodes with average or reconstructed values between -55 and 70 (minimum temperature of -5.5°C to 7.0°C), whereas open circles indicate values between 71 and 200 .

also show that the extensive intercontinental dispersal within *Hyla* occurred within a relatively similar climatic regime, including the reinvasion of high montane Middle America (i.e., *H. euphorbiacea* and *H. plicata*).

The estimated maximum likelihood value of phylogenetic correlation (λ) for Bio6 is 0.9337 , which suggests strong

phylogenetic signal. The log likelihood of the data under the null hypothesis of no conservatism ($\lambda = 0$) is -339.276 and under the alternate hypothesis (estimated $\lambda = 0.9337$) is -305.575 . The likelihood ratio test statistic is 67.4008 ($P < 0.000001$). These results indicate that there is significant phylogenetic conservatism in this trait.

DISCUSSION

Patterns of Species Richness

The tendency for species richness to increase from poles to the equator is one of the oldest known patterns in ecology and biogeography (Brown and Lomolino 1998). Although hylids show this pattern overall (i.e., most hylid species live in the New World tropics), we show that a very different pattern of species richness develops in parallel in Asia, Europe, and North America, with the highest species richness at midlatitudes (~30–35°). Our phylogenetic results show that this pattern has developed four times (i.e., twice in North America). In each of these four cases, species richness appears to have built up largely through in situ diversification on each continent, although repeated dispersal between continents also contributes to the pattern in Asia.

This repeated pattern has several interesting implications for studies of the latitudinal gradient in species richness. Many authors contend that high energy and/or productivity explain the species richness of many higher taxa in wet tropical regions and the overall latitudinal diversity gradient (e.g., Francis and Currie 2003; Hawkins et al. 2003; Willig et al. 2003). If this were the case in hylids, we would expect hylid faunas in North America, Europe, and Asia to show their highest species richness in the warmest and wettest regions of each continent, not at midtemperate latitudes. Surprisingly, the lineages in Middle America and Asia fail to invade major regions of lowland tropical rainforests, even though they are geographically adjacent to them. The observed peaks of species richness at temperate latitudes suggests that energy and productivity alone are insufficient to explain patterns of species richness in these lineages of hylid frogs and that these factors cannot be a universal explanation for global species-richness patterns. This pattern is also surprising given that hylids overall show a strong latitudinal diversity gradient in the New World, with the highest local species richness in lowland tropical rainforests of South America (Duellman 1988, 2001; Duellman and Sweet 1999).

Other authors have suggested that patterns of species richness may be generated by stochastic patterns of dispersal bounded by the shape of continents, with the highest richness near the center of the continent (the mid-domain effect; Colwell and Lees 2000). Although hylids do indeed appear to have their highest species richness on each continent near the middle of their latitudinal ranges (Fig. 3), their latitudinal ranges do not seem to be bounded by the shape of continents alone. If they were, we would expect these hylid lineages to range further south into Asia and Middle America. Randomizations also showed that in North America and Asia hylids have a peak in diversity in midtemperate latitudes significantly higher than predicted by the mid-domain effect alone (Fig. 3). We do not claim that the mid-domain effect is unimportant in hylid frogs. However, if constraints on dispersal are generating patterns of species richness, these constraints do not appear to be related to the shape of the continents alone but are more likely associated with other ecological factors (e.g., climatic tolerances, competition).

We hypothesize that patterns of species richness in temperate hylids have been strongly influenced by the combination of niche evolution (associated with the shift from trop-

ical to temperate climate regimes in the two hylid lineages that dispersed from Middle America) and niche conservatism (limiting dispersal out of the temperate niche, especially in North America and Asia). Within the temperate zone, there has been extensive dispersal between continents and speciation within continents. Several authors have suggested that the pattern of high tropical species richness in many groups of organisms may be related to these same processes, with climatic niche conservatism limiting dispersal of most tropical clades into temperate regions and leading to a buildup of species richness in tropical regions over time (e.g., Ricklefs and Schluter 1993; Brown and Lomolino 1998; Futuyma 1998; Ricklefs 2004; Wiens and Donoghue 2004). The niche conservatism hypothesis is unusual in that it can explain both the high species richness of many groups in tropical regions and the pattern seen in many temperate clades (such as Holarctic treefrogs). Studies are presently underway to evaluate the role of niche conservatism in generating the high tropical species richness of New World hylids, using phylogenetic analysis (i.e., to test whether hylids originated in the New World tropics and compare diversification rates at different latitudes) and ecological niche modeling (i.e., to address whether climatic factors limit dispersal of clades from tropical to temperate regions).

