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Clonality and Genetic Diversity Revealed by AFLPs in Schisandra glabra (Brickell) Rheder (Schisandraceae), a Rare Basal Angiosperm

Matthew J. Valente
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Matthew J. Valente entitled "Clonality and Genetic Diversity Revealed by AFLPs in Schisandra glabra (Brickell) Rheder (Schisandraceae), a Rare Basal Angiosperm." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology and Evolutionary Biology.

Joseph H. Williams, Major Professor

We have read this thesis and recommend its acceptance:

James A. Fordyce, Randall L. Small

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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A Thesis
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Degree
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Matthew J. Valente
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Abstract

Rare species with fragmented distributions often exhibit reduced levels of genetic diversity within populations. However, life history traits such as long lived perennial habit and outcrossing mating system, are associated with high levels of within species genetic variation being partitioned within populations. *Schisandra glabra* (Schisandraceae) is a rare basal angiosperm with a fragmented distribution across the southeastern US and in a disjunct population in cloudforest of Mexico. The species' clonal reproduction by rhizomes, confounds the delineation of genetically distinct individuals in the field. The patterns of genetic diversity and clonality in 10 populations of *S. glabra* were investigated using AFLP markers. I found a surprising number of distinct genetic individuals in the two populations sampled on 3m grids, with 31 unique genotypes out of 42 samples at Wolfpen Creek, KY, and unique genotypes in all 48 samples from Panther Creek, GA. AMOVA of 237 individuals from 10 populations revealed that the largest portion of the genetic variation is found within populations (58.0%; $P<0.0001$), and 27.7% ($P<0.0001$) of the genetic variation is partitioned between the US and Mexico *S. glabra* populations. Population structure was also detected between the US and Mexico populations, but no structure was detected between the majority of the US populations. The genetic differentiation of the disjunct population in Mexico, may be the result of a Pliocene or Miocene vicariance hypothesized for many species with similar distributions. The high levels of genetic diversity found within populations are evidence of historical gene flow between the US populations, and the preservation of genetic diversity by the long lived species in its present fragmented distribution.

Table of Contents

List of Tables

Table **Page**

List of Figures

Figure **Page**

1. Introduction.

Schisandra glabra (Brickell) Rehder (Schisandraceae), is a rare woody vine occurring throughout the southeastern United States, and in a single known disjunct population in central Mexico (Stone 1968; Duncan 1975; Ettman 1980; Kral 1983; Patrick *et al*. 1995; Panero and Aranda 1998). Despite its wide historical distribution from the coastal Carolinas, Georgia Piedmont, Blufflands of the Mississippi River, to cloud forest in Hidalgo, Mexico, *Schisandra glabra* is considered threatened or endangered in all US states. Many "populations" consist of a single contiguous patch that flowers infrequently (Taylor 1994; Liu *et al*. 2006, M. Valente *pers. obs.*). Additionally, several historical populations, including those in the coastal counties of South Carolina, and several around Atlanta, GA visited in 2005, appear to have been extirpated by athropogenic habitat destruction, to which *Schisandra glabra* is especially sensitive (Kral 1983; Patrick *et al.* 1995).

Very little is known about the mating system, breeding system, population genetics, or life history in general of the species. Sexual reproduction observed in the species appears to be rare at many sites (Ettman 1980; M. Valente pers. obs.). Lui *et al.*(2006) show that both staminate and pistillate flowers of *S. glabra* are thermogenic, the pollinators include various Coleoptera and Diptera, and flowers may function as brood sites for many insects. It is not known what disperses *S. glabra* seeds, but its large fleshy red fruits are suggestive of ornithichory (van der Pijl 1982; Figure 1).

These observations raise several questions regarding the conservation status of *S. glabra* and the history of this species in North America. Wilson *et al.* (2004) show that

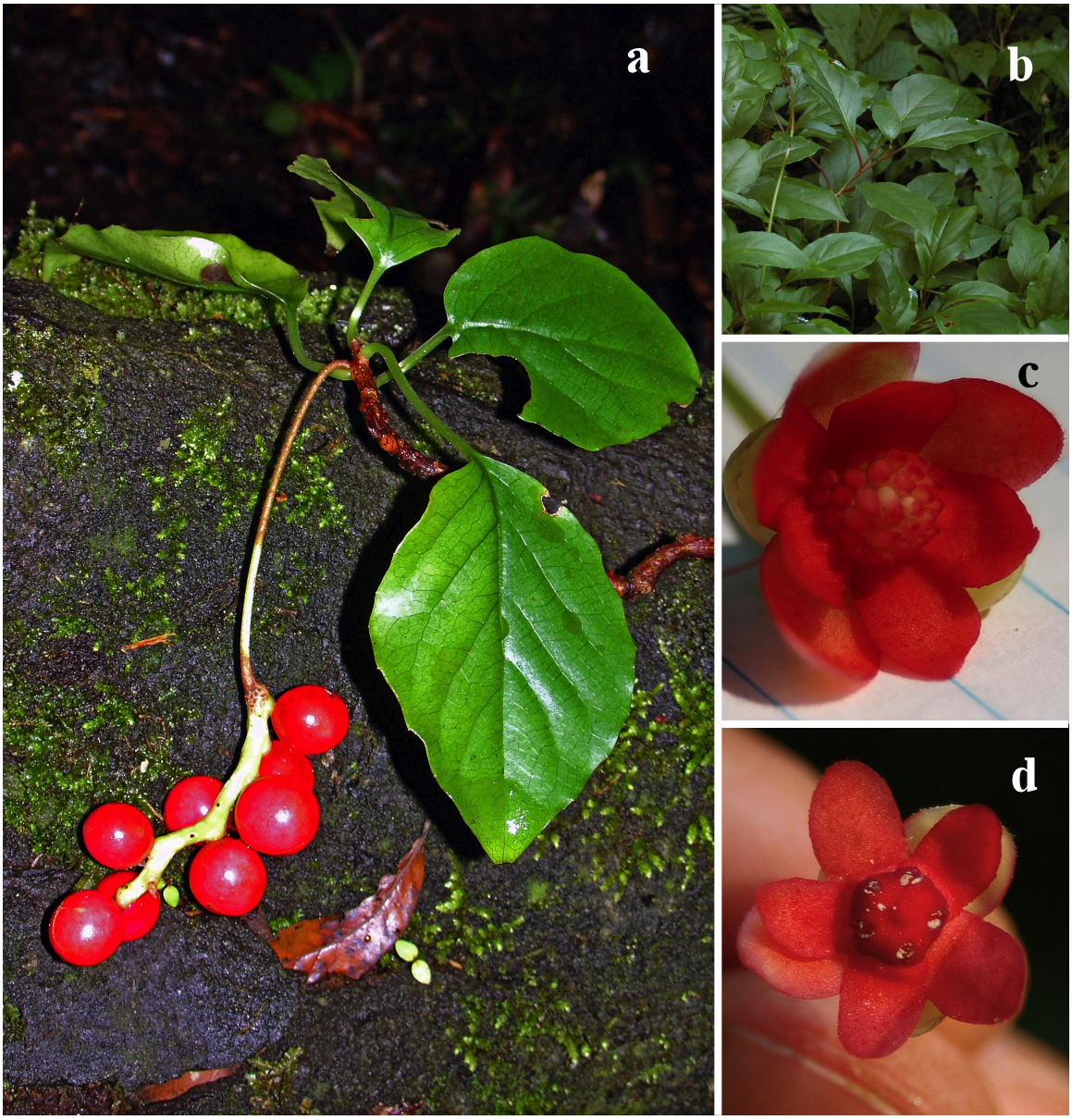

Figure 1. Photographs of *Schisandra glabra* fruits (a), habit (b), carpellate flower (c), and staminate flower (d). The photograph of fruits (a) was taken at the Zacualtipan, Mexico (MX) study site by Alvaro Campos. The remaining photographs were taken at the Panther Creek, Georgia (PC) study site by Matthew Valente.

sparse, fragmented distributions over relatively large geographic areas likely indicate a declining species. The distribution of *S. glabra* (Figure 2) is widespread in the Southeastern U.S. with a disjunct population in the state of Hidalgo, Mexico, and fits the Type C rarity classification (wide range, specific habitat, small local population size) of Rabinowitz (1986). The species however is described as endangered or threatened in most U.S. states where it occurs and is known from a single location in Mexico (Ettman 1980; Patrick *et al.* 1995; Panero and Aranda 1998). Present assessments of the conservation status of *S. glabra* also assume that known localities constitute populations (*i.e.* more than one genetic individual). However, the vine is capable of asexual reproduction by long rhizomes (Smith 1947; Feild *et al.* 2004; M. Valente *pers. obs.*) and many sites consist of large contiguous patches of *S. glabra* that could be large clones, a genetically diverse population, or a mixture of both. Vines that have fallen out of trees also may root at the nodes and continue reproducing asexually by layering (Taylor 1994; M. Valente *pers.obs*.). Therefore, estimating the actual population size (*i.e.* number of genetically distinct individuals) of *S. glabra* is impossible in the field. This determination however is essential to conservation decisions that are made regarding the species.

The distribution of *S. glabra* has received attention from biogeographers, specifically in the context of Pleistocene refugium theory (Delcourt and Delcourt 1975). They conclude that the Blufflands of the Mississippi alluvial valley provided a pathway for the migration of Mixed Mesophytic Forest species, of which *S. glabra* is associated (Ettman 1980), southward during the Pleistocene. Similar topography and microclimates along major river systems of the Coastal Plain also may have provided the environment necessary for many Mixed Mesophytic Forest species during the Pleistocene (Delcourt

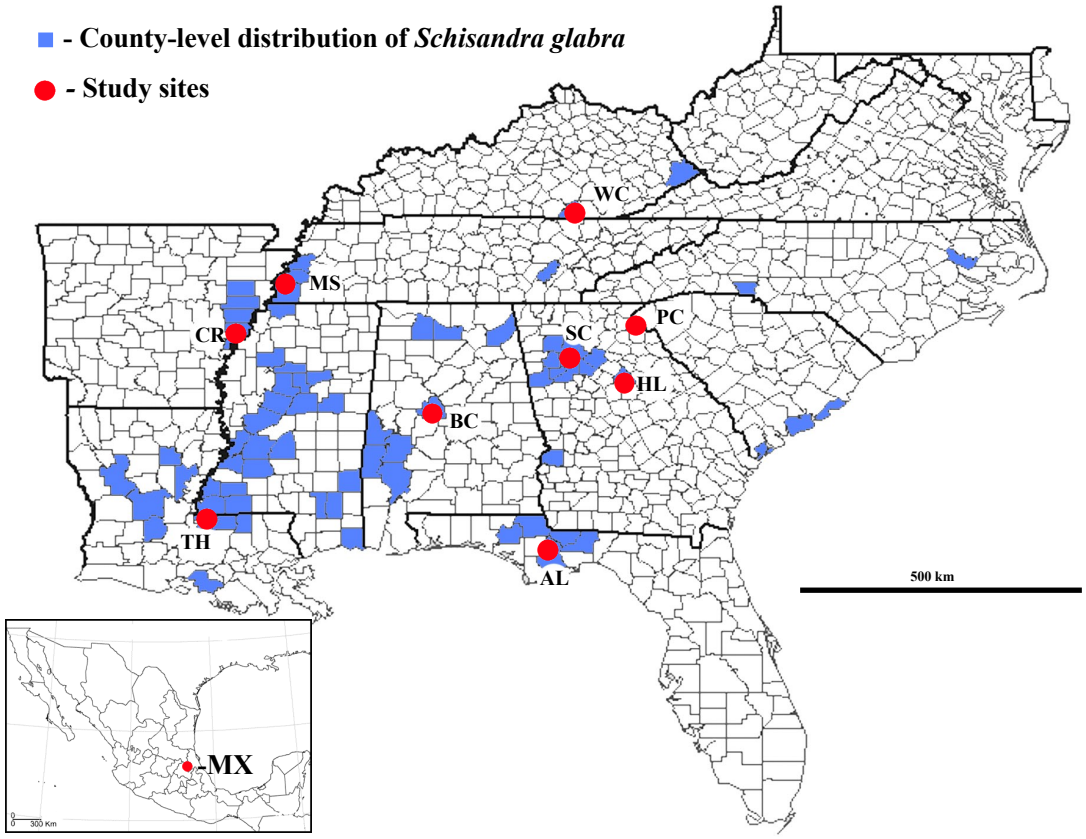

Figure 2. County-level distribution of *Schisandra glabra* and location of study sites. Counties where the species has been documented are shaded in blue (NCU Southeastern Flora Atlas 2007). Location of sample sites are indicated by red dots. The 10 study sites shown are MX-Zacualtipan, Mexico; TH-Tunica Hills, Louisiana; CR-Crowley's Ridge, Arkansas; MS-Meeman Shelby Forest, Tennessee; BC-Bibb County, Alabama; WC-Wolfpen Creek, Kentucky; AL-Aspalaga Landing, Florida; SC-Sope Creek, HL-Hard Labor Creek, and PC-Panther Creek, in Georgia. The location of population in Hidalgo, Mexico (MX) is indicated on the inset map.

and Delcourt 1975). However, there is evidence that some temperate deciduous taxa survived as least as far north as 35°N latitude, just 500 km south of the Wisconsin Glacier (Jackson *et al.* 2000). If the distribution of *S. glabra* was fragmented (even more than it is today) during the Pleistocene, genetic divergence between refugial populations may have occurred due to differential partitioning of ancestral variation via genetic drift (Knowles and Richards 2005).

The first goal of this study is to determine the number of genetically distinct individuals (*i.e.* not clones) in typical *S. glabra* populations. Secondly, the population structure and genetic diversity within and among populations will be assessed to determine if the fragmented distribution of the species is contributing to divergence between isolated populations. The geographical patterns of population structure and relationships of populations will be used to identify the genetic signature of significant demographic events, such as Pleistocene migrations and isolation of the Mexican population, in the history of *S. glabra.* Additionally, the conservation implications of these results will be discussed.

2. Materials and Methods.

2.1 Study sites, sampling protocol, and DNA extraction.

Leaf samples and herbarium vouchers were collected at 10 sites across the range of *Schisandra glabra* (Table 1 and Figure 2), in the summers of 2005 and 2006. In Georgia, samples were collected at Hard Labor Creek State Park, Morgan County (HL), Chattahoochee National Forest, Panther Creek, Stephens County (PC), and Sope Creek, Cobb County (SC). In Louisiana, leaves were collected at Tunica Hills Wildlife Management Area, West Feliciana Parish (TH). In Arkansas, leaf samples were collected at Crowley's Ridge, St. Francis National Forest, Lee County (CR). In Tennessee leaves were sampled at Meeman-Shelby State Forest (MS). In Florida, leaves were collected at Aspalaga Landing, Gadsden County (AL), and in Kentucky at Wolfpen Creek, Daniel Boone National Forest (WC). In Alabama, plants were sampled in Bibb County on Pratt Creek (BC). In Mexico, leaves were collected at the disjunct population near Zacualtipan, Hidalgo (MX). Leaf samples were collected from vines that were separated by >10 m to reduce the chance of sampling ramets of the same genet. Voucher specimens from each sample site were deposited in the University of Tennessee Herbarium (TENN), Knoxville, Tennessee.

Leaf material was stored on ice (<48 h) until it could be flash frozen in liquid nitrogen, then stored at –80ºC. This method was chosen because yield of total genomic DNA extracted from *S. glabra* leaves was higher from frozen leaves when compared to extractions from material dried in silica gel. Extraction of genomic DNA from leaf

Table 1. Location of *S. glabra* study sites. Number of individuals sampled, and voucher herbarium specimen collection number and herbarium acronym in parentheses.

Location	Coordinates	ID	Sample Size	Voucher
Panther Creek, Stephens Co., GA	34.6733°N, 83.3533°W	PC	48	*M. Valente 311* (TENN)
Wolfpen Creek, McCreary Co, KY	36.6843°N, 84.6036°W	WC	42	*M. Valente 318* (TENN)
Sope Creek, Cobb Co., GA	33.9369°N, 84.4362°W	SC	9	*M. Valente 313* (TENN)
Meeman-Shelby Forest, Shelby Co., TN	35.3426°N, 90.0438°W	MS	20	*M. Valente 320* (TENN)
Tunica Hills WMA, West Feliciana Parish, LA	30.9424°N, 91.5173°W	TH	21	*M. Valente 321* (TENN)
Aspalaga Landing, Gadsden Co., FL	30.6171°N, 84.9077°W	AL	19	*M. Valente 322* (TENN)
Hard Labor Creek SP, Morgan Co., GA	33.6645°N, 83.5909°W	HL	12	*M. Valente 314* (TENN)
Crowley's Ridge, St. Lee Co., AR	34.6941°N, 90.6797°W	CR	20	*M. Valente 315* (TENN)
Cahaba River, Bibb Co., AL	33.0475°N, 87.0917°W	BC	26	*M. Valente 323* (TENN)
Zacualtipan, Hidalgo, Mexico	20.6896°N, 98.6756°W	MX	19	*A. Campos 6107* (MEXU, TENN)

material was accomplished using Quiagen Dneasy plant kits following the manufacturer's protocol with the following modification: membrane bound DNA was eluted in 200µl ddH2O, concentrated in a spin-vac, then diluted in 20µl Tris-Hcl pH 7.5 to achieve higher DNA concentration. DNA resuspended in Tris-HCl pH 7.5 buffer was quantified on DNAquant Fluorometer (Hoefer), then stored at -20ºC.

2.2 Genotyping by AFLP.

AFLP analysis is a fast and efficient way to generate multilocus genotypes without prior knowledge of the genome of a species (Vos *et al.* 1995), and can be more reproducible than other dominant markers such as RAPDs (Albert *et al.* 2003; Kjolner *et al.* 2004) or ISSRs (Bahulikar *et al.* 2004; Nybom 2004). The AFLP technique is a PCR-based amplification of a subset of restriction fragments from the digestion of total genomic DNA (Vos *et al.* 1995). The resulting fragments are labeled with fluorescent dyes for sizing on automated sequencers and polymorphisms at the sequence level are detected by presence or absence of amplicons of specific lengths. The amplification of a fragment depends on successful restriction enzyme digestion, ligation of adapters, and the annealing of selective primers. Polymorphisms are generated by mutations at the restriction sites or the selective primer binding sites (adjacent to restriction sites), which prevent the amplification of a particular fragment.

Thirty-six AFLP selective primer-pairs were screened on five individuals using a modified protocol (Appendix 1) of Vos *et al*. (1995) with fluorescently labeled *Eco*RI-primers. The fragments were analyzed on an ABI 3100 genetic analyzer with a ROX-500

(ABI) standard. Of the selective primer-pairs screened, three were selected for further analysis based on overall clarity of bands and level of polymorphism.

AFLP profiles were scored with the software GENOGRAPHER v1.6 (Benham 2001). GENOGRAPHER is a free program that provides a graphical interface for the binning of AFLP fragments and allows the user to set a relative fluorescent unit (RFU) threshold for the presence of a band. Fragments in the size range of 80-400 base pairs were included in the analysis and have been informative in previous studies (Escaravage *et al.* 1998; Lamote *et al.* 2002; Albert *et al.* 2003; Sullivan *et al.* 2004;). To assess the repeatability of the AFLP markers, profiles were scored with GENOGRAPHER implementing a RFU threshhold of 100 from independent extractions and genotyping of 20 individuals.

2.3 Analysis of clonality.

AFLP markers have been used extensively in the analysis of clonality in populations of many plant species (Tsyusko *et al.* 2005: Kjølner *et al.* 2004: Albert *et al.* 2003; Lamote *et al.* 2002; Escaravage *et al.* 1998, Van Der Hulst *et al.* 2000). The extent of clonality at the PC and WC sites was determined with AFLP fragments generated from plants sampled on a 3 m grid. These populations were selected because they were large, well documented, and contrasting in physiognomy; for example, many vines at the PC site are large (>3cm) and produce many flowers and fruits (Ettman 1980), while the WC site consists of mostly ground patches and small vines, very few flowers, and no reports of mature fruit (Taylor 1994).

For the purpose of this study, a clone will be defined as a ramet of the same genet that is not physically connected by an above ground stem. This definition includes plants

that are still physically connected by rhizomes, and those where rhizomes have been severed or decomposed, since the distinction of such would require uprooting the plants. Clones in the populations were identified with the R software package AFLPDAT (Ehrich 2006), implementing a threshhold which corresponds with the error rate obtained in the reproducibility experiments; *i.e.* individuals that differ by more than the error rate were designated as different genets.

However, multiple genotypes occurring more than once in a population could be the product of clonal reproduction or sexual reproduction. To determine the likelihood of the latter, *i.e.* that the observed clonal genotypes were the product of random mating and not clonal reproduction, theoretical progeny were simulated with program MLGSIM (Stenburg *et al.* 2003). MLGSIM allows dominant genetic data and uses a Monte Carlo simulation to find significance values for the likelihood of multilocus genotype occurring more than once in a population by sexual reproduction (P_{sex}) assuming Hardy-Weinberg and linkage equilibrium. Allele frequencies required for the calculation of P_{sex} were obtained from ARLEQUIN v3.11 (Excoffier *et al.* 2005). A critical value for the significance of P_{sex} at the $P = 0.05$ level was obtained in MLGSIM with 100,000 simulations.

2.4 Analysis of genetic diversity and population structure.

Gene diversity, H_e (Nei 1987) with a 95% confidence interval (CI), was calculated for the individuals at each sample site in the R package AFLPDAT (Ehrich 2006) by 10,000 bootstrap replicates over loci. An unrooted neighbor-joining phylogram was constructed from AFLP profiles based on a pairwise distance matrix of restriction site distances of

Nei and Li (1979) implemented in the program PAUP* (Swofford 2002) to visualize the similarity between individuals. Rooting of the tree with the Asian species *S. chinensis* was attempted, but few bands were amplified and these may not have been homologous to bands from *S. glabra* and therefore inappropriate for rooting. The underpinnings of this method disregard the effects of reticulation (Magri *et al.* 2006), and were interpreted as such.

Genetic structure was analyzed using the Bayesian clustering method of Zhivotovsky (1999) implemented in the program STRUCTURE V2.1 (Pritchard *et al.* 2000). This method uses the assumption of Hardy-Weinberg and linkage equlibrium within subpopulations to define the number of subpopulations that best fits the data, and has become an increasingly popular method of identifying the presence of genetic structure with AFLPs (Wang *et al.* 2003; Nybom 2004; Pearse and Crandall 2004; Tsuda and Ide 2005; Gompert *et al.* 2006; Van Ee *et al.* 2006). This method, in contrast with traditional genetic structure analyses, makes no assumption of population identity based on sampling sites (Pritchard *et al.* 2000). The appropriate number of clusters (K) was determined using the ΔK method (Evanno *et al.* 2005). The analysis was run using the admixture model for K = 1-12 and no prior information regarding sampling location. AFLP genotypes were coded as (1,missing data) for presence and (0,0) for absence as suggested by Evanno *et al.* (2005). Individuals were then assigned probabilistically to K populations with 500,000 generations after a burnin of 50,000 generations. AFLPs have been shown to be a reliable marker for population assignment using STRUCTURE (Campbell *et al.* 2003).

To test for isolation by distance (IBD) between populations, Mantel tests were conducted for all populations and for US populations excluding Mexico. Pairwise great-

circle distances between populations were calculated using the *rdist.earth* function in the software package FIELDS (Nychka 2005) in R. Genetic distances between populations used were pairwise F_{ST}'s obtained with ARLEQUIN v3.11 (Excoffier *et al.* 2005). Mantel tests of correlation of the pairwise genetic and geographic distance matrices were carried out in the R package APE (Paradis *et al.* 2004).

Spatial analysis of molecular variance (SAMOVA), based on a simulated annealing algorithm, was used to identify geographic barriers that had the largest effect on genetic differentiation (Dupanloup *et al.* 2002). The program SAMOVA uses a defined range of groups (*K*) of geographically adjacent populations to seek groupings that will maximize F_{CT} (Dupanloup *et al.* 2002). *K* groups of populations identified to maximize F_{CT} were subjected to analysis of molecular variance (AMOVA) using the locus-by-locus AMOVA option in ARLEQUIN v3.11 (Excoffier *et al.* 2005) with 1000 permutations to explore the partitioning of genetic variation among *K* groups, among populations within *K* groups, and within populations.

To infer the geographic locations of genetic discontinuities in the sampled range, a Bayesian approach implemented in the R package GENELAND v2.0 was used (Guillot *et al.* 2005b). This method analyzes geo-referenced multilocus genotypes in a Bayesian framework and assumes (a) the number of subpopulations is unknown, (b) the spatial distribution of subpopulations is unknown, (c) Hardy-Weinberg equilibrium, and (d) allele frequencies in subpopulations are unknown (Guillot *et al.* 2005a). A literature search yielded no examples of AFLP data being analyzed in GENELAND, however the algorithm is similar to that of STRUCTURE and genotypes were coded again as (1,missing data) for presence and (0,0) for absence of AFLP fragments. Initial runs to determine the

number of subpopulations computed the mode of the posterior distribution of subpopulation number, using the Dirichlet model with 50,000 generations, which provided consistent results in replicate runs. Using this mode, the model was run for 50,000 generations with a fixed number of subpopulations to assess the posterior probability of subpopulation membership for any pixel in the geographic range of the samples, which again provided consistent results in successive runs.

3. Results.

3.1 AFLP repeatability and polymorphism.

Of the 36 selective primer pairs screened, the *Eco*RI-aagt(HEX)/*Mse*I-cgag, *Eco*RI-aaca(FAM)/*Mse*I-cctg, and *Eco*RI-atat(NED)/*Mse*I-cgag were selected for further analysis based on levels of polymorphism. The use of four selective base pairs for each selective primer produced a greater number of scorable and polymorphic loci than fewer selective base pairs, which is often the case for species with relatively large genome sizes, such as other members of the Schisandraceae (Fay *et al.* 2005; Bennett and Lietch 2005).

A repeatability rate of 97.8% was achieved with AFLP data scored in GENOGRAPHER with a RFU threshold of 100 from independent extractions, similar to AFLP repeatability rates previously reported (Kjolner *et al.* 2004; Lamote *et al.* 2002; Chung *et al.* 2004; Vos *et al.* 1995). Genotyping of 237 *S. glabra* samples from 10 populations using the three primer pairs above yielded a total of 117 polymorphic loci (Table 2), which were used for all analyses.

3.2 Clonality at the PC and WC sites

In the identification of clonal genotypes at the PC site, AFLP genotypes of all 48 samples differed by >3%, therefore no clones were detected. However, at the WC site, 3 clonal genotypes were identified. Two of the clonal genotypes were recovered in 2 samples and 1 clonal genotype was shared by 10 samples (Figure 3). P_{sex} calculated for each clonal genotype was very low ($P_{sex} = 1.33 \times 10^{-15}$), and well below the P=0.05 critical

Table 2. Polymorphism of AFLP selective primer pairs. Columns show the number of AFLP fragments generated, the number of polymorphic loci recovered, and the proportion of polymorphic loci. (n = 237)

Selective primer 1	Selective primer 2	Fragments generated	Polymorphic loci	% polymorphic
*Eco*RI-aagt	*Mse*I-cgag	63	40	63.5%
*Eco*RI-aaca	*Mse*I-cctg	49	35	71.4%
*Eco*RI-atat	*Mse*I-cgag	62	42	67.7%
	TOTAL	174	117	67.3%

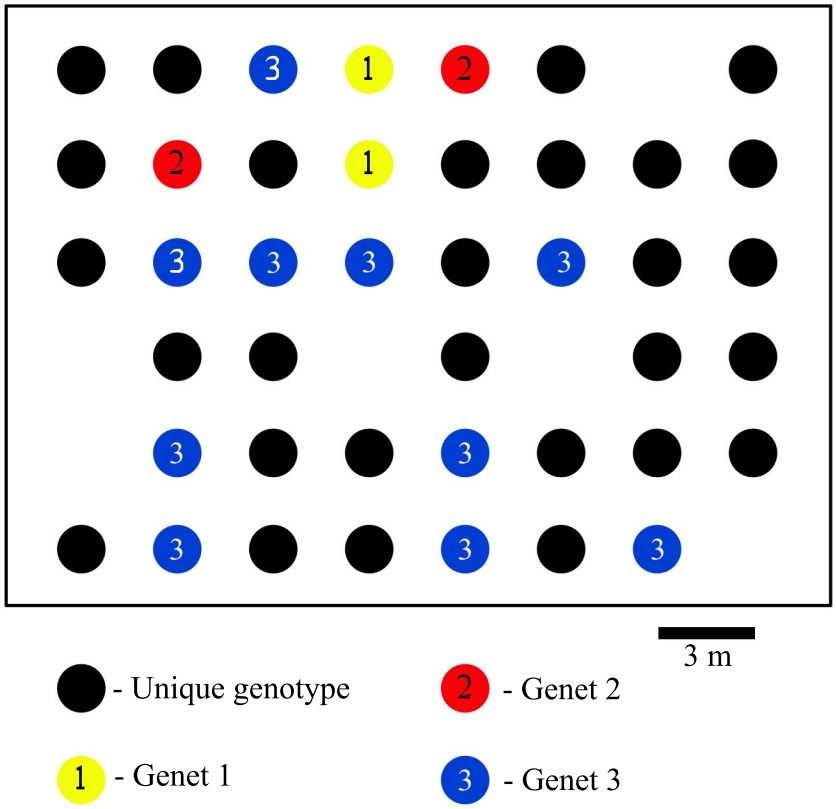

Figure 3. Spatial distribution of clones identified at the WC site. Of the 42 samples collected on a 3m grid, 28 were unique genotypes. Three genotypes were recovered in more than one sample from the grid, genet 1 (2 samples), genet 2 (2 samples), and the larger genet 3 (10 samples). Sampling points on the grid where no *S. glabra* plants were found are indicated by a blank space.

value ($5.93x10^{-8}$) probably due to the high exclusionary power of multilocus AFLP genotypes. Therefore, it is unlikely that any of the identified clonal genotypes were the result of sexual reproduction. Unique genotypes, *i.e.* those differing by > 3%, were found in the 28 remaining samples collected on a 3m grid at the WC site. In addition, no identical AFLP profiles were generated in the samples from the other eight sites.

3.3 Genetic structure and diversity.

Total gene diversity estimates for the species (H_E = 0.271; 95% CI = 0.241-0.302) and mean gene diversity within populations (H_E = 0.207; 95% CI = 0.171-0.243) were relatively high at each sample site (Table 3). Bootstrapped 95% CIs for within population H_E were overlapping for all sample sites except between SC (H_E = 0.181; 95% CI = 0.144-0.218) and PC (H_E = 0.253; 95% CI = 0.219-0.285) and these sites had the highest and lowest gene diversity estimates respectively. The unrooted neighbor-joining tree (Figure 4) generated from Nei-Li genetic distances in PAUP*, supports the monophyly of the MX population, but individuals from all other populations appeared on more than one major branch of the tree.

AFLP data was best described with K = 3 clusters by the Bayesian clustering algorithm of STRUCTURE (Figure 5). All samples from the MX population were assigned with high probability to one cluster (cluster 1), and the remaining samples (*i.e.* the US populations) were assigned among the other 2 clusters. Within the southeastern US populations, the PC and CR individuals primarily were assigned to cluster 2 and individuals from the remaining populations (HL, AL, BC, MS, WC, TH, and SC) primarily were assigned to cluster 3. However, many individuals from the PC site were

Table 3. Within population and total gene diversity estimates for sampled *Schisandra glabra* populations. The sample size, number of polymorphic AFLP loci, gene diversity, and bootstrapped 95% CI for gene diversity are given for each study site.

Population	Sample size	Polymorphic loci	Gene diversity	Gene diversity 95% CI lower bound	Gene diversity 95% CI upper bound
AL	19	71	0.215	0.179	0.250
BC	25	85	0.203	0.168	0.237
CR	36	81	0.199	0.164	0.232
HLC	9	68	0.205	0.169	0.241
MS	16	68	0.232	0.191	0.274
MX	19	84	0.192	0.150	0.229
PC	45	91	0.253	0.219	0.285
SC	9	53	0.181	0.144	0.218
TH	19	75	0.203	0.169	0.237
WC	40	83	0.191	0.158	0.225
Within population mean	23.7	75.9	0.207	0.171	0.243
Total	237	117	0.271	0.241	0.302

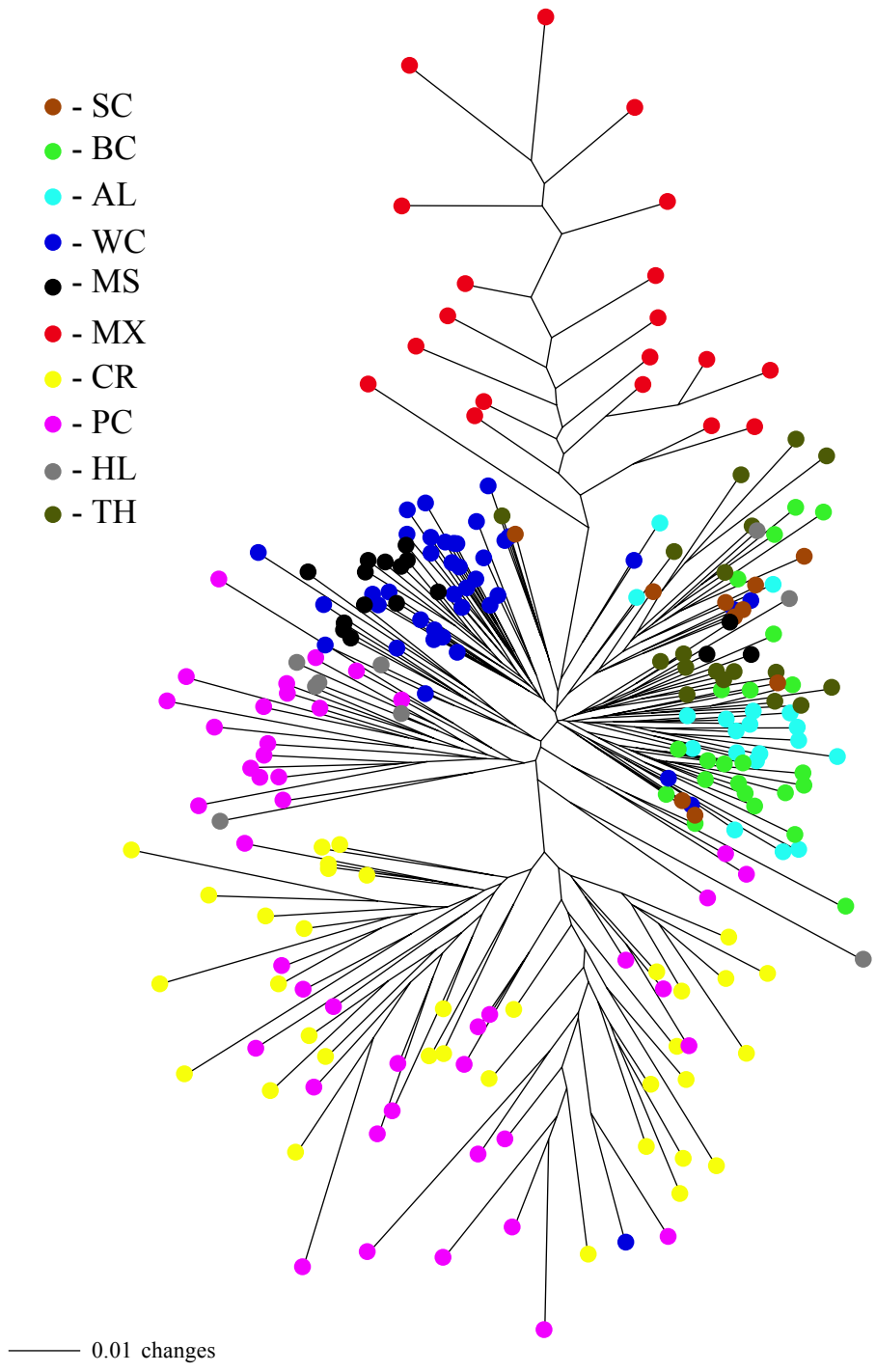

Figure 4. Unrooted neighbor-joining tree of 237 individuals from 10 populations. Neighbor-joining tree was generated using the Nei-Li genetic distances based on 117 AFLP markers. Individuals are color coded by sample site.

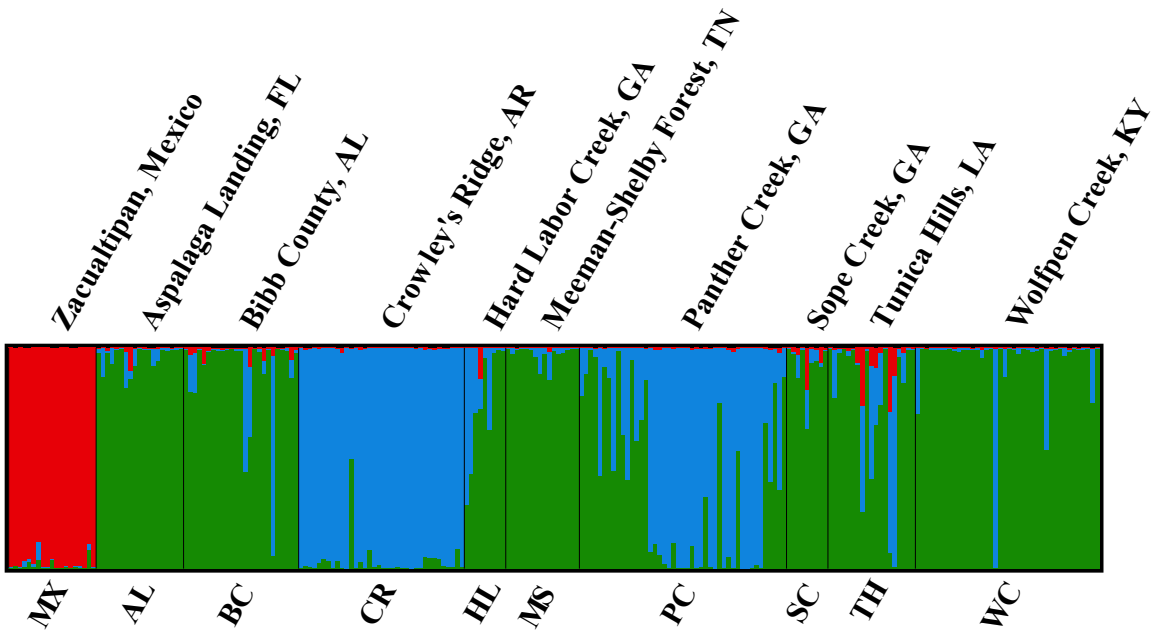

Figure 5. Population assignments for 117 individuals of *S. glabra* from 10 populations. Each vertical line represents an individual and the color reprensents the assignment probability to each of the 3 populations inferred by STRUCTURE.

not assigned with high probability to either cluster 2 or cluster 3.The Mantel test for IBD revealed a significant correlation (r = 0.8, *P* = 0.035) between genetic distance and geographic distance for all populations (Figure 6). However, since the disjunction between the Mexico and US populations is greater than the distance between any two of the sampled populations in the US and that the pairwise F_{ST} values are high in the Mexico-US comparisons, the correlation appears to be driven by a line fit through two clusters of points. Mantel test for IBD within the US populations, with the MX population removed, revealed no significant correlation between genetic distance and geographic distance (r = 0.41, P = 0.45).

The results of the SAMOVA analysis of all populations showed that F_{CT} was maximized when the populations were divided into 2 geographically contiguous groups: group 1 consisting of the MX population and group 2 consisting of the remaining nine populations from the southeastern US. Locus-by-locus AMOVA between these two groups revealed that 27.7% (P < 0.0001) of the genetic variation is explained by the SAMOVA defined groups (Table 4). AMOVA partitioning among groups within populations, *i.e.* within the US populations, explained 14.3% (P < 0.0001) of the variation. The largest proportion of the total genetic variation is found within populations (57.9%, P < 0.0001).

The posterior mode of the number of subpopulations in GENELAND indicated that *K*=4 best described the genetic discontinuities in the sampled range of *S. glabra*, when the geographic location of samples was considered. Again, the MX population was assigned to one group (group 1), while the US populations were assigned to the 3 remaining groups (Figure 7). Within the southeastern US, group 2 included the AL, TH,

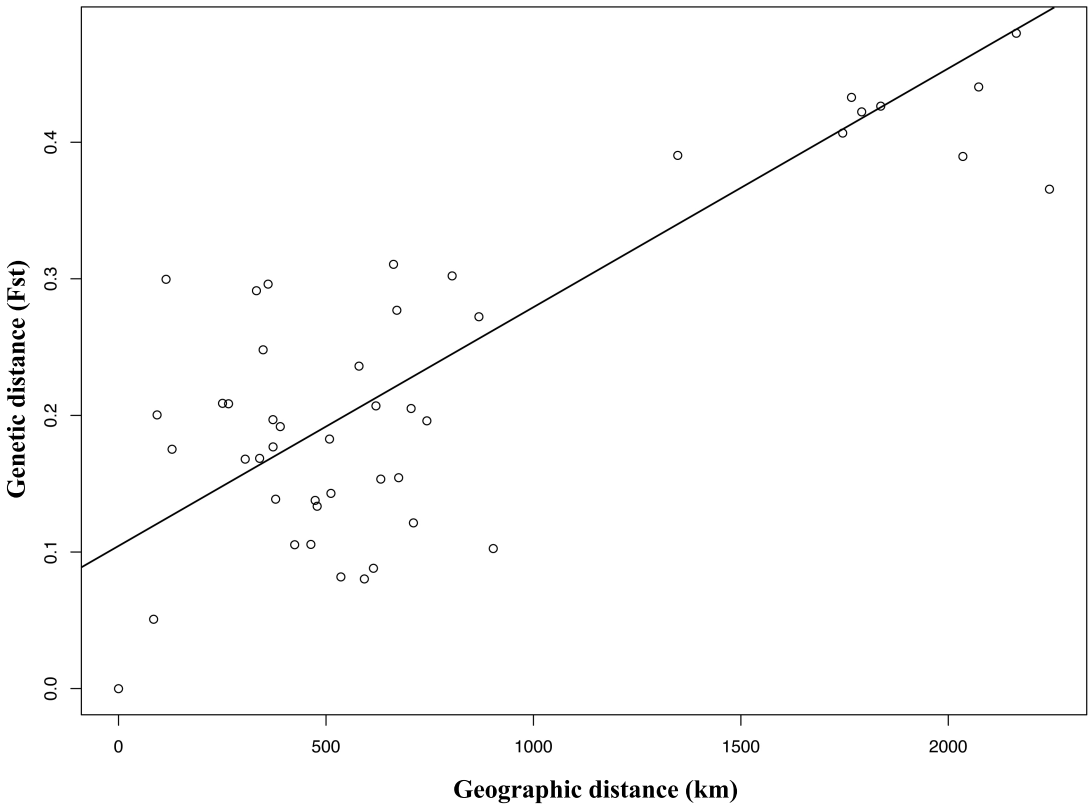

Figure 6. Plot of genetic distance versus geographic distance of 10 populations of *S. glabra.* Geographic distance was calculated by great circle distance in km and genetic distances by pairwise Fst estimates. A mantel test revealed a significant correlation (r = 0.80, $P < 0.05$) when all populations were included. Within the southeastern US (i.e. excluding pairwise comparisons with the MX population), the correlation breaks down (r = 0.41, $P = 0.45$).

Table 4. Results of locus-by-locus AMOVA. Genetic variation was partitioned among SAMOVA inferred groups (US vs. Mexico), among groups, among populations, and within populations. All variance components were significant, $P < 0.0001$.

Source of variation	Sum of squares	Variance components	Percentage variation
Among groups	278.84	5.89	27.71**
Among populations within groups	668.32	3.04	14.31**
Within populations	2797.51	12.32	57.98**
Total	3744.67	21.26	

** $P < 0.0001$

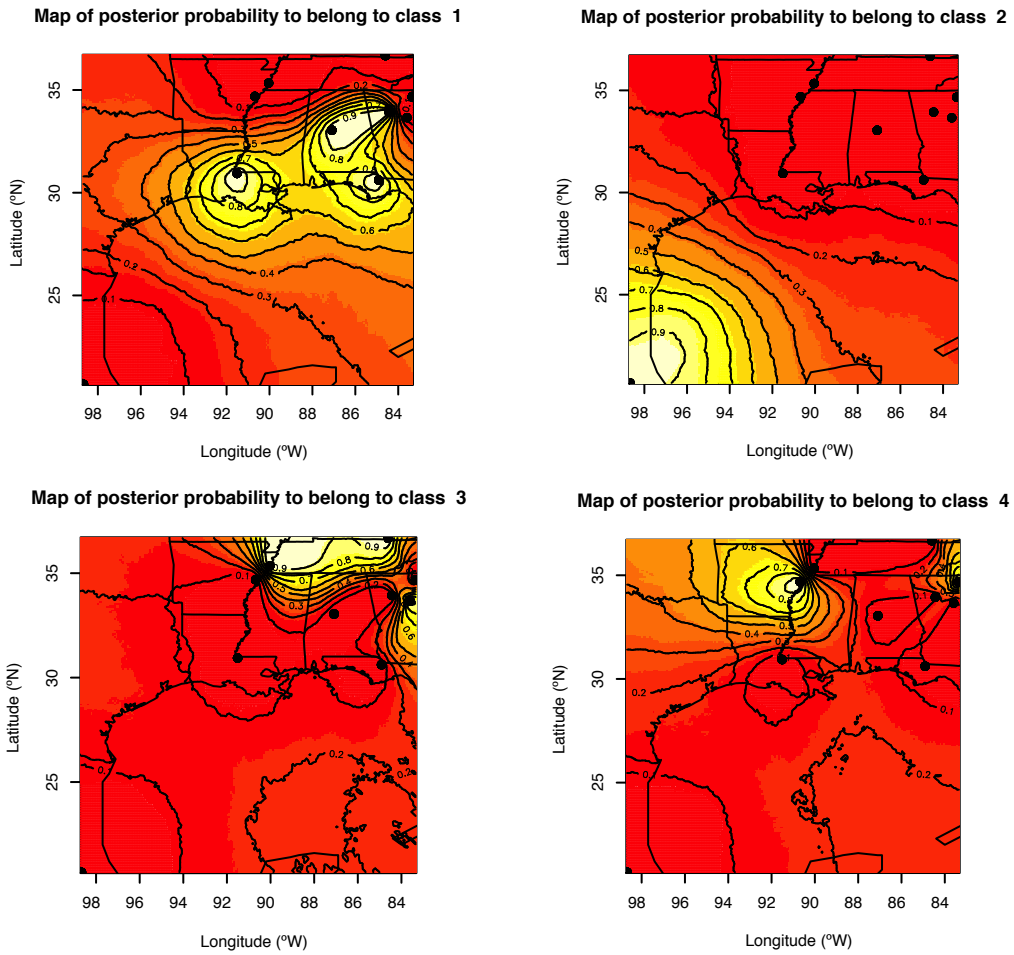

Figure 7. Maps of posterior probability of belonging to GENELAND inferred populations. Maps were generated from GENELAND analysis for *K* = 4 and 50,000 MCMC generations with a burnin of 10,000.

BC, and SC; group 3 included the HL, WC, and MS; and group 4 included the CR and PC populations. The map of geographic location of the GENELAND inferred populations constructed from the posterior mode of population membership of each pixel in the sampled range of *Schisandra glabra* shows the location of group 1, restricted to Mexico; group 2 on the coastal plain of Mississippi, Louisiana, Alabama, and the SC site near the Chattahoochee River in the piedmont of Georgia; group 3 in Tennessee, Kentucky, and the HL population in Georgia; and group 4 with a disjunct distribution of the PC population in Georgia and the CR population in Arkansas (Figure 8).

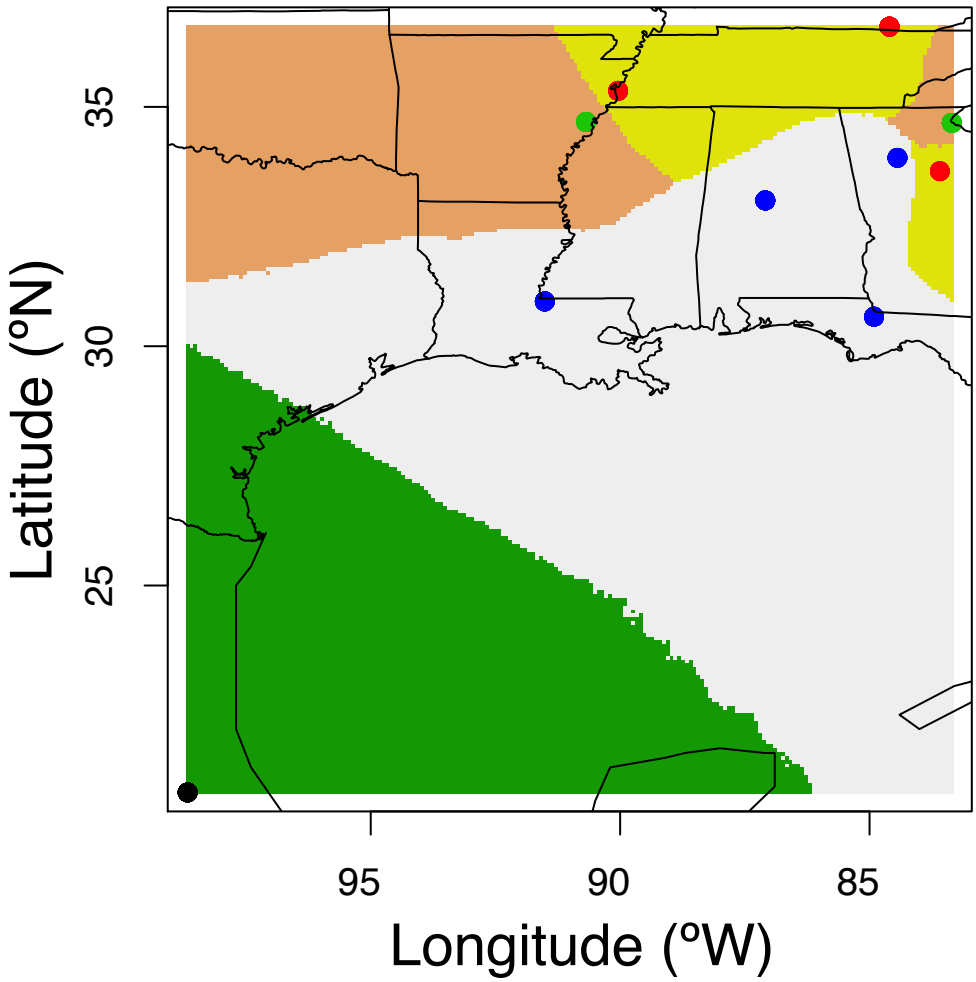

Figure 8. Map of posterior probability of population membership for each pixel in the sampled range determined by GENELAND. The analysis was based on MCMC run of 50k generations with a burnin of 10k generations for K = 4. Each pixel on the map is color coded to show the most probable population membership.

4. Discussion

4.1 The extent of clonality in Schisandra glabra *populations.*

The identification of no clonal genotypes in the PC population, and only 3 clonal genotypes in the WC population were surprising results, suggesting that many *S. glabra* populations, though isolated and small, consist of many genetically distinct individuals. The abundance of unique genotypes observed at the WC and especially the PC population are the result of either regeneration by sexual reproduction or somatic mutations in dispersed meristems. At the PC study site, many *S. glabra* vines reached into the canopy and produced seeds, so the abundance of genotypes can easily be attributed to regeneration by sexual reproduction. However, at the WC population, no flowers were observed in Summer 2006, and no vines reached the canopy. Since contemporary sexual reproduction does not appear to be frequent at the WC site, the abundance of distinct genotypes may have been generated at a time in the past when the *S. glabra* plants produced more flowers and fruits. The role of somatic mutations in generating genetic diversity detectable with AFLP's is not established, but the high levels of genetic diversity observed at all the sample sites would not be expected to be maintained by somatic mutations alone.

The recovery of some clonal genotypes confirms field observations and of vegetative reproduction via rhizomes by *S. glabra*. Other modes of vegetative reproduction reported in the Schisandraceae include basal sprouting and layering (Taylor 1994; Feild *et al.* 2004), and all of these modes were observed at study sites where *S.*

glabra vines reached >3cm in diameter. Such vegetative reproduction is viewed as a life history strategy adapted to deal with the light and disturbance regimes of understory habitats common to many basal angiosperms (Feild *et al.* 2004).

4.2 Levels of gene diversity and partitioning of genetic variation.

Given the fragmented distribution and apparent small sizes of *S. glabra* populations, it would be reasonable to expect that genetic drift would cause reduced gene diversity. The species, however, possesses a suite of life history traits that might mitigate the effects of small population size. *Schisandra glabra* is a long-lived woody species, regionally distributed, potentially outcrossing, clonally reproducing, and its seeds are dispersed by animals, all of which are predictors of high levels of gene diversity in allozyme studies (Hamrick and Godt 1996). Such results were also found in a comparison of intraspecific gene diversities obtained from RAPDs and AFLPs (Nybom and Bartish 2000; Nybom 2004). For long-lived woody plants, average within species gene diversity estimates of H_E=0.283± 0.009, and within population gene diversities of H_E=0.253± 0.008 have been reported (Hamrick *et al.* 1992). The within species gene diversity estimate for all *S. glabra* individuals sampled were not significantly different (H_E=0.271± 0.030), and the largest portion of genetic variation (57.9%) was found within populations by AMOVA. Thus, *S. glabra* surprisingly high levels of genetic diversity.

The life span of *S. glabra* individuals is not known, but the lianas grow slowly and may be able to persist in the understory for many years before the opportunity arises to climb into the canopy, where flowering is most frequent. It is possible that high levels of genetic diversity within populations are then preserved, not only by the long lives of

the individual lianas, but also by the extreme long lives of genets, which may have many reproducing lianas not necessarily overlapping in space or time. The mating and breeding systems of the species are not known, but the reports of beetle and fly pollination (Liu *et al.* 2006) of this monoecious species suggest a mixed or primarily outcrossing breeding system, as does the pattern of high within and low between population genetic diversity. These life history traits of *S. glabra*, especially its potential for long genet life, may allow the species to maintain high levels of genetic diversity within its populations.

4.3 Genetic structure and phylogeography.

The disjunction of over 2000 km from the Louisiana populations to the single known locality in Mexico (Panero and Aranda 1995), fits a long recognized biogeographic pattern shared by many North American plants and animals (Sharp and Miranda 1950; Martin and Harrell 1957). This disjunction of many temperate taxa was hypothesized to have been the result of a Pleistocene migration associated with glacial advance and subsequent retreat at the onset of the Holocene (Miranda and Sharp 1950; Sharp 1953: Dressler 1954). An alternative hypothesis was advanced by Dobzhansky and Epling (1944) and especially Braun (1955), proposing an expansion of the range of widespread temperate taxa into Mexico by the mid-Cenozoic and a Miocene or Pliocene vicariance associated with the aridification of the Western Gulf Coastal region. The latter hypothesis is supported in Martin and Harrell's (1957) review of plant and animal species with this distribution, and more recently in a review by Graham (1999).

All analyses support the conclusion that the disjunct MX population of *S. glabra* is a strongly genetically differentiated population. Results from the SAMOVA found that

the geographic discontinuity that explained the most genetic variation was the US-Mexico disjunction, explaining 27% of the variance, which was greater than the proportion of the genetic variation explained by divergence among the 9 populations of the southeastern US (14.3%). Additionally, both the Bayesian population structure analyses, GENELAND and STRUCTURE, assigned individuals from the MX site with a high probability to a single inferred subpopulation. The test for IBD also illustrates the genetic divergence between the US and Mexico *S. glabra* populations, which influence the significant positive correlation of geographic distance and genetic distance when all study sites are considered.

Genetic analyses of a few southeastern U.S. taxa with disjunct populations in montane cloudforests of Mexico, are adding support to the Miocene-Pliocene vicariance hypothesis. Cluster analysis of allozyme variation of *Liquidambar styraciflua* in the U.S. and Mexico revealed a deep split between the southeastern U.S. populations and the cloudforest populations in Mexico (Hoey and Parks 1994), concurrent with adaptive differentiation between the populations reported by McMillan and Winstead (1976). For the sister species *Fagus grandifolia* and *F. mexicana*, Manos and Stanford (2001) reported a molecular clock estimated divergence of ca. 7 million years, consistent with the Miocene vicariance hypothesis. In *Illicium*, the divergence between *I. floridanum* and *I. mexicanum* was estimated to have occurred between the Late Miocene and Late Pliocene based on multiple molecular clock calibration scenarios, with a minimum estimated divergence of 2.98 MYA (Morris *et al.* 2007). Future investigations of sequence divergence and molecular clock analyses would be necessary to estimate the time to coalescence for these populations.

Within the U.S. *S. glabra* populations, all analyses reveal low genetic divergence between most populations, but with a few geographically complex exceptions, such as the CR and PC populations in Arkansas and Georgia, which cluster together in the STRUCTURE, GENELAND, and neighbor-joining analyses. Several scenarios could account for these observations, such as founder effect in the establishment of these somewhat peripheral populations or genetic divergence during the Pleistocene with these sites as possible isolated refugia. However, these scenarios do not account for the consistent grouping of these populations together in multiple analyses. Phylogeographic patterns of temperate species in the unglaciated southeastern US often are the result of a mosaic of demographic events played out on a heterogeneous landscape (Soltis *et al.* 2006). Recent studies have also shifted away from the paradigm that most temperate species migrated far south during the Wisconsin glaciation exclusively and may have survived a low densities near the glacial front, as is the case for *Fagus grandifolia*, one of *S. glabra*'s most closely associated species (McLachlan and Clark 2004).

The high levels of genetic diversity within and lack of genetic structure between the US populations indicate that gene flow between populations was once higher than is suggested by the species' present rarity and fragmented distribution. The extensive deforestation and impoundment of river courses in the southeastern US in the early 20th century undoubtedly reduced greatly the amount of suitable habitat for *S. glabra*. But, since *S. glabra* is long lived, the rate of genetic drift is slow, and not enough time has passed for drift to significantly impact the genetic structure and diversity of the species (Hamrick 2004).

4.4 Conservation of Schisandra glabra.

The pre-Columbian distribution and abundance of *S. glabra* is unknown due to an absence of Holocene fossils attributable to the species in the US and Mexico (Saunders 2000). However, the accounts of 18th and 19th century naturalists in the southeastern US provide some clues. William Bartram may have been the first botanist to document *S. glabra* in the Tunica Hills, north of Baton Rouge, Louisiana in 1777 (Bartram 1791). He describes a liana reaching the high into the canopy of the forest with vines as "thick as a man's leg" with many diagnostic features of *S. glabra* such as spongy, cinnamon-colored bark, spicy odor, and notably flexible vines. He was unable to identify the plant to genus and declined to collect any specimens, possibly due to the fact that the species was not described until 1803 by Brickell (see Smith 1947). John Lyon also made notes on the species in the vicinity of Savannah, Georgia, near the type locality in 1803 (Ewan and Ewan 1963). However he states only that the plant is found also on the Flint River in Georgia and provides a vivid description of the abundant flowers near Savannah, where no extant populations are known.

None of the populations examined here fit the dramatic accounts of Bartram and Lyon, but several observations made in the course of this study are relevant to the conservation of the species. Kral (1983) viewed the most eminent threats to *S. glabra* populations as deforestation and encroachment by invasive exotic species. Evidence of major disturbance by recent logging and abundance of invasive exotic species were notably absent from all of the sites where *S. glabra* was found in this study. No plants were found at several sites where *S. glabra* was historically reported and at these sites there was evidence of both recent logging and the presence of invasive species

(especially *Ligustrum sinense, Hedera helix,* and *Lonicera japonica*). Consequently, conservation strategies for protected populations should include monitoring and removal of exotic invasive species.

The preservation of genetic diversity and protection of unique genetic entities are often used to inform modern conservation strategies (Ellstrand and Elan 1993). For *S. glabra*, the high levels of genetic diversity found within populations suggests that a reduction in genetic diversity expected in fragmented, small populations (via genetic drift and bottlenecks) has not occurred. As mentioned above, the long life, presumably outcrossing mating system, and other life history traits of *S. glabra* may have contributed to the preservation of genetic diversity within populations. However, the current fragmented distribution of *S. glabra* almost certainly is a barrier to future gene flow between populations, which emphasizes the importance of maintaining the sizes of existing populations.

The MX study site, in cloudforest habitat, is the only known population of *S. glabra* in Mexico. While the population is large compared to others reported from the US, it only covers a few hectares of unprotected land. No cloudforest habitat in the state of Hidalgo is currently protected despite a high concentration of rare and endemic taxa and rapid deforestation in the region (Vega *et al.* 2000). Panero and Aranda (1998) found no apparent morphological features to distinguish the Mexican specimens from the species in the US, however, the transition to high-elevation cloudforest habitat may be associated with divergence in a range of physiological and leaf anatomical variables. The evidence of substantial genetic structure between the US and Mexico populations support

the recognition of the Mexican *S. glabra* population as an independent lineage deserving special conservation status and possibly subspecies, variety, or species taxonomic status.

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Appendices.

Appendix 1.

AFLP protocol

This AFLP protocol was adapted from a protocol developed by Chris Nice and Zach Gompert at Texas State University. The sequences for all custom oligonucleotides used in the protocol are listed in table a1.

Table a1. Oligonucleotides used in the AFLP protocol. All sequences are listed in the 5' to 3' direction. The fluorescently labeled selective primers were HPLC purified by the manufacturer (Operon).

Primer	**Sequence**
MseI adapter 1	5'-GACGATGAGTCCTGAG-3'
MseI adapter 2	5'-TACTCAGGