

## Combining Historical Biogeography with Niche Modeling in the *Caprifolium* Clade of *Lonicera* (Caprifoliaceae, Dipsacales)

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**Abstract.**—The *Lonicera* clade *Caprifolium* contains ~25 species distributed around the Northern Hemisphere, including in the Mediterranean climates of California and Europe. We sequenced the second intron of *LFY* to help resolve relationships within the clade where the internal transcribed spacer and chloroplast markers had previously failed to do so. Divergence time estimation and biogeographic analyses over the posterior distribution of dated trees suggest that a widespread ancestor was distributed across the Northern Hemisphere some 7–17 million years ago. Asian species form a sister group to a clade in which the European species are sister to the North American species. We use climatic niche modeling and divergence time estimates to explore the evolution of climate variables in the group. Principal component analyses help to identify instances of convergence, especially between distantly related species in the Mediterranean basin and in the chaparral of California. We document several cases of significant divergence between sister species in eastern North America and western North America. Climatic models were projected from one continent into the others (e.g., North American species projected into Asia and Europe) to examine whether species living in these areas occupy similar climates. This study demonstrates the utility of combining niche modeling with historical biogeographic analyses and documents significant climatic niche evolution within a group of species distributed throughout the Northern Hemisphere. These results suggest a possible model for the origin of the Madrean–Tethyan disjunction pattern. [Biogeography; *Caprifoliaceae*; *Caprifolium*; disjunction; *Lonicera*; Madrean–Tethyan; niche modeling; phylogeny.]

Patterns of distribution around the Northern Hemisphere have been of particular interest to biogeographers as they include clades that are disjunct across large boundaries—in particular, between eastern North America and East Asia, and in some cases between western North America and Europe (i.e., Fritsch 1996; Wen 1999; Fritsch 2001; Hileman et al. 2001; Donoghue and Smith 2004). Many questions remain, however, as to the timing and the routes of movement between these areas. Two major paths for dispersion were available during the Tertiary: the Bering land bridge and North Atlantic land bridge (Tiffney 1985), but the viability of these routes for movement of particular clades of plants and animals was intermittent, fluctuating with changes in physical connectivity and climate (Wen 1999; Tiffney and Manchester 2001). Although the paleoclimate of these land bridges has been investigated, there is still uncertainty as to the conditions during various geologic periods. Furthermore, it is still unclear how various changes in land connectivity and climate influenced routes of movement of organisms with particular tolerances. In order to better understand the historical biogeography, and Northern Hemisphere disjunction in particular, we need to include information about the relevant organisms and the environments that they have occupied now and in the past.

Most previous studies on Northern Hemisphere plant biogeography (e.g., Wen 1999; Donoghue et al. 2001; Xiang and Soltis 2001; Milne and Abbott 2002; Donoghue and Smith 2004; Xiang et al. 2004; Winkworth and Donoghue 2005) have focused on mesic temperate plants; the biogeography of dry-adapted plants around the Northern Hemisphere is less well understood. Here,

we examine the *Lonicera* clade *Caprifolium* (*sensu* Smith 2009)—a clade of ca. 25 species distributed throughout the Northern Hemisphere. This group of honeysuckles is readily distinguished from the other clade of ca. 155 *Lonicera* species in having perfoliate leaves and flowers in sessile cymes forming terminal heads or spikes. Six of the *Caprifolium* species occupy xeric Mediterranean climates, three in western North America and three in Europe. Others are found in more mesic habitats in eastern Asia and eastern North America. Also, there are clear disjunct distributions of populations within a number of the *Caprifolium* species. Close examination of niche models in these cases can shed light on speciation mechanisms within the clade.

Based on previous dating analyses (Bell and Donoghue 2005; Smith 2009), *Caprifolium* originated by the Miocene and diversified around the Northern Hemisphere throughout the Neogene (23 million years ago [ma] to present). During the Neogene, geological and climatic fluctuations were frequent around the Northern Hemisphere (Graham 1999; Tiffney and Manchester 2001). Geologic events that may have played a role in diversification include the continued uplift of the Rocky Mountains and erosion of the Appalachian Mountains (Graham 1999). Mountain ranges in Europe, formed during the Paleogene, continued to rise during this time (Thompson 2005). From the Paleogene to the Neogene, there was a general cooling trend (Graham 1999; Tiffney and Manchester 2001; Thompson 2005), and drying continued from the Miocene on, creating more seasonal climates (Tiffney and Manchester 2001). Forest composition also changed throughout the Neogene—tropical elements receded and more

temperate elements, adapted to seasonal cold, spread (Graham 1999; Fine and Ree 2006). Given these changes in climate throughout the Neogene in the Northern Hemisphere, we need to understand the evolution of climate niche variables within the clades that occupied these regions in order to fully understand their geographic evolution. The *Lonicera* clade *Caprifolium* presents an excellent opportunity to explore the integration of phylogeny, biogeography, and the modeling of climatic niches.

In addition to the drying, especially in western North America, Mediterranean climates, characterized by dry and hot summers, were established in what is now the chaparral region of the California Floristic Province and around the Mediterranean basin of Europe (Axelrod 1975; Thompson 2005). In some plant clades, closely related extant species occur in these two disjunct areas, some with distinctive adaptations to the new climate, which may have evolved in situ or may have already evolved within their lineages to accommodate drier climates (Ackerly 2004). This so-called Madrean–Tethyan pattern has long puzzled biogeographers. Many hypotheses have been proposed for the cause of disjunctions between the chaparral and Mediterranean basin of Europe. Europe and North America were connected by a land bridge by as late as the late Eocene (see Tiffney 1985), and Axelrod (1975) suggested the existence of a continuous vegetation of dry-adapted plants spanning the Atlantic. This would require disjunct species between these two areas to have diverged at least by the late Eocene. However, several studies show younger divergences between these disjunct taxa (e.g., Fritsch 1996; Hileman et al. 2001; Coleman et al. 2003). Other hypotheses for these distributions include long-distance dispersal or convergent evolution to the specific climate (e.g., Wolfe 1975). However, neither the origin of the geographic distributions nor the evolution into drier climate are well understood.

Phylogenetic biogeographic analyses that infer ancestral geographic ranges provide a means of tracing the evolution of geography and ecological tolerances (e.g., Ronquist 1997; Ree et al. 2005). Recently, species distribution models, or niche models, have been recognized as a powerful tool for understanding the evolution and ecology of species (Kozak et al. 2008 and references within). More than traditional approaches, niche modeling allows for the incorporation of climate data—past, present, and future. Integrating such analyses with phylogenetics, we can employ continuous character analyses (i.e., Graham et al. 2004; Yesson and Culham 2006a, 2006b; Evans et al. 2009) to help elucidate the evolution of climate niche variables and to identify cases of the convergent and divergent evolution of these variables. It is important to recognize that studying climatic niche variables is not the same thing as studying niches in general (niches have many more dimensions), nor is it sufficient to fully understand climate tolerances. Nevertheless, analyzing these climatic variables can shed some light on the nature and evolution of species niches and climate tolerances.

In this study we present a comprehensive biogeographic study of *Caprifolium*, with special emphasis on the evolution of the climatic niche. We explore the large-scale biogeographic evolution of the clade, integrating over divergence time uncertainty with parametric biogeographic reconstruction methods. We reconstruct ancestral niche conditions through time, allowing us to examine the extent to which sister species (and disjunct populations within species) have diverged in niche space and the extent to which species in different regions have converged. These results help us understand a Northern Hemisphere radiation that has involved major biome shifts, and they support a revised model for the origin of the Madrean–Tethyan disjunction pattern.

## MATERIAL AND METHODS

### *Sampling and Sequencing*

*Lonicera* (Caprifoliaceae, Dipsacales) contains about 180 species (Rehder 1903; Hara 1983) and is traditionally separated into two subgenera: *Lonicera* (with ca. 155 species) and *Caprifolium* (with ca. 25 species). Both subgenera are distributed around the Northern Hemisphere and are well supported in phylogenetic analyses (Theis et al. 2008; Smith 2009). Here, we focus on the smaller clade, *Caprifolium*. The most comprehensive previous studies (Theis et al. 2008; Smith 2009) found few well-supported relationships within this group. We use previously published internal transcribed spacer (ITS) and chloroplast *trnS-trnG* sequences (Smith 2009) and have added sequences of the nuclear gene *LEAFY* (*LFY*; Howarth and Baum 2005; Kim et al. 2008) second intron (Table 1). Genomic DNA was isolated from silica dried leaf tissue or herbarium samples using the Qiagen DNeasy Plant Kit (La Jolla, CA). Primers and polymerase chain reaction (PCR) protocols were modified from those developed by Howarth and Baum (2005) (LoniINTFSH: 5'-CTCCAAATTAGGGTGATAGTATAAG-3' and Lfy-CaprF1a:5'-CCAAGTTCAGAATATTGCCAAG-GAGA-GAGG-3'). Amplification used the following protocol: 94 ° 5 min; 94 ° 45 s; 54 ° 1 min; 72 ° 2 min; repeat 39 cycles. PCR products were cloned using the Invitrogen TOPO kit (Carlsbad, CA). At least eight colonies were picked for each PCR product, grown in Lysogeny broth medium, and plasmids were isolated and cleaned using the Qiagen Mini-Prep Kit (La Jolla, CA). Automated sequencing reactions, using the PCR primers, used the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit and were analyzed using an ABI 3730 Genetic Analyzer. Consensus sequences were then made from each successfully sequenced clone for each specimen. Our sampling of 19 species of the 25 represented all of the major biogeographic regions and ecological zones occupied by these plants. *Lonicera morrowii*, from *Lonicera* subgenus *Lonicera*, was included as a representative of the sister group of *Caprifolium* (Theis et al. 2008; Smith 2009).

TABLE 1. *Lonicera* species and voucher information

Species	Source	Voucher	LFY	ITS	trnS-trnG
<i>L. albiflora</i>	HUH	Charles H. Perino 1441		FJ217862	FJ217893
<i>L. albiflora</i>	TX	Turner 21-774	GU269257		
<i>L. arizonica</i>	YH	S. A. Smith 152	GU269260	FJ217881	FJ217934
<i>L. caprifolium</i>	YH	N. Cellinese 1117	GU269250		FJ217891
<i>L. carnosifolia</i>	HUH	J. Li 3303	GU269252		EU265341
<i>L. ciliosa</i>	NY	Bernard Boivin 13604	GU269263		FJ217907
<i>L. dioica</i>	YH	S. A. Smith 153	GU269259	GU269247	FJ217892
<i>L. etrusca</i>	ED	19871161	GU269256	FJ217842	FJ217899
<i>L. flava</i>	GA	Coile 4019		FJ217875	FJ217943
<i>L. hirsuta</i>	NY	C.E. Garton 8533	GU269262	FJ217821	FJ217913
<i>L. hispidula</i>	RSA	8523	GU269264	FJ217855	FJ217889
<i>L. implexa</i>	NY	S. Castroviejo 14-VI-1975		FJ217861	FJ217897
<i>L. implexa</i>	ED	19801440	GU269255		
<i>L. interrupta</i>	NY	Karen Reichhardt 90-01	GU269266		FJ217909
<i>L. morrowii</i>	AR	525-84A	GU269249	FJ217859	FJ217917
<i>L. periclymenum</i>	ED	19980453	GU269254	FJ217825	FJ217928
<i>L. pilosa</i>	MI	Medina & Barrios 2576		GU269248	FJ217900
<i>L. reticulata</i>	UMO	Kennedy PK99-30	GU269258		
<i>L. sempervirens</i>	NY	Thomas J. Rawinski 8528	GU269261	FJ217845	FJ217886
<i>L. stabiana</i>	YH	N. Cellinese 1105	GU269253		
<i>L. subspicata</i>	RSA	11744	GU269265	FJ217843	FJ217915
<i>L. tragophylla</i>	HUH	J. Li 3066	GU269251	FJ217874	EU265377

Note: Source refers to the herbarium where the voucher specimen is located. For herbarium specimens, collector and collection number are given in the voucher column. For living plants (sources AR, ED, and RSA), accession numbers are given.

### Phylogenetic Analyses

Sequences for each of the three gene regions (ITS, *trnS-trnG*, and *LFY*) were aligned using MUSCLE with default parameters (Edgar 2004). Large indel regions of *LFY* within *Lonicera* were found to largely be in single species and therefore not informative. To minimize large indel regions for phylogenetic analyses and to increase repeatability of the phylogenetic analyses, sites with at least 50% missing data were trimmed and the sequences were concatenated using Phyutility (Smith and Dunn 2008). The posterior distribution of phylogenetic trees was explored using the Metropolis-coupled Markov chain Monte Carlo (MC<sup>3</sup>) algorithm as implemented in MrBayes (ver. 3.1.2; Ronquist and Huelsenbeck 2003; Altekar et al. 2004). The three gene regions were run under GTR+I+G model of substitution, with all three regions unlinked and partitioned. We replicated the MrBayes analysis three times to verify convergence to the same posterior distribution. Convergence to the same posterior distribution was determined by examining trace plots of each parameter in Tracer (ver. 1.4; Rambaut and Drummond, 2007). In each analysis, we ran four chains for 10<sup>7</sup> generations, sampling every 10<sup>3</sup> steps. Consensus trees were calculated after removing burn-in as determined by examining effective sample size, which were all at least 1500, and trace plots in Tracer (ver. 1.4; Rambaut and Drummond, 2007). Because relationships within the North American clade were poorly resolved when including *L. flava* and *L. pilosa*, we ran a maximum-likelihood analysis with RAxML (ver. 7.0.4; Stamatakis 2006) to produce the best-resolved phylogeny for the principal components and analysis of variance ANOVA comparisons that included these species (Figure S4, available from <http://www.sysbio.oxfordjournals.org/>). All

three gene regions were partitioned and the GTR+I+G model of substitution was used for each. First, 100 rapid bootstraps were conducted, then a final maximum likelihood search was conducted using 10 random bootstrap topologies as starting trees. All alignments and trees are available in TreeBASE under the freebase study number S2611.

### Divergence-Time Analysis

Divergence-time analyses were conducted with the Bayesian method implemented in BEAST (ver. 1.4.7, Drummond and Rambaut 2007) in order to accommodate uncertainty in the placement of the fossils and topology. Gene regions were parameterized with the GTR+I+G model of substitution; however, because of failure to converge due to missing data in some species, the substitution model was linked across gene regions. We employed the uncorrelated log-normal model of rate evolution, which does not require rates to be heritable (Drummond et al. 2006). This may be essential when accommodating for rate changes through the tree and can help lessen the effects of rate heterogeneity correlated with differences in life history (Smith and Donoghue 2008). However, there are no clear life-history differences within *Caprifoliaceae*—all are perennial woody vines. In order to estimate divergence times, we used the previously published posterior divergence time estimate for the clade (Smith 2009), setting the root of the tree to be normally distributed between 7 and 17 ma. This previous analysis considered the entire *Caprifoliaceae*, including *Lonicera*, and multiple fossil calibrations were used in that analysis within *Lonicera* subgenus *Lonicera*. The previous study also found that meaningful biogeographic analyses could be conducted despite large confidence intervals. Although

the estimate used here is based on previous analyses, *Caprifolium* lacks known fossils, making this the best available information. BEAST analyses were run for  $20^7$  generations, sampling every  $10^4$  steps. We replicated the analyses 4 times verifying convergence to the same posterior distribution by examining the posterior distribution of parameters in Tracer (ver. 1.4; Rambaut and Drummond 2007).

#### Biogeographic Analysis

In order to determine the broad scale geographic evolution of the clade, we conducted parametric likelihood analyses using Lagrange (Ree and Smith 2008). Lagrange implements the likelihood-based geographic range reconstruction method using biogeographic speciation scenarios and parameters for dispersal and extinction (Ree et al. 2005). We considered species to be distributed within 4 broad areas: eastern North America (eNA), western North America (wNA), Europe (Eu), and Asia (As). Previous analyses of Northern Hemisphere biogeography using the parametric method for ancestral range reconstruction have employed area connectivity scenarios (Ree et al. 2005). Because *Caprifolium* species generally appear to have different climatic tolerances than the species examined by Ree et al. (2005), the availability of movement corridors around the Northern Hemisphere (e.g., North Atlantic land bridge, Beringia) is less well understood. Therefore, we did not employ geological constraints. We ran these analyses by sampling 1800 trees from the posterior distribution of dated trees generated from the BEAST analyses (Smith 2009).

#### Niche Modeling and Projections

We used ecological niche modeling to estimate the climatic niches of species within *Caprifolium*. For environmental layers, the 19 "Bioclim" variables developed by Hijmans et al. (2005, <http://www.worldclim.org>) were used; these summarize temperature and precipitation dimensions (often monthly) of the environment into biologically relevant layers and have been used in previous evolutionary studies using niche models (Graham et al. 2004; Kozak and Weins 2006; Evans et al. 2009). We used all 19 of these variables and a grid size of 30 arc-seconds that corresponds to  $\sim 1 \text{ km}^2$  at the equator.

Locality data for *Caprifolium* species is variable. A variety of sources were used to collect localities, including labels on herbarium specimens (on loan from NY, HUH, MO, LL, FLAS, and PE), relevant herbarium databases (ARIZ, UNM, and WTU), and other databases (CONABIO, Consortium of California Herbaria, and GBIF). The final number of points per species ranged from 10 (*L. carnosifolia*) to  $>42,238$  (*L. periclymenum*), with an average (excluding *L. periclymenum*) of 270 localities for each species (Table S1). These locality data can be obtained from Supplementary Material. The number of recorded localities for *L. carnosifolia* is limited in part due to its relatively recent description (Hsu and Wang 1979). As in Evans et al. (2009), we

sampled within the known localities (China; Jinfushan, Nanchuan, Chongqiong and Xianyi, Chengkou) for *L. carnosifolia* until a minimum of 10 points was reached.

We estimated niche models with the maximum entropy method, as implemented in Maxent (ver. 3.2.1, Phillips et al. 2006). This works with presence data and has performed well in recent comparisons of methods (Araujo and Rahbek 2006; Elith et al. 2006). It also performs relatively well with small samples (down to as few as 10 points; Hernandez et al. 2006; Pearson et al. 2007), which is important for at least 2 of the more narrowly distributed species: *L. stabiana* and *L. carnosifolia*. To build niche models we used all available points and to evaluate the quality of the model, we partitioned the locality data into training and testing data sets (75% vs. 25%, respectively). To measure the degree to which the models produced differed from random, we used the AUC, the area under the receiving operating characteristic curve (but see Lobo et al. 2008; Table S1). We also projected predictions into other continental regions to evaluate the extent to which species occupy similar niches in disjunct areas. We create a model with Maxent but instead of visualizing the model on the area where the species is currently distributed, we project the model onto disjunct areas for visualization.

#### Climate Reconstructions

A variety of approaches have been used to reconstruct climate niches on phylogenies. Some have used the results from niche modeling methods (e.g., Graham et al. 2004; Yesson and Culham 2006a, 2006b), whereas others have extracted climate data from localities (Evans et al. 2005; Ackerly et al. 2006; Knouft et al. 2006; Stockman and Bond 2007). Here, we use both, emphasizing the use of all the data as in Evans et al. (2009). Specifically, whenever possible, we attempt to adequately represent the distribution of data and not just mean values.

To examine ancestral climate evolution in *Caprifolium*, we reconstructed maximum-likelihood ancestral states for the continuous characters over the posterior distribution of dated trees (Schluter et al. 1997; Knouft et al. 2006). We sampled from the probability distribution functions produced by combining the niche occupancy profile from Maxent with each Bioclim layer for the extant taxa (this procedure is described in detail in Evans et al. 2009). In other words, we combine the suitability profile created by Maxent with individual Bioclim layers to produce a probability distribution for each Bioclim layer. We sample from this distribution for the data that are reconstructed. This method reconstructs the distribution of climatic values for each Bioclim variable, instead of reconstructing the mean tolerances (in contrast to e.g., Graham et al. 2004; Ackerly et al. 2006; Yesson and Culham 2006a, 2006b). That is, unlike the methods developed recently by Felsenstein (2008) and Ives et al. (2007), we are reconstructing the distributions of values as opposed to single values. This should better

represent extant and ancestral climatic niches, especially as many species have niches that do not approximate a normal distribution (i.e., are multimodal), making the mean and the variance poor summaries. We calculated ancestral values on the posterior distribution of dated trees thus taking into account uncertainty in the phylogeny and in divergence times. This code is open source and available at <http://code.google.com/p/pebls>.

### *Niche Comparisons*

Niche overlap was quantified by comparing predicted climate occupancy profiles using the method described in Evans et al. (2009, see equation and Feinsinger et al. 1981) as well as with Schoener's *D* (1968) and the *I* statistic described by Warren et al. (2008). The measure described in Evans et al. (2009) differs from the *D* and *I* statistics by using the Bioclim values individually after the creation of the niche model, whereas the *D* and *I* statistics use only the niche model itself for comparisons. For the comparisons similar to those of Evans et al. (2009), we examined differences over multiple Bioclim variables and added the absolute differences of each variable. Bioclim variables (or any climate variables) are not completely independent, and therefore, by adding absolute differences between species for each variable, values may tend to be inflated for similar species and deflated for dissimilar species. Here we do not emphasize the values themselves, instead, we note the most similar and least similar species.

We also compared niches as described by the primary axes obtained from principal component analyses (PCAs) of extracted data. Instead of using information extracted from the Maxent analyses, we extracted the climate data for the specimen localities directly from the Bioclim layers. We then conduct PCAs of these data and compared these results to sister species. The species included in the PCAs were based on the majority rule consensus tree produced from the MrBayes analyses. Values for all 19 bioclim variables were included in each PCA. Differences between sister species were compared using an ANOVA of first and second principal components (Graham et al. 2004).

The PCAs and subsequent tests provide a detailed description of the differences between the climate spaces occupied by the species. They use only the occurrence data and so are not subject to all of the same criticisms as analyses relying on the niche models. However, they also do not take advantage of the recent advances in testing of niche equivalency and niche similarity as described by Warren et al. (2008). Therefore, in addition to the descriptive measures of the PCAs described above, 2 additional tests were conducted to measure niche equivalency and similarity of species that make use of the niche models. The first test, niche equivalency, is meant to test whether the climatic niches are equivalent (Warren et al. 2008). This test was conducted on all species pairs. The second test, niche similarity, is meant to test whether the niche of one species is more or

less similar to another using a null based on study-specific criteria (Warren et al. 2008). This second test is less conservative than the equivalency test, though the background environment on which to test the species' niches can be hard to define (Warren et al. 2008). Because this test is more relevant for understanding speciation, it was conducted only on sister species or using very closely related species.

In order to conduct these tests, measures of the overlap of the predicted niche models are required. These measures, Schoener's *D* (Schoener, 1968) and the *I* statistic of Warren et al. (2008), were calculated on niche models created with 10 km<sup>2</sup> Bioclim layers. The use of less resolute layers (10 km<sup>2</sup> as opposed to 1 km<sup>2</sup>) was necessary because of the time required to conduct replicates for the aforementioned tests. The niche equivalency and niche similarity tests were performed as described in Warren et al. (2008). For the equivalency tests, 100 pseudoreplicate data sets were created from the pooled occurrence data from a pair of species. Niche models were estimated with these new data sets and the *D* and *I* statistics were calculated on these niche models to create a null distribution for comparison with the *D* and *I* scores calculated from the real data. For the similarity tests, comparisons are made by creating niche models based on random background cells chosen from the area available to the two sister species. This was done 100 times and this null distribution of *D* and *I* scores was compared with those from the actual data. The choice of background area available to the sister species can be important and difficult (Warren et al. 2008). For these analyses, we assume that a bounding box encompassing the area in which any samples were found represented the available background environmental space.

### *Evaluating Disjunctions Within Species*

A number of *Caprifolium* species have intraspecific disjunct distributions (e.g., *L. albiflora*, *L. subspicata*, *L. interrupta*, *L. flava*, *L. pilosa*, and *L. ciliosa*). In order to determine the extent to which climate variables differ between disjunct populations within these species, we conducted PCA and ANOVA comparisons between populations within *L. ciliosa*, *L. flava*, *L. hirsuta*, and *L. interrupta*, the species with the most distinct disjunct distribution patterns. We also compared niches with the *D* and *I* statistics described in Warren et al. (2008) using the procedure described above for sister species. The background environment available to a species was considered to be the bounding box encompassing all observations of the disjunct population. We also conducted niche reconstructions using each disjunct population to determine whether these would project into the missing populations. Because Maxent results (and other presence-based methods) reflect observed climate variables (Phillips and Dudik 2008), niche modeling with disjunct populations can test whether disjunct ranges are different.

## RESULTS

## Phylogenetic Analyses

Sequencing of *LFY* was successful for all species examined except *L. flava* and *L. pilosa*. Typical fragments were ca. 1800 bases in length. The addition of *LFY* sequences to the ITS and cpDNA sequences provided the additional phylogenetic information needed to resolve previously unresolved nodes (Fig. 1). Because *LFY* could not be sequenced from *L. flava* or *L. pilosa*, all analyses were conducted with and without these species. In all analyses (including those with *L. flava* and *L. pilosa*), there was good support for an Asian clade, a European clade, a North American clade, the European clade sister to the North American clade, and within a Californian “chapparal” clade. Analyses supported the placement of both *L. pilosa* and *L. flava* within the North American clade. Relationships remained poorly resolved within the North American clade, exclusive of a well-supported chapparal clade. Relationships were weakly supported within the European clade as well.

## Divergence-Time Estimation

The prior used to date the phylogeny was based on previous molecular dating analyses on Caprifolieae (Smith 2009). Those analyses supported the monophyly of *Caprifolium* but did not confidently resolve relationships within this clade. Based on this prior (normally distributed between 7 and 17 ma), the European clade of *Caprifolium* originated between 4 and 11 ma (95% highest probability density [HPD]), and the North American clade between 5 and 13 ma (95% HPD). The Mediterranean species originated from 0.3 to 3 ma (95% HPD) and the chaparral species in California originated

some 3–9 ma (95% HPD). The covariance statistic, which measures the autocorrelation of rates throughout the tree, was found to be  $-0.00722$  (95% HPD of  $-0.353, 0.352$ ). Although this indicates that there is little autocorrelation of rates, the utility of this statistic for this measurement has not yet been explored. The coefficient of variation, which measures the proportion of rate variation around the mean was 0.45 (95% HPD 0.273, 0.65), indicating extensive rate heterogeneity through the tree.

## Biogeographic Analyses

Biogeographic reconstructions for 8 major nodes are presented in Figure 2 (see Fig. S1 for results with *L. flava* and *L. pilosa* included). The root was found to be either widespread across all areas or widespread across all areas excluding eastern North America. The data set excluding *L. pilosa* and *L. flava* favored a widespread ancestor more frequently, but some reconstructions excluded eastern North America. The data set including *L. pilosa* and *L. flava* also found both scenarios, but the most frequent reconstruction excluded eastern North America. The ancestor of the crown of the European clade was reconstructed to Europe in both data sets. The ancestor of the crown North American clade was inferred to have been spread across North America in both data sets; an ancestor in both North America and Europe, or just in western North America was recovered with less frequency. The ancestor of the North America plus Europe clade was reconstructed as being in both North America and Europe in both data sets, but with less frequency in the tree excluding *L. pilosa* and *L. flava*, which also yields different speciation scenarios. Specific speciation scenarios (i.e., within-area vs. between-area speciation) are displayed in Figure 2. Note that there

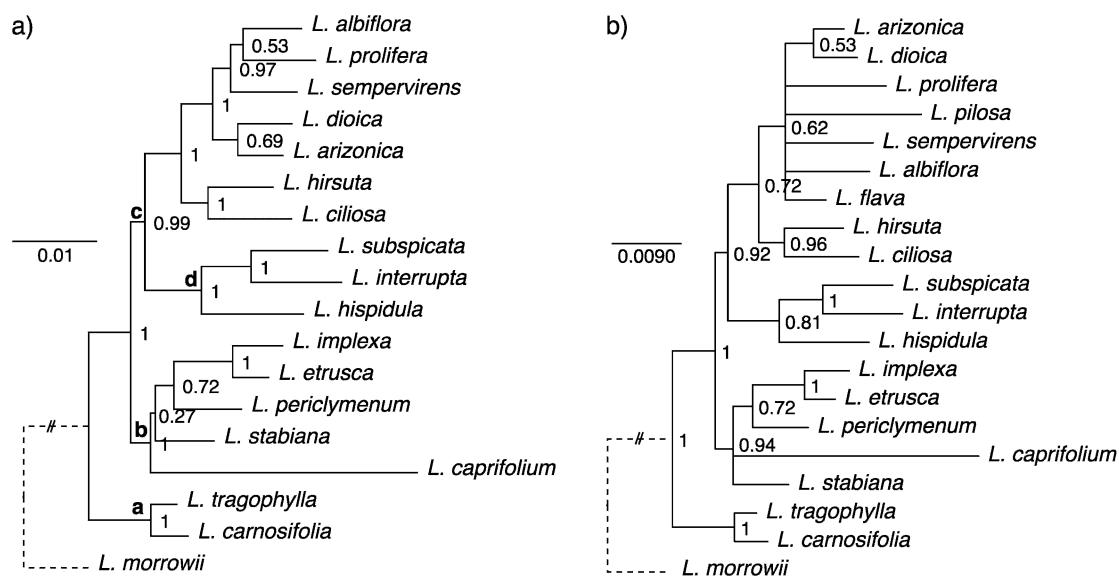


FIGURE 1. a) Bayesian consensus tree showing all compatible groups. Major clades are labeled: Asian **a**, European **b**, North American **c**, and chaparral **d**. b) Majority rule (50%) consensus tree from Bayesian analysis including *L. flava* and *L. pilosa*. The dashed line indicates the connection to the outgroup, *L. morrowii*. Numbers associated with clades are Bayesian posterior probabilities.

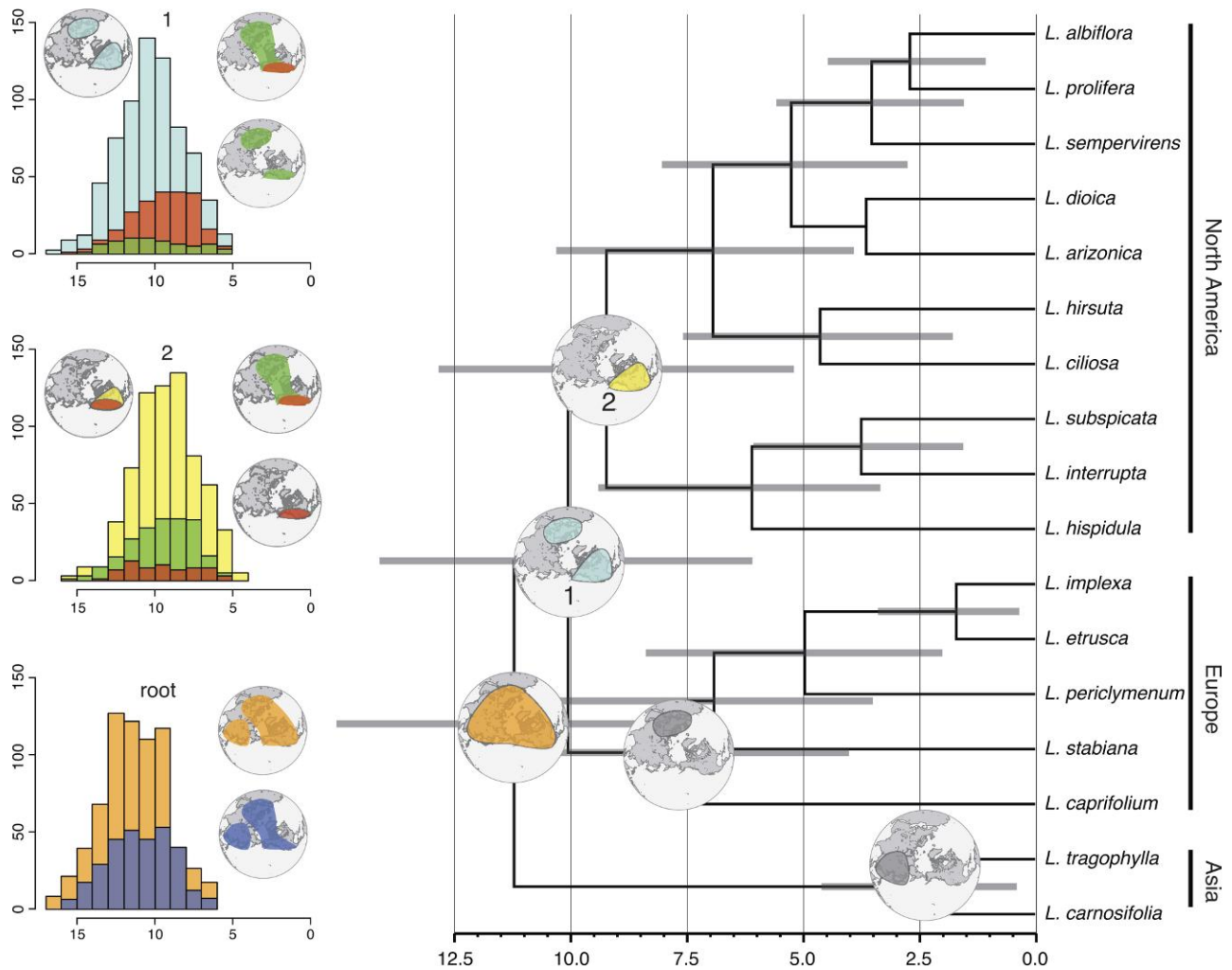


FIGURE 2. Historical biogeography of the *Caprifolium* clade of *Lonicera*. The phylogeny is the maximum credibility clade from the Bayesian divergence time analysis with *L. flava* and *L. pilosa* included. Inferred ancestral ranges for 5 major well-supported clades are shown. Speciation scenarios are shown in the histograms and associated maps. The x-axis on the phylogeny and in the histograms represents time in millions of years; histograms display variance in the inferred ancestral area as a function of time.

were no discernable differences between scenarios as a function of inferred divergence times.

#### Niche Modeling

AUC values for our models range from 0.968 to 1.000 (Table S1), which were similar to values reported previously (Elith et al. 2006; Hernandez et al. 2006; Evans et al. 2009). Climatic niche modeling documented the diversity of habitats occupied by *Caprifolium*. Weighted mean values for each species and Bioclim variable are presented in Tables 2 and 3. Environments of the North American species that occur the farthest west (*L. interrupta*, *L. hispidula*, and *L. subspicata*), primarily in chaparral areas, were characterized by temperatures ranging from 28–32 °C in the warmest month (Bioclim variable 5) to –0.4 to 1.9 °C in the coldest month (Bioclim variable 6). The annual precipitation for the same species ranged from 46.7 to 97.3 cm, again with high

variance. The precipitation for the warmest quarter (Bioclim variable 18) ranged from 2.7 to 7.2 cm and for the coldest quarter ranges from 23.4 to 46.1. The remaining western North American species occur from the Pacific Northwest (*L. ciliosa*), with a maximum temperature in the warmest month of 25 °C and in the coldest month of –3 °C, and precipitation of 116 cm, to the southwest and Mexico (*L. arizonica*, *L. albiflora*, and *L. pilosa*), with an average temperature in the warmest month of 28 °C and in the coldest month of –6 °C, and precipitation of 41–76 cm. The eastern North American species (*L. hirsuta*, *L. flava*, *L. sempervirens*, *L. dioica*, and *L. prolifera*) occupied temperatures ranging from 26 to 32 °C in the warmest month to –2.4 to –15 °C in the coldest month. The annual precipitation for these species ranged from 85 to 123 cm.

The European species (*L. caprifolium*, *L. etrusca*, *L. implexa*, *L. periclymenum*, and *L. stabiana*) ranged from central and northern European environments to the

TABLE 2. Weighted mean values of North American *Caprifolium* species for the 19 Bioclim variables given the predicted niche models (T = temperature in °C; P = precipitation in cm)

	North America											
	<i>albiflora</i>	<i>arizonica</i>	<i>ciliosa</i>	<i>dioica</i>	<i>flava</i>	<i>hirsuta</i>	<i>pilosa</i>	<i>prolifera</i>	<i>sempervirens</i>	<i>hispidula</i>	Chaparral <i>interrupta</i>	<i>subspicata</i>
1. Mean annual T	15.4	9.7	8.2	8.5	13.9	5.5	16.7	10.2	14.9	12.2	13.8	14.7
2. Mean diurnal range <sup>ma</sup>	16.1	15.8	12.5	12.1	13.1	11.5	15.7	11.9	12.5	13.2	15.3	14.6
3. Isothermality <sup>b</sup>	4.8	4.6	4.2	3.0	3.6	2.7	6.1	3.0	3.7	4.9	4.7	5.0
4. T seasonality <sup>c</sup>	6.2	6.7	6.0	9.8	8.3	10.3	3.2	9.7	7.7	5.0	6.2	5.2
5. Max T warmest month	31.9	28.1	25.7	28.4	31.8	26.0	29.1	29.7	31.5	28.0	32.0	31.0
6. Min T coldest month	-1.3	-6.4	-3.9	-11.8	-4.6	-15.5	3.4	-9.8	-2.4	0.8	-0.4	1.9
7. Annual range T <sup>d</sup>	33.2	34.5	29.7	40.2	36.4	41.5	25.7	39.5	33.9	27.1	32.5	29.1
8. Mean T wettest <sup>a,d</sup>	22.2	14.3	1.6	17.2	12.8	16.2	19.4	19.1	17.7	6.8	11.2	9.2
9. Mean T driest <sup>a,d</sup>	13.3	11.7	15.8	-2.2	10.1	-7.3	14.6	-1.9	12.2	18.3	19.4	20.1
10. Mean T warmest <sup>a,d</sup>	23.2	18.4	16.1	20.6	24.3	18.2	20.4	22.2	24.4	18.8	21.9	21.6
11. Mean T coldest <sup>a,d</sup>	7.2	1.4	1.0	-4.8	2.8	-8.5	12.2	-2.9	4.5	6.4	6.4	8.5
12. Annual P	41.4	54.9	115.8	91.5	122.5	85.1	75.8	95.9	122.9	97.3	65.5	46.7
13. P wettest month	8.2	9.2	18.9	10.8	13.7	10.1	16.8	11.1	13.7	17.6	12.2	9.0
14. P driest month	0.8	1.3	1.9	4.5	6.6	3.9	0.9	4.2	7.0	0.7	0.5	0.2
15. P seasonality <sup>e</sup>	6.8	5.2	5.8	3.0	2.0	3.0	8.7	2.9	1.9	7.6	7.3	8.1
16. P wettest <sup>a,d</sup>	20.4	23.4	53.8	30.1	37.2	28.5	43.2	31.5	37.7	48.8	32.3	24.5
17. P driest <sup>a,d</sup>	3.4	5.5	8.4	15.4	23.1	13.6	3.8	14.9	23.9	3.8	2.6	1.2
18. P warmest <sup>a,d</sup>	17.2	18.5	9.2	28.1	30.7	27.6	30.2	29.7	32.9	4.7	7.2	2.6
19. P coldest <sup>a,d</sup>	6.7	13.9	50.0	16.4	27.7	14.3	6.4	15.3	29.1	46.2	29.0	23.4

<sup>a</sup>Mean of the difference between monthly maximum temperature and minimum temperature.

<sup>b</sup>(Bioclim 2/Bioclim 7)\*100.

<sup>c</sup>The standard deviation of monthly mean temperature multiplied by 100.

<sup>d</sup>Bioclim5-Bioclim6

<sup>e</sup>The coefficient of variation of monthly mean precipitation.

Mediterranean. The Mediterranean basin species (*L. etrusca*, *L. implexa*, and *L. stabiana*) occurred in temperatures ranging from 28 to 31 °C in the warmest month to 2 ° to 3 °C in the coldest month. The annual precipitation for the same species ranged from 60 to

69 cm. The central European species (*L. caprifolium* and *L. periclymenum*) grow in temperatures 21 to 22 °C in the warmest month to -2 to -1 °C in the coldest month. The annual precipitation for the same species ranged from 73 to 87 cm. The 2 Asian species represented here

TABLE 3. Weighted mean values of European and Asian *Caprifolium* species for the 19 Bioclim variables given the predicted niche models (T = temperature in °C; P = precipitation in cm)

	Europe					Asia	
	Mediterranean basin					<i>carosifolia</i>	<i>tragophylla</i>
	<i>caprifolium</i>	<i>periclymenum</i>	<i>etrusca</i>	<i>implexa</i>	<i>stabiana</i>		
1. Mean annual T	8.4	8.9	14.2	15.0	13.7	14.5	12.9
2. Mean diurnal range <sup>a</sup>	7.6	7.7	9.8	10.3	7.5	7.3	9.7
3. Isothermality <sup>b</sup>	3.2	3.3	3.6	3.7	3.2	2.6	2.9
4. T seasonality <sup>c</sup>	5.9	5.5	6.0	5.9	5.9	7.2	8.4
5. Max T warmest month	21.5	21.5	29.1	30.5	26.9	28.7	29.1
6. Min T coldest month	-2.5	-1.4	2.3	3.1	2.9	0.6	-4.4
7. Annual range T <sup>d</sup>	24.0	22.8	26.9	27.4	24.0	28.1	33.5
8. Mean T wettest <sup>a,d</sup>	10.8	9.9	10.0	10.4	10.9	22.1	21.9
9. Mean T driest <sup>a,d</sup>	5.0	7.4	20.3	22.2	21.0	5.4	2.1
10. Mean T warmest <sup>a,d</sup>	16.0	16.0	22.0	22.8	21.5	23.5	23.3
11. Mean T coldest <sup>a,d</sup>	1.0	2.0	6.7	7.8	6.5	4.8	1.6
12. Annual P	73.2	87.4	65.5	60.3	69.0	115.9	91.5
13. P wettest month	8.1	9.8	10.1	8.8	10.0	20.2	17.1
14. P driest month	4.3	5.0	1.5	1.0	2.3	2.0	1.6
15. P seasonality <sup>e</sup>	2.0	2.0	5.2	5.1	4.1	6.5	7.1
16. P wettest <sup>a,d</sup>	22.8	27.6	27.3	24.1	26.8	54.7	44.5
17. P driest <sup>a,d</sup>	13.9	16.4	6.2	4.7	8.2	6.9	6.0
18. P warmest <sup>a,d</sup>	19.5	21.3	7.0	5.6	10.5	51.0	41.6
19. P coldest <sup>a,d</sup>	17.7	22.6	24.5	21.6	22.1	7.2	6.5

<sup>a</sup>Mean of the difference between monthly maximum temperature and minimum temperature.

<sup>b</sup>(Bioclim 2/Bioclim 7)\*100.

<sup>c</sup>The standard deviation of monthly mean temperature multiplied by 100.

<sup>d</sup>Bioclim5-Bioclim6.

<sup>e</sup>The coefficient of variation of monthly mean precipitation.

(*L. carnosifolia* and *L. tragophylla*) were characterized by temperatures ranging from 28 to 29 °C in the warmest month to 0.5 to –4 °C in the coldest month, and annual precipitation from 92 to 116 cm.

Many species, especially those in North America, had multimodal distributions for many variables. This especially characterizes those species with disjunct geographic distributions: *L. albiflora*, *L. arizonica*, *L. ciliosa*, *L. flava*, *L. interrupta*, and *L. pilosa*. *Lonicera hispidula*, which occurs along the west coast of North America, had multimodal distributions for many variables but was not obviously disjunct in its geography.

#### Niche Projections

Niche projection results are presented in Figures 3 and 4 (Figs. S2 and S3). Many species predicted with relatively high probability into widely disjunct regions around the Northern Hemisphere. The North American species occurring in the chaparral region were projected with high probability mostly into the Mediterranean region of Europe, reflecting the climate similarities in the 2 regions. Three major regions of Asia were projected into by North American species: higher altitude areas surrounding the Himalayas, southern east Asia, and northern east Asia. *Lonicera albiflora*, *L. pilosa*, and *L. arizonica* projected into the Himalayan region with high probability, especially the drier and colder climate in those regions. *Lonicera dioica*, *L. hirsuta*, and *L. ciliosa* projected into a more northern east Asian region as well as central Europe. These projections highlighted the more intermediate dry and cold climate of the regions. *Lonicera sempervirens* projected into southern east Asia emphasizing the generally warmer and wetter environments. The other North American species projected with low probability variably throughout the Northern Hemisphere. European species tended to project into western North America, but farther north than typical chaparral ranges, with greater frequency than Asia. The Asian species *L. tragophylla* projected into central North America and central Europe with relatively high probabilities. Maxent employs “clamping,” which restricts the range of climate values used when projecting a species into an area outside of the current distribution to values found while training the data in the locations of the current distribution. Values that fall outside of the training range may have a large effect on the predicted suitability and are marked by Maxent. *Lonicera implexa*, *L. stabiana*, and *L. carnosifolia* all show high amounts of clamping. Areas with values falling outside of the empirical range (areas where clamping occurred) were considered suspect.

#### Ancestral Reconstructions

Ancestral reconstructions of climate variables are presented in Figure 5 and Figure S7 (for results with *L. pilosa* and *L. flava* included, see Figure S8). In all but a few reconstructions (for Bioclim variables 2, 5, 7, and 18), the North American species encompass the full

range of values, with ancestral values often intermediate between European and Asian values. In many of the reconstructions related to temperature (Bioclim variables 5, 6, 7, and 11), North American ancestral values are closer to Asian values despite the European clade being more closely related to the North American clade. This is even more prominent in the variables related to precipitation. Patterns of divergent evolution (within clades; Fig. 5, right panel), convergent evolution (among clades), and trait conservation (Fig. 5, left panel) are apparent throughout.

#### Niche Comparisons

*Niche overlap.*—Values for niche overlap between species are presented in Tables S2–S6. Niche overlap as calculated in Evans et al. (2009) across all Bioclim variables was generally equivalent across species. In these analyses, when all Bioclim variables were considered together, for the Asian species (*L. carnosifolia* and *L. tragophylla*), for those in Mediterranean environments (*L. etrusca* and *L. implexa* in Europe, and *L. interrupta*, *L. subspicata*, and *L. hispidula* in western North America), and for all the European species (*L. caprifolium*, *L. etrusca*, *L. implexa*, *L. periclymenum*, and *L. stabiana*), the most closely related species were also seen to be the closest in terms of niche overlap. In other cases, environmentally similar species occur within the same broad biogeographic region (e.g., North America or Europe), though these are not the most closely related species. Segregating the results by the Bioclim variables related to temperature (Bioclim variables 1–11) showed a slightly different pattern. The eastern North American *L. hirsuta* was the least overlapping species for 15 of the 19 species and is notable in experiencing the lowest temperatures overall (Bioclim 6) and the lowest temperatures in the driest quarter (Bioclim 9). Focusing just on these variables, the patterns of niche overlap mimic those of the overall comparison, but with *L. tragophylla*, an Asian species, being most similar to *L. sempervirens*, a southeastern US species. Segregating the results by variables related to precipitation (Bioclim variables 12–19), *L. albiflora* and *L. flava* were seen to be the most different from the other species. *Lonicera albiflora* of the desert Southwest is notable in experiencing the lowest precipitation, and *L. flava* of eastern North America shows the second highest precipitation after *L. sempervirens*. *Lonicera flava* also has a disjunct distribution between the southeastern US and central eastern US, and the precipitation values differ widely between these areas. The *D* and *I* statistics as calculated by Warren et al. (2008) produced results similar to those just described using the methods of (Evans et al. 2009; Tables S5 and S6).

*PCAs and niche equivalency.*—PCA plots are presented on the majority rule consensus tree in Figure 6 (Figure S4). These are not ancestral reconstructions; rather a PCA diagram at a node indicates that all species descended from that node were included in the analysis. The first

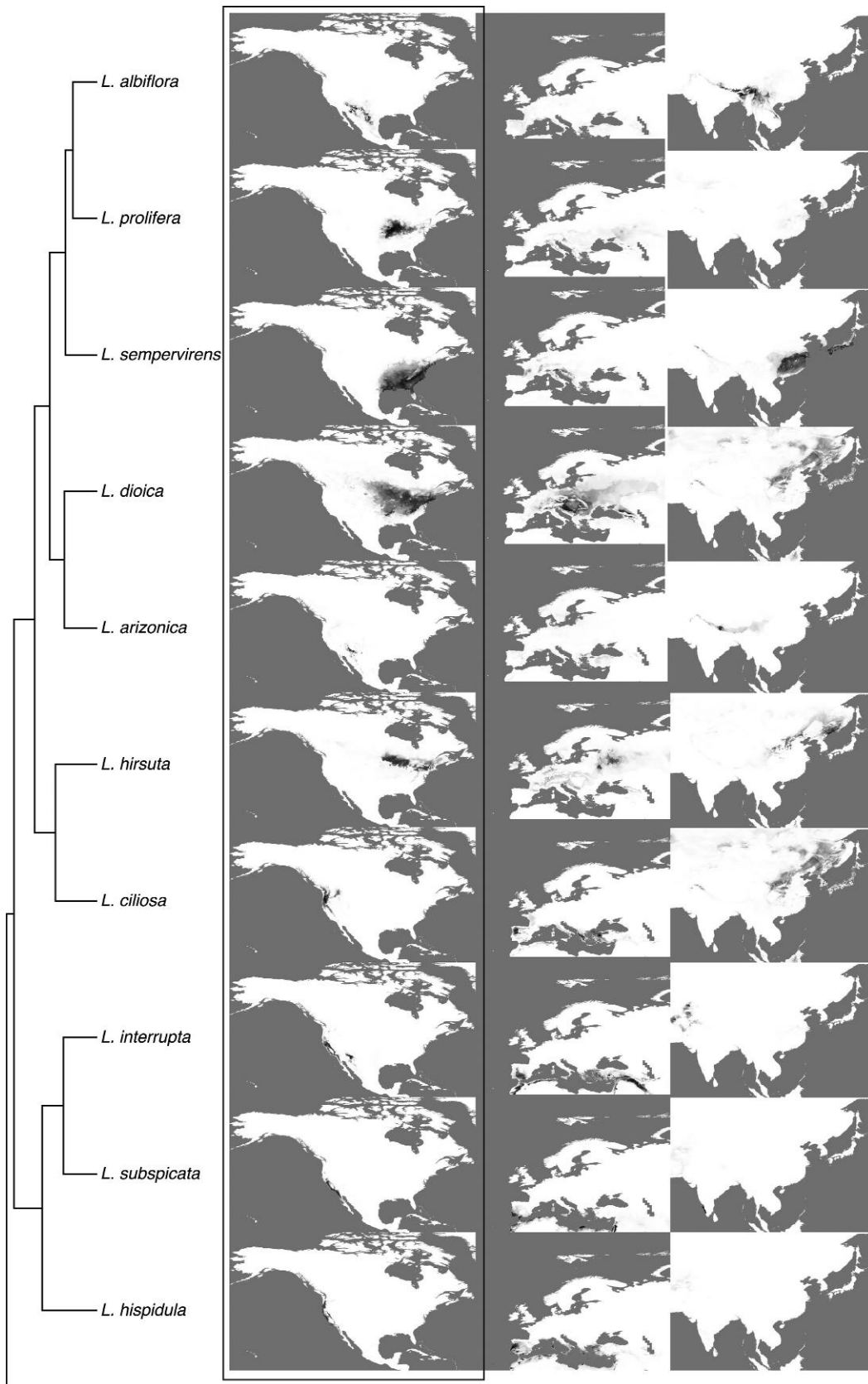


FIGURE 3. Niche models for North American species. The black box surrounds the continent where the species are currently distributed; the other projections represent areas in Europe and Asia with significantly similar climatological conditions to those produced in the niche model.

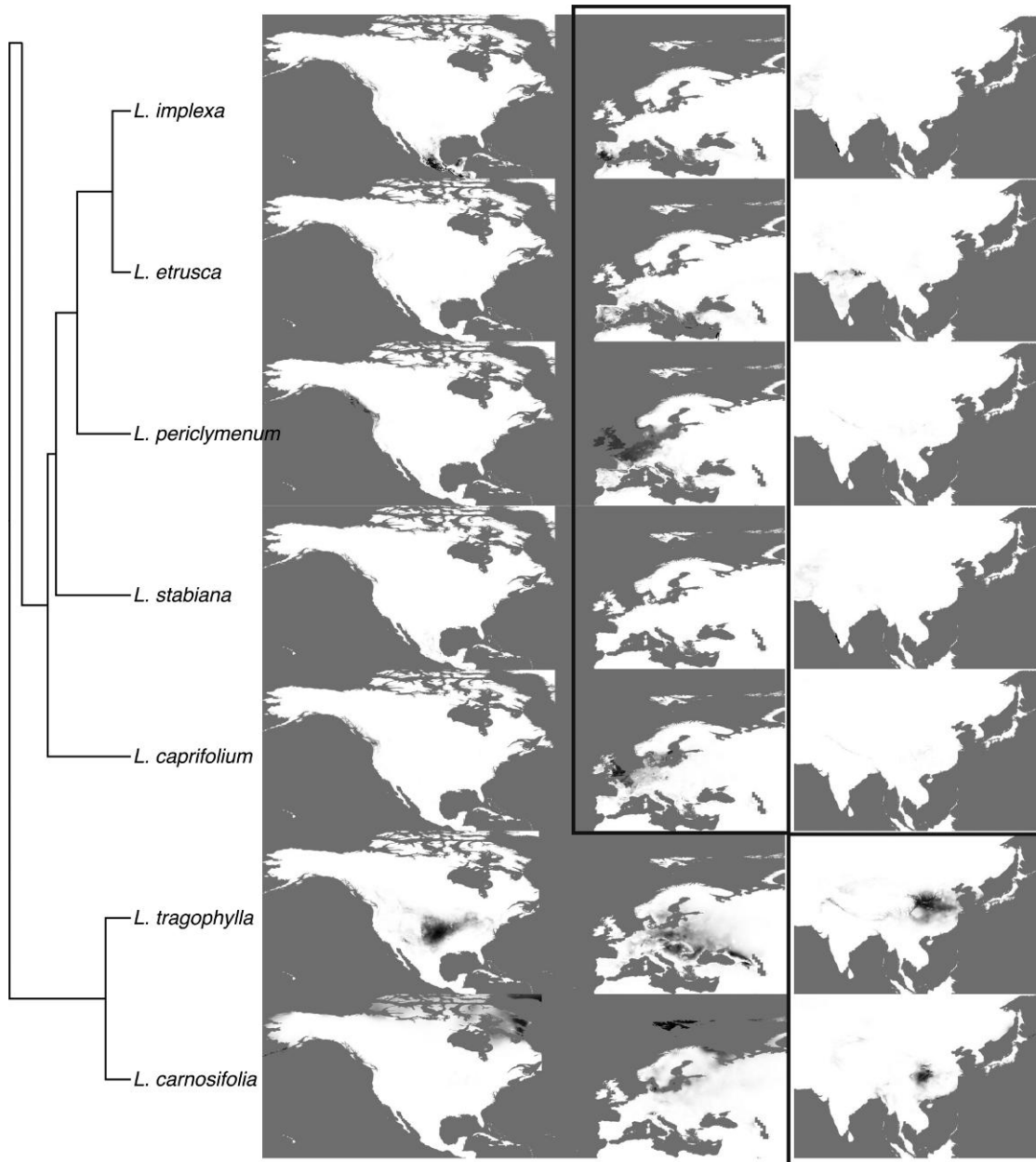


FIGURE 4. Niche models for European and Asian species. Black boxes surround the continent where the species are currently distributed; the other projections represent areas on other continents with significantly similar climatological conditions to those produced in the niche model.

and second principal components (PC1,  $x$ -axis and PC2,  $y$ -axis) explained between 66% and 83% of the variation among the 19 Bioclim variables in most analyses. The first 2 principal components accounted for 60% of the variation in the 2 largest analyses, and PC1, PC2, and PC3 together explained  $\sim 80\%$  of the variation. For PC1, the directionality of loadings generally corresponded to a temperature axis, where annual mean temperature (Bioclim variable 1), mean temperature of the coldest quarter (Bioclim variable 11), and minimum temperature of the coldest month (Bioclim variable 6) were often positively related, and temperature seasonality (Bioclim variable 4) and the annual range in temperature (Bio-

clim variable 7) were often negatively related. Variation in PC2 largely corresponded to a precipitation axis, with loadings much more variable (e.g., Bioclim variables 15, 16, 17, and 19); temperature variables such as Bioclim variables 5 and 10 were also reflected in the PC2 loadings. In contrast to the results above, PC1 in the analysis including just *L. arizonica* and *L. dioica* was dominated by precipitation of the coldest quarter (Bioclim variable 19) and PC2 was dominated by mean diurnal range and isothermality (Bioclim variables 2 and 3).

PCAs involving more than pairs of sister species are provided here for illustrative purposes as phylogenetic distances are not considered. ANOVAs were calculated

on both PC1 and PC2 on sister species. ANOVA analyses on PC1 for *L. albiflora*/*L. prolifera*, *L. hirsuta*/*L. ciliosa*, *L. interrupta*/*L. suspicata*, and *L. implexa*/*L. etrusca* all produced *F* values greater than 150 and showed greater differences between groups than within groups. The ANOVAs between *L. tragophylla*/*L. carnosifolia* and *L. arizonica*/*L. dioica* did not show significant differences in PC1, with *F* values of 3.2 and 0.6, respectively. All comparisons were significantly different with PC2.

Niche equivalency tests as described by Warren et al. (2008) yielded similar results. These tests demonstrated that only *L. carnosifolia* and *L. tragophylla* (with *D*, *P* = 0.96 and with *I*, *P* = 0.44) and *L. stabiana* and *L. etrusca* (with *D* and *I*, *P* = 0.24) have niches that are statistically equivalent. This may be due to smaller sample sizes in both cases, a pattern noted also by Warren et al. (2008). For all other comparisons, niches were not equivalent based on either *D* or *I* statistics (*P* < 0.05).

*Niche similarity.*—Niche similarity tests demonstrated that all comparisons were significantly similar except *L. arizonia* with *L. dioica* background (with *D* and *I*, *P* < 0.01), *L. hirsuta* with *L. ciliosa* background (with *D* and *I*, *P* = 0.01), and *L. sempervirens* with *L. prolifera* background (with *D* and *I*, *P* < 0.01). Several comparisons were more similar than expected including *L. dioica* with *L. arizonica* background (with *D* and *I*, *P* < 0.01), *L. etrusca* and *L. implexa* (with *D* and *I*, *P* < 0.01), *L. hispidula* and *L. interrupta* (with *D* and *I*, *P* < 0.01), *L. hispidula* and *L. subspicata* (with *D* and *I*, *P* < 0.01), *L. interrupta* with *L. subspicata* background (with *D* and *I*, *P* < 0.01), and *L. prolifera* with *L. sempervirens* background (with *D* and *I*, *P* < 0.01).

#### Disjunctions Within Species

Although many of the species of *Caprifolium*, especially in North America, showed at least some disjunction

within species, a few show very strong patterns (Fig. 7). Here we tested the difference between populations within *L. ciliosa*, *L. flava*, *L. hirsuta*, and *L. interrupta*. PCA and ANOVAs all showed significant differences between disjunct populations of these species. *Lonicera ciliosa* showed the weakest difference (*F* = 57.1) and *L. flava* the strongest (*F* = 2253.5). The strongest components of PC1 and PC2 included both precipitation and temperature-related variables, except for PC1 in *L. ciliosa*, which showed the strongest relationships with temperature. Ecological niche models conducted using each disjunct population failed to predict the other ("missing") disjunct populations, with the exception of weakly predicted partial ranges for both populations of *L. ciliosa*.

Niche equivalency tests demonstrated that *L. ciliosa* populations, using only the *D* statistic (with *D*, *P* = 0.36 and with *I*, *P* = 0.04), have niches that are statistically equivalent. For all other comparisons, niches were not considered equivalent based on either *D* or *I* statistics (*P* < 0.05). Niche similarity comparisons demonstrated that all comparisons were significantly similar except the *L. ciliosa* eastern population with the *L. ciliosa* western population background (with *D* and *I*, *P* < 0.01), the *L. flava* eastern and western populations (with *D* and *I*, *P* < 0.01), and the *L. hirsuta* western population with the *L. hirsuta* eastern population background (with *D* and *I*, *P* < 0.01). The *L. ciliosa* western population compared with the *L. ciliosa* eastern population background was found to be more similar than expected (with *D* and *I*, *P* < 0.01).

#### DISCUSSION

Several recent papers have highlighted phylogenetic niche conservatism as a factor responsible for broad scale biogeographic patterns (Donoghue 2008; Crisp et al. 2009). This is based on the observation that many

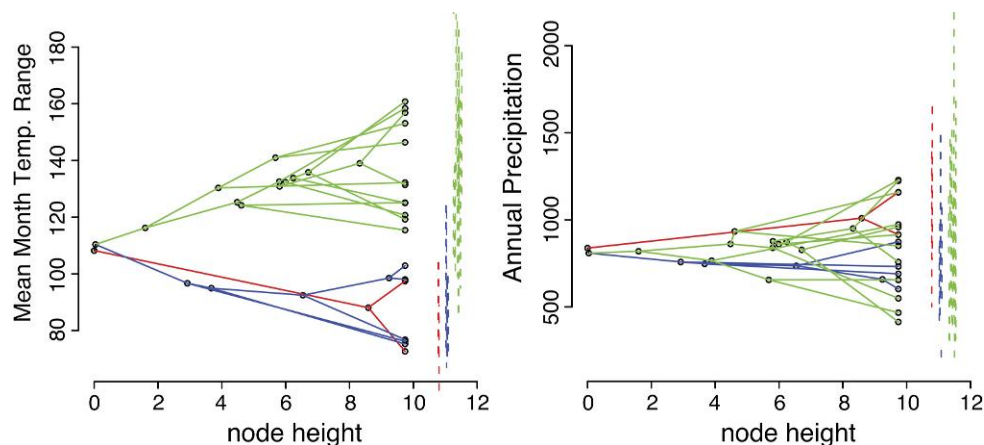
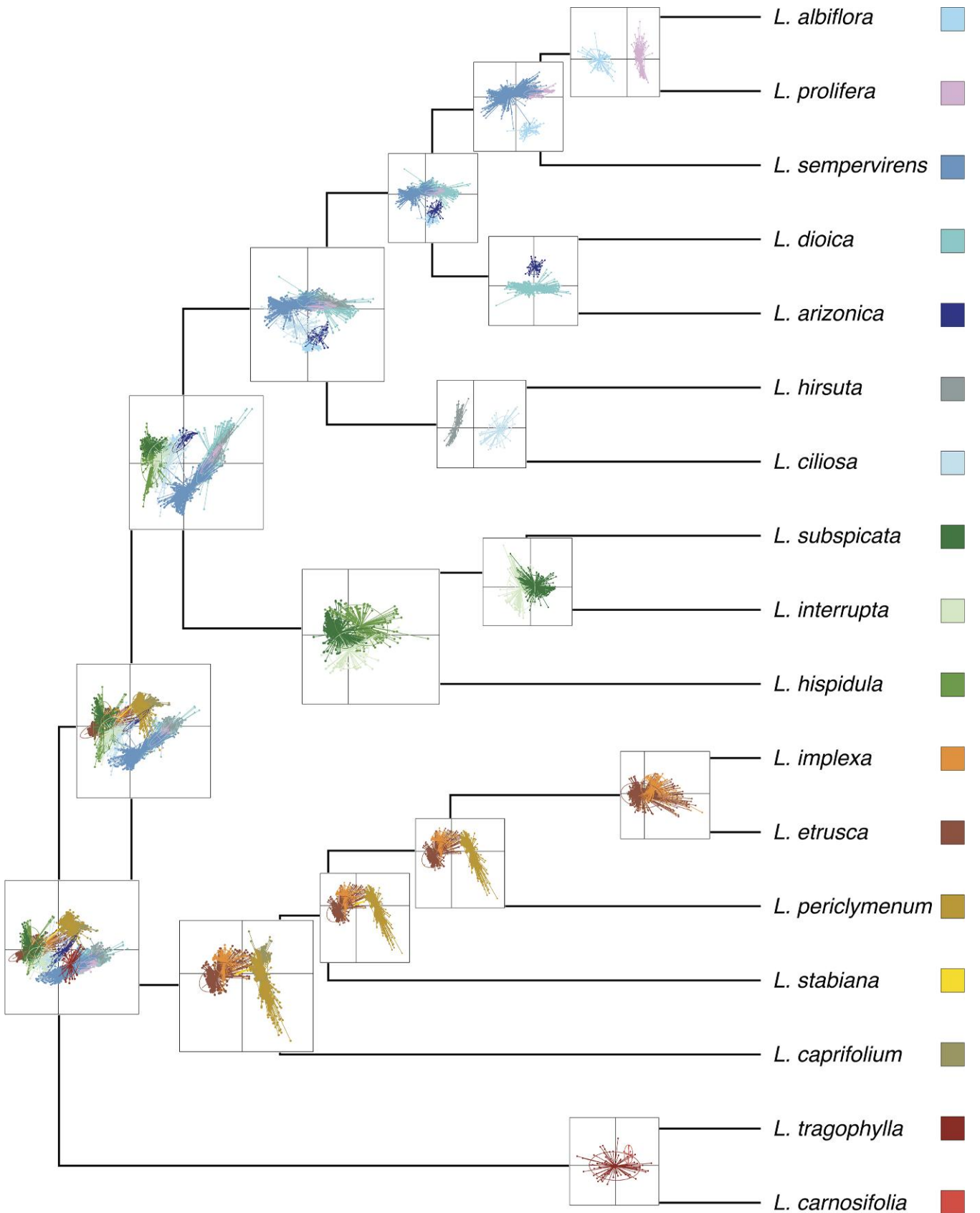


FIGURE 5. Examples of ancestral reconstructions of climate niches. Green branches represent species in North America, red branches in Asia, and blue branches in Europe. Extant disparity is represented on the right; the *x*-axis represents time. The plot on the left represents mean monthly temperature range; this exhibits greater differences among clades than within clades, and greater niche conservatism. The plot on the right represents annual precipitation; this exhibits greater differences within clades than between clades, and less niche conservatism. Results for all climate traits are presented in the Supplemental Figures.



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FIGURE 6. Principal components analyses. Plots at each node do not show reconstructed ancestral values. Instead they display principal components results for all species stemming from the node.

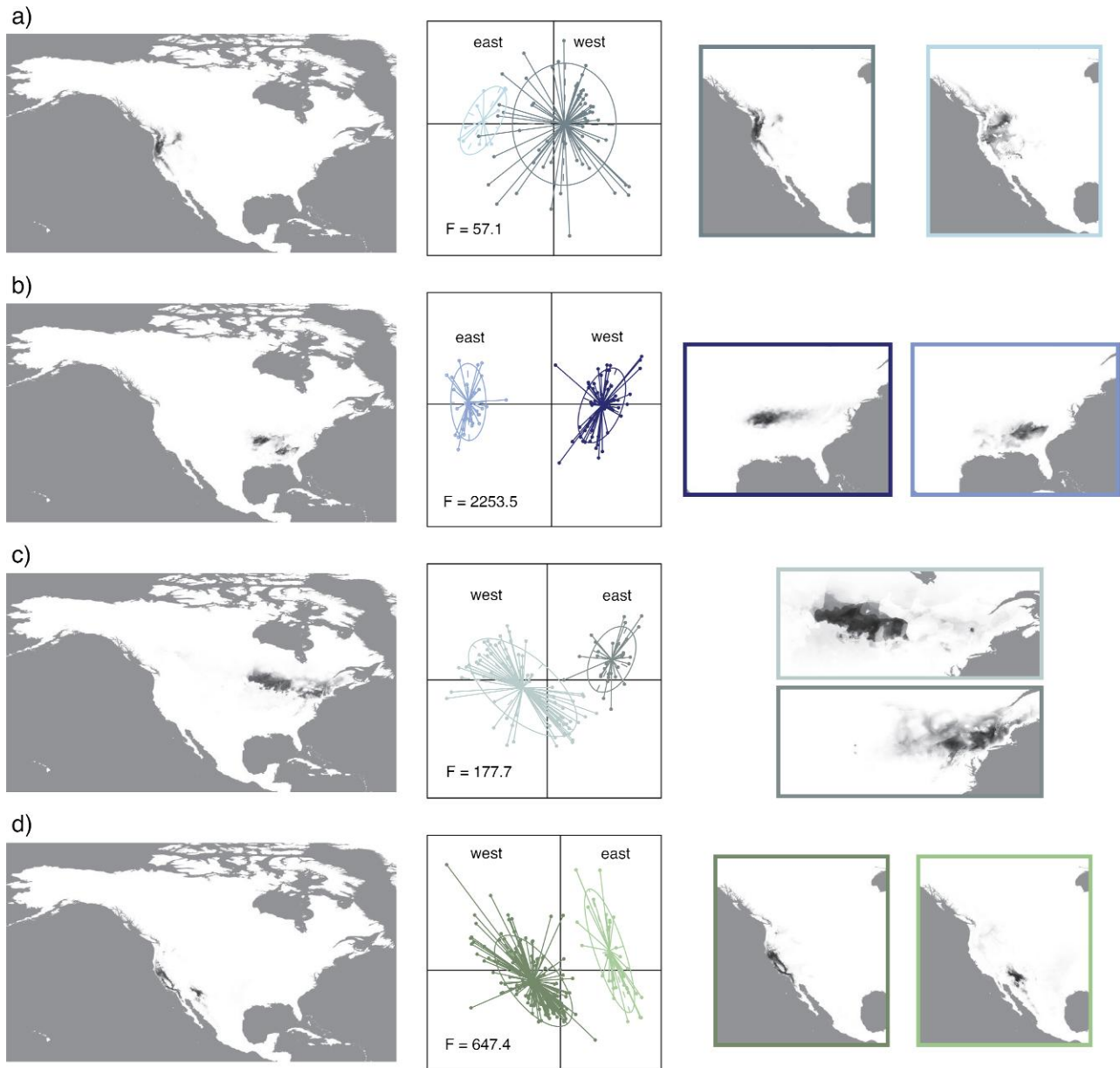


FIGURE 7. Niche models for four *L. caprifolium* species with disjunct populations (left), PCAs (center), and niche models using each disjunct population (right). a) *L. ciliosa*, b) *L. flava*, c) *L. hirsuta*, and d) *L. interrupta*.  $F$  values in the PCA plots are for ANOVAs conducted on PC1.

plant clades appear to have diversified within a particular biome, and the corollary that shifts between biomes, which generally involve substantial physiological adjustments (e.g., tolerance of prolonged freezing), are relatively rare. In the context of Northern Hemisphere biogeography, many clades have retained their presence in mesic temperate forests and are now found to be disjunct between eastern Asia and eastern North America (Wen 1999; Donoghue and Smith 2004). In some cases, these disjunct species have experienced relative stasis with regard to their morphological evolution (Wen 1999, 2001). The *Caprifolium* clade is of special interest

in representing a much less common alternative pattern, wherein biome shifts have accompanied spread around the Northern Hemisphere. Specifically, some *Caprifolium* species occupy temperate forest habitats, whereas others occupy the significantly drier climates that originated more recently in western North America and around the Mediterranean basin. The analyses presented here, combining phylogenetics, biogeography, and niche modeling, are intended to elucidate the sequence of events underlying this distribution, especially to identify the directionality and the number of shifts in key climate parameters. Our findings

suggest convergent shifts to drier climates in both the Old World and the New World, accompanied by shifts in morphological characters. As outlined below, these findings bear directly on the origin of the so-called Madrean–Tethyan biogeographic pattern.

#### *Phylogenetic Relationships*

The present study confirms that *LFY* second intron can be helpful in resolving closely related plant species that show little to no molecular divergence in traditionally variable markers such as ITS and chloroplast intergenic spacers (e.g., Howarth and Baum 2005; Kim et al. 2008). However, additional molecular regions are still needed to confidently resolve the phylogeny of *Caprifolium*. It will also be important to complete the *LFY* sequences of *L. flava* and *L. pilosa* and to obtain material of the remaining species from Asia.

In his monographic treatment of the North American species of *Caprifolium*, Perino (1978) circumscribes species groups based on characters associated with pollination: species with long corollas (e.g., *L. sempervirens* and *L. pilosa*) were separated from those with short corollas (e.g., *L. albiflora* and *L. dioica*). Our analyses instead support divisions based on large-scale geography—we find support for an Asian clade, a European clade, and a North American clade, and for a link between the European and North American species. This implies that corolla and pollination characters have evolved independently in different geographic regions. This can be seen, for example, in the long corollas of *L. sempervirens* in eastern North America and in *L. etrusca* in Europe. Within North America, instead of finding western and eastern clades, one clade of 3 species (*L. hispidula*, *L. subspicata*, *L. interrupta*) is found mainly in the chaparral of the far west, and a second clade contains both eastern and western North American elements in diverse ecological settings.

#### *Niche Evolution and Speciation*

Niche conservation, evolution, and convergence are all seen in *Caprifolium*. Within North America, there is extensive evolution of the niche. For example, the closely related *L. sempervirens* and *L. albiflora* encompass the largest and smallest values for annual precipitation in the group. Likewise, the sister species *L. hirsuta* and *L. ciliosa* show dramatic differences in many temperature variables. This was also reflected in the ANOVA, PCA, and niche equivalency analyses that showed differences in every pair of sister species excluding the Asian clade and *L. arizonica* plus *L. dioica*. Unfortunately, we had few specimens of *L. carnosifolia*, which might have affected this comparison. The PCA involving *L. arizonica* and *L. dioica* demonstrated that species can be very similar along one niche axis (precipitation), where *L. dioica* encompasses a wider range in precipitation, but markedly different along another (temperature). These 2 species also occur in widely separated geographic areas: the southwest in the case of *L. arizonica*, and north

central and eastern North America for *L. dioica*. Only the Asian clade and *L. stabiana* and *L. etrusca* were found to be equivalent based on the niche model equivalency tests as measured by the *D* and *I* statistics (Warren et al. 2008). This result could also reflect the small sample size in *L. stabiana* and *L. carnosifolia* (Warren et al. 2008).

The niche similarity tests demonstrated that the sister species *L. albiflora* and *L. prolifera*, and *L. dioica* with the *L. arizonica* background (but not the converse), have similar niches. The chaparral species and the European species were also found to have similar niches, but this is expected as they share geographic spaces. In the case of niche similarity, geographic separation (failure to span intervening environments) may have driven the divergence between species. However, the failure to reject the null may also be the result of other factors, as Warren et al. (2008) noted. Specifically, in the context of the present study, the niche similarity tests present 2 methodological problems. First, high-resolution climate data cannot be practically used because of the time required to conduct these analyses. Therefore, despite the fact that the occurrence data used here is of good resolution, we must use lower resolution climate layers for these tests. This alone may bias in favor of finding niche similarity. Second, limited sampling in one or both of the species may well influence the results. Finally, interpretation of these similarity results with respect to speciation depends on other factors, such as the vagility of the organisms under consideration and consequent gene flow. As *Lonicera* species have evidently traversed great distances, it is not entirely obvious that geographic separation alone is responsible for divergence.

Graham et al. (2004) discussed patterns of geography and ecology in relation to speciation of frogs and found evidence of ecologically mediated speciation (divergent selection) in all species pairs, 2 of which were allopatric. The data presented here support a similar scenario—speciation in isolation (i.e., sister species often being distinct geographically), perhaps along with selection for divergence (i.e., they differ in ecological space). Warren et al. (2008) discuss the difference between tests of “niche equivalency” and “niche similarity,” and our PCA and ANOVA analyses are more similar to niche equivalency tests where principal components of sister species are tested for equivalency. We also conducted niche equivalency tests directly on the niche models (as described by Warren et al. 2008) and these yielded much the same results as those based directly on the occurrence data. The value of the PCAs is that they provide more specific information about how the niches differ, as opposed to identifying if they differ significantly. The “niche similarity” tests conducted in this study complement these analyses and bear on the relative influence of geographical separation versus climatic differences in driving divergence. Our results are in many cases consistent with a role for climate differences, but geographic isolation itself has also undoubtedly been a factor. Here it is also important to remember that even where climatic niches differ significantly, there are likely to be other factors involved (e.g., range restrictions due

to competitors; Kozak and Weins 2006). Therefore, although one possible explanation for species differences is divergent selection on climatic variables themselves, there may well be other explanations for realized species ranges and other drivers of divergence.

Additional patterns are found within *Caprifolium*. For example, both the chaparral clade (*L. interrupta*, *L. subspicata*, and *L. hispidula*) and the Mediterranean clade (*L. implexa*, *L. etrusca*, and *L. stabiana*) suggest speciation with overlapping geography and ecology, and warrant careful study of potential reproductive barriers or microecological differentiation. It is also possible that these cases of current range overlap of closely related species resulted from secondary contact.

There is also noteworthy conservation of traits within some clades and in some variables. The most striking variable showing conserved differences among clades is mean diurnal range (Bioclim variable 2). The Asian species overlap with the Mediterranean and central European species, but the North American species all tend to have higher values. A similar pattern, but with the Asian species overlapping the North American species, is seen in the annual temperature range. Many other variables in the European species show noticeable conservation: temperature seasonality, temperature in the wettest quarter, precipitation in the coldest quarter, and precipitation in the wettest quarter. Much of this apparent conservatism in Europe is likely driven by the 3 Mediterranean species, but it appears that the central European species only differ in a few variables or by small amounts in many variables. The niche overlap analyses as measured across Bioclim variables suggest that the central European species differ from the Mediterranean species mainly in temperature and precipitation of the wettest and driest quarters. The Mediterranean species tolerate higher temperatures and less precipitation than do the central European species.

#### Niche Convergence in Mediterranean Climates

In addition to clear cases of niche divergence between sister species and of the conservation of niche variables across whole clades, we also see clear instances of convergence to similar habitats in different regions. The most obvious case is convergence on the Mediterranean climate (with dry summers) in the chaparral of western North America and in the Mediterranean basin in Europe. An in-depth analysis of leaf functional traits in *Lonicera* would help to determine whether various “adaptations” (or elements of these) evolved before or after the origination of the Mediterranean climate itself (cf. Ackerly 2004). However, at this stage, it seems that evergreen leaves evolved in the *L. hispidula*/*L. interrupta*/*L. subspicata* clade in the chaparral of western North America and independently in the *L. implexa*/*L. stabiana* clade of Europe. Evergreen leaves are evolutionarily derived within *Caprifolium* and are associated only with the species that grow in this

climate. Chaparral species also differ from other North American species in having longer inflorescences with more verticils, but flowering times do not appear to have shifted (Perino 1978); all overlap in flowering times from May to July. The 3 chaparral species do differ from one another in leaf characters: *L. interrupta* leaves lack cilia, *L. hispidula* has long petioles, and *L. subspicata* leaves are not perfoliate. The Mediterranean species also differ from one another by other characters: as noted, *L. implexa* and *L. stabiana* are evergreen, *L. etrusca* flowers are pedunculate, and *L. stabiana* has yellow berries as compared with the red berries of the other species. Because the species in the chaparral and those in the Mediterranean overlap in geography and with respect to climate variables, finer scale differences (e.g., pollinators and microecological factors) probably serve to limit gene flow.

Divergence time estimates for the North American chaparral species (including *L. hispidula*, which also occurs north of the chaparral) are between 3.2 and 9.5 ma and between 3.3 and 10.6 ma for the Mediterranean clade (including *L. perichlymenum*, which occurs outside the Mediterranean climate region). For the North American species, this corresponds well with the inferred onset of chaparral climate (i.e., summer drought) between 2 and 5 ma (Axelrod 1975). The time period for the European species corresponds with the uplift of mountain ranges around the Pliocene and Pleistocene (Zohary 1973) and the onset of the Mediterranean climate around 3 ma (Thompson 2005). Axelrod (1975) noted that the summer dry climate in North America is harsher than it is in the Mediterranean. This is reflected in the species studied here (Bioclim variables 14, 17, and 18), which may explain why the climate overlap of the species in these regions is not significant.

#### Biogeographic Reconstructions and Divergence Time Analyses

Previous analyses with a broader sampling of *Caprifoliae* showed little resolution within the *Caprifolium* clade and reconstructed a widespread ancestor between the Old World and the New World (Smith 2009). Our results support this biogeographic hypothesis but further resolve biogeographic events within the clade. The base of the North American clade is primarily reconstructed to be widespread between eastern and western North America; however, in the 2 most frequently reconstructed alternatives, the ancestor is not present in eastern North America. If eastern North America is not an ancestral area, then this represents a disjunction between western North America and Europe (cf., Fritsch 1996, 2001; Hileman et al. 2001). We infer the split between North America and Europe to be 6–14 ma, too recent for the Madrean–Tethyan hypothesis but consistent with other plant groups exhibiting a disjunct between western North America and Europe (e.g., Fritsch 1996, 2001; Hileman et al. 2001).

The reconstructions at the base of the *Caprifolium* tree and for the most recent common ancestor of the

European and North American clade show a widespread ancestor occupying areas that at the inferred time (Late Miocene) were no longer physically connected. There was a connection through Beringia (Tiffney and Manchester 2001), a presumed common route for Northern Hemisphere disjuncts (Wen 1999; Donoghue and Smith 2004), but no direct connection across the North Atlantic (Tiffney 1985). The reconstruction implies a series of vicariance events at the nodes separating Asia from Europe plus North America and Europe from North America. Specifically between the root node and node 1 (Fig. 2) and the node subtending the Asian clade, there was probably a lineage splitting event within Asia. One clade remained only in Asia, whereas the other remained widespread around the Northern Hemisphere. Subsequently, the widespread lineage may have become extinct in Asia, leaving the observed disjunction between Europe and North America.

Our results from the niche reconstructions and the niche projections suggest that despite extensive niche evolution within *Caprifolium*, the ancestor of the entire clade was tolerant of a somewhat dry climates and moderate temperatures, similar perhaps to the Asian *L. tragophylla*, which projects into areas in both North America and Europe where extant *Caprifolium* species occur. Ancestral reconstructions of annual precipitation favor results similar to extant values of *L. tragophylla*, *L. dioica*, *L. periclymenum*, and *L. hirsuta*—between 75 and 95 cm. Mean annual temperature reconstructions favor values similar to extant *L. tragophylla* and *L. hispidula*—between 12 and 13 °C. If the ancestor of the North America plus Europe clade were widespread, as our reconstructions imply, extinction in Asia could explain the disjunction between Europe and North America without requiring movement across the North Atlantic. The initial movement to establish the widespread ancestor could have been through Beringia. This scenario suggests that a spectrum of habitats, from mesic to moderately xeric, must have existed through Beringia during this time. Following the disjunction, some *Caprifolium* species adapted to wetter climates in eastern North America and some to drier climates in the far west and southwest North America. In Mediterranean Europe and in the chaparral region of California, these plants independently adapted to summer drought, accompanied by the evolution in both regions of evergreen leaves.

#### *Disjunct Species*

The 4 species that exhibit the strongest intraspecific disjunction patterns (*L. ciliosa*, *L. flava*, *L. hirsuta*, and *L. interrupta*) also show differences in climate variables between disjunct populations. This has implications for niche conservation/evolution and speciation. *Lonicera ciliosa* has populations in both the coastal forests of Washington and Oregon and in the northern Rocky Mountains, a pattern seen in other plant groups (Brunsfeld et al. 2001). Although we lack phylogeographic data, there is a difference in climatic variables

between the 2 populations that is consistent with the view that either the Idaho population is a relict of an older more widely distributed population, or relatively recent colonization from the western population (Brunsfeld et al. 2001). Plants in the eastern population differ from those in the western population in apparently tolerating colder climates (Bioclim variables 6, 8, and 11). However, niche equivalency tests show some support for the 2 populations to be statistically equivalent (only in the *D* statistic), and niche similarity comparisons suggest that geographic isolation (the inability to survive in intervening environments) may be driving divergence, as opposed to climatic difference between the areas occupied by these populations. Of course, both factors could be at work. *Lonicera flava*, *L. hirsuta*, and *L. interrupta* all show even stronger patterns with less geographic and environmental overlap. *Lonicera flava* eastern populations experience more precipitation than western populations (Bioclim variables 12 and 19), *L. hirsuta* western populations experience warmer climates (Bioclim variables 6 and 11), and *L. interrupta* eastern populations experience more precipitation than western populations (Bioclim variable 15). None of these 3 species had populations that were statistically equivalent; however, in the cases of *L. interrupta* and *L. hirsuta*, the niches of the eastern population and the western background were found to be statistically similar. As noted above, such findings are not easy to interpret, but, taken at face value, they suggest a primary role for geographic separation in driving divergence in this case.

Geographic disjuncts are expected to occupy, at least initially, quite similar climates on the two sides of a barrier (e.g., Peterson et al. 1999). In some cases such disjunctions might arise by changing climates and the lack of an evolutionary response (Wiens 2004). In *Lonicera*, geographic disjunctions that might have been initiated in this fashion are also now generally marked by ecological differences that might signal the beginnings of speciation. A similar pattern of geographic and climatic differentiation characterizes several pairs of sister species in *Caprifolium*, suggesting that speciation has been driven by the climatic divergence of isolated populations (Graham et al. 2004). Overall, we would argue that climate change has been the driver of evolution and speciation in this group. Climate changes (coupled with the lack of an evolutionary response enabling movement into intervening climates) may have served to geographically separate populations initially, and subsequent climate changes may then have driven niche differentiation in separated populations. In those cases where closely related allopatric species or populations are similar with respect to climate niche, geographic separation may primarily be the driving divergence. But, in general, we suppose that geographic separation and climate change have worked together to promote divergence. Finally, it is possible that a different model applies to the several *Caprifolium* species whose ranges now overlap, such as *L. interrupta* and *L. subspicata*. However, it is also possible that these species initially

diverged in isolation and have come into secondary contact.

*Toward a Model for Geographic Evolution in the Northern Hemisphere*

Most studies of Northern Hemisphere biogeography have focused on plant clades found in more mesic temperate climates, including the well-known eastern Asia–eastern North America disjuncts (reviewed in [Wen 1999](#); [Donoghue and Smith 2004](#)). The results presented here, from both large-scale and small-scale analyses, suggest a general model of biogeographic movement and evolution that can help explain the distribution of Northern Hemisphere clades that occupy more xeric habitats. We suggest that some plant groups that were adapted to somewhat drier conditions moved around the Northern Hemisphere through such environments, which must have existed to some extent in Beringia through the Miocene. Climate changes subsequent to this dispersion drove range disjunctions and adaptations in the direction of more xeric conditions, and also in some cases, including within *Caprifolium*, into more mesic environments.

Adaptation to drier climates in western North America and in Europe led to convergence, especially in response to the more recent origin of summer drought ([Axelrod 1975](#); [Thompson 2005](#)). The end result, especially in cases that also involve extinction in Asia and eastern North America, is what has been referred to as the Madrean–Tethyan disjunction pattern ([Axelrod 1975](#); [Fritsch 1996, 2001](#); [Hileman et al. 2001](#)). Instead of being the remnants of a widespread sclerophyllous forest around the Northern Hemisphere, these patterns may be the result of more recent convergent evolution to drier climates from a semi-xeric ancestor. Related adaptations may also have evolved independently in western North America and in Mediterranean Europe, such as evergreen leaves in *Caprifolium*. This model would explain why many of these disjunctions appear to be much more recent than suggested initially. Subsequent extinctions related to a failure to adapt to changing climates in the Northern Hemisphere may result in clades with species only in the chaparral and in the Mediterranean basin.

In *Caprifolium* extant species growing in other climates shed light on the evolution of this ecological and biogeographic pattern. [Hileman et al. \(2001\)](#) found that the diversification of the *Arbutus* complex probably did not coincide with the timing proposed for the Madrean–Tethyan hypothesis; *Arbutus* appears to be younger. Therefore, [Hileman et al. \(2001\)](#) proposed movement of the group around the Northern Hemisphere followed by changes in geographic range due to climate change. However, they imagined movement across the Atlantic, while we suggest movement across Beringia for *Caprifolium*, and other groups showing the Madrean–Tethyan pattern. Additionally, in *Arbutus*, it is unclear how climate niches evolved in the group

as a whole. Divergence time estimates for *Styrax* are also younger than suggested by the Madrean–Tethyan hypothesis ([Fritsch 1996](#)). The alternative explanations postulated for that clade include long-distance dispersal or extinction. Focusing on clades like *Caprifolium*, with species distributed in Europe, western North America, eastern North America and Asia, and coupling biogeographic analyses with studies of the evolution of climate niches may provide the best insights into the origins of this enigmatic pattern. We hope that the hypothesis presented here will be tested by applying the suite of approaches used here to other lineages distributed in all of the major biogeographic regions of the Northern Hemisphere.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.sysbio.oxfordjournals.org/>.

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