Our results also raise the intriguing possibility that the geographic decrease in species richness from temperate to polar regions may be disconnected from the decrease from temperate to tropical regions. In addition, parts of the latitudinal diversity gradient within a continent may be composed of groups that differ radically from the overall pattern. For example, Asian amphibians show a strong diversity gradient from the equator to the poles (GAA; ICUN et al. 2004). Hylid frogs clearly contribute to this gradient from temperate to polar regions (Fig. 3) but not from temperate to tropical regions (because they are largely absent from tropical Asia). Glaciation may be important in explaining the low species richness of hylids in boreal versus temperate regions but not temperate versus tropical regions. There are no endemic hylids on any continent above 50° latitude. Instead, the northern part of the range of hylids on each continent is made up of relatively wide-ranging species (e.g., *H. arborea* in Europe; *H. japonica* in Asia; *Pseudacris triseriata*, *H. chrysocelis* and *H. versicolor* in the New World). These northern populations presumably represent recent recolonization of glaciated areas. However, glaciation may have little to do with the disparity in species richness between warm-temperate and tropical regions, given that this interface occurs well south of glaciated regions and the clades that are affected by glaciation may not even occur in tropical regions (e.g., Asian hylids). Recent biogeographic studies demonstrate extensive interchange of temperate plant and animal lineages among continents in the Northern Hemisphere (Sanmartín et al. 2001; Donoghue and Smith 2004), and the independence of different portions of the latitudinal diversity gradient may be a common phenomenon.

Why Are There Temperate Clades?

Many clades of organisms are spread across the temperate regions of the Northern Hemisphere (e.g., Sanmartín et al.

2001; Donoghue and Smith 2004), and may show patterns of species richness similar to those observed in hylids. Why are there clades confined to temperate regions? For example, why have these temperate lineages of *Hyla* not invaded the tropical forests of Southeast Asia or lowland Middle America? One hypothesis is that temperate hylid lineages have become so specialized for temperate climates that tropical regions are largely outside their range of acceptable environmental conditions (i.e., the niche conservatism hypothesis of Ricklefs and Latham 1992). Another hypothesis is that their dispersal into tropical communities is prevented by competition.

Our results cannot rule out either hypothesis, but they nevertheless offer several lines of support for the hypothesis of niche conservatism. First, the similarity in distribution patterns of *Hyla* on different continents may be explained most simply by a single shared factor (i.e., climatic tolerances inherited from a common ancestor) rather than postulating that different suites of competing species on each continent create parallel distribution patterns by chance. Second, temperate hylids show a weak tendency to shift to higher elevations at lower latitudes on all three continents (but especially in Middle America), suggesting that they track a similar climatic niche across their ranges. Third, for most of the species examined, a limited set of climatic variables does predict the range limits of Holarctic hylids at the edge of tropical regions. Finally, in Asia, patterns of distribution of hylid treefrogs and rhacophorid treefrogs do not match the pattern expected if the presence of one clade excludes the other.

A significant negative relationship was found between the number of tropical hylid species and the number of species derived from temperate North America in local communities in Mexico, however, suggesting a possible role for competition. But this result could also be caused by the failure of tropical lineages to invade cooler high montane habitats rather than by the failure of temperate lineages to invade species-rich tropical communities. We found that of the seven communities that included species derived from the temperate North American lineage, only one species from this lineage was present at each site and two of the sites were high-elevation localities that contained no tropical lineages; the high-elevation temperate species coexist with lower-elevation tropical species in five communities; and a species of the temperate North American clade (*H. eximia*) occurs at one of the most species-rich local sites in Mexico (Cuatlapán, Veracruz, with nine hylid species; Duellman 2001).

If environmental niche conservatism does explain these biogeographic patterns, how might climatic tolerances actually limit dispersal? Ecological niche modeling suggests that high summer temperatures and/or the absence of low winter temperatures may limit invasion of tropical lowland habitats by temperate lineages of *Hyla* in adjacent montane regions (Table 3). Species that are adapted to cool summer temperatures in tropical montane regions may have limited ability to tolerate high summer temperatures at low elevations (Janzen 1967), and amphibians may be particularly heat sensitive (Duellman and Trueb 1986). It is much less obvious how the absence of cold winter temperatures in the lowland tropics limits dispersal. Yet, this variable seems to predict

the relevant range limits in more species than does high summer temperatures (Table 3). One hypothesis is that winter cooling may play an important role in cueing reproductive cycles in temperate amphibians (e.g., Reading 1998) and that the absence of these cues in the lowland tropics may cause populations that disperse into these regions to fail. Clearly, detailed local-scale ecological studies are needed to elucidate the mechanisms that prevent these clades from invading tropical regions. These studies should also shed light on the relative roles of climatic tolerances and competition and possible interactions between them.

In theory, some of the patterns of distribution and diversity that we describe herein could have been generated by population extinctions and climate change (see also discussion of glaciation above). However, the fossil record in temperate North America and Europe suggests that the hylid clades present there today are the only clades that successfully invaded these regions (Holman 1998, 2003). Furthermore, climate change and population extinctions seem unlikely to explain the failure of Holarctic hylid clades to invade tropical regions, because the regions south of their ranges have presumably been tropical throughout their evolutionary history.

Patterns of Dispersal

The results of this study show that hylid frogs can disperse remarkable distances within a similar climatic regime but may be unable to disperse even short distances if major transitions in climatic regimes are involved. We found that one clade of hylids seemingly dispersed from Middle America to North America (~3500 km distance between the approximate midpoint of the range of hylids in each region), then to Europe and Asia (8500 km and 12,000 km, respectively, as estimated above) over about 20 million years (based on the times between the origin of the clade and the first inferred splitting in each region). We also found that one clade of hylids dispersed from eastern North America to temperate climate regions of Japan (~11,000 km) and Middle America (~2500 km) over the course of about 10 million years. Yet, over the same time period, species in this clade seemingly failed to invade tropical climate regions that were only a few kilometers distant (i.e., in tropical Middle America). This result suggests that ecology may be far more important than distance in determining large-scale biogeographic patterns. More specifically, we hypothesize that the failure of these lineages to evolve (or re-evolve) ecological traits suited to the tropics has limited their dispersal.

Our results also suggest striking differences in the dispersal tendencies of the two hylid clades in North America. Although both have similar ages and patterns of distribution within North America, one clade has remained confined to North America (*Acris-Pseudacris*), whereas the other (*Hyla*) has spread to Europe, Asia (twice), and montane Middle America. The difference in dispersal tendency of these clades would be an interesting topic for future research.

Assembly of the North American Hylid Fauna

Our results suggest that the two clades of temperate North American hylids have undergone similar patterns of diversification, despite their extensive sympatry. For example,

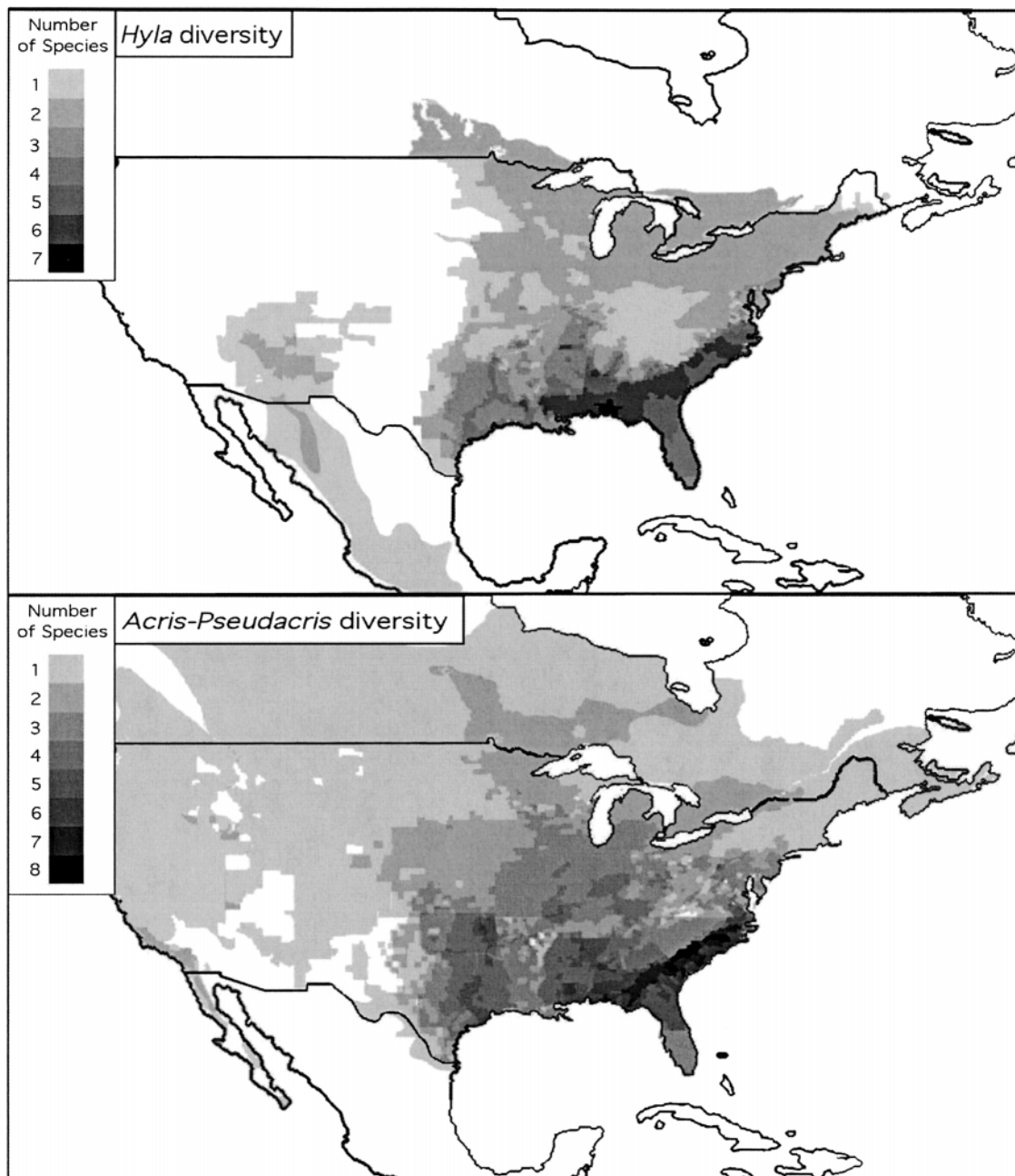


FIG. 5. Pattern of species richness in the *Acris-Pseudacris* clade and the North American clade of *Hyla*, based on range maps from the Global Amphibian Assessment (IUCN et al. 2004; see also Duellman and Sweet 1999).

both clades show high species richness, sympatry, and endemism in the southeastern United States (Fig. 5). They also show very similar numbers of species in each region and may have diversified over similar time scales (*Acris-Pseudacris* beginning 30–43 million years ago; *Hyla* ~33 million years ago).

These patterns suggest that there has been little competitive exclusion of *Hyla* and *Acris-Pseudacris* in North America and that the diversification in these two clades has proceeded over roughly the same time and place, with seemingly little influence of one clade on the other. These two clades do have several differences that may reduce potential competition be-

tween them. The *Acris-Pseudacris* clade consists mostly of small-bodied (adults of most species range up to only ~32 mm snout-vent length; mean maximum size = 31 mm; range = 17–48 mm) terrestrial species, many of which breed during the early spring and winter (Conant and Collins 1991). In contrast, the 10 species of North American *Hyla* tend to have larger body size (maximum adult size of most species > 40 mm; mean = 54 mm; range = 40–70 mm), are arboreal, and breed during summer (Conant and Collins 1991; Duellman 2001). But even within these two clades, there appears to be extensive sympatry in regional assemblages (Fig. 5), especially in the southeastern United States. These patterns sug-

gest the possibility that competition may not strongly limit biogeographic patterns in temperate hylids.

Conclusions

In this study, we have shown that hylid frogs have repeatedly developed similar patterns of species richness on different continents in the Northern Hemisphere, with the highest species richness at midtemperate latitudes rather than in tropical regions. These patterns contrast sharply with expectations based on many current explanations for global species-richness patterns and even expectations based on hylids overall (i.e., most hylid species occur in the New World tropics). We suggest that these patterns can be explained by the combination of niche evolution (i.e., transition from tropical to temperate climate regimes) and niche conservatism (i.e., maintenance of the temperate climatic niche across different continents). Specifically, there were two major invasions of temperate regions from tropical regions, followed by extensive dispersal between different continental regions with similar (temperate) climates, and *in situ* diversification in each continental region. Our results suggest that evolution and conservatism in ecological factors (e.g., climatic tolerances) may strongly influence patterns of dispersal and biogeography, and that these patterns of dispersal and biogeography may influence global patterns of species richness. We anticipate that similar patterns will be found in many other groups of organisms, as historical biogeographers begin to incorporate ecological information and researchers interested in species richness patterns incorporate historical biogeography.

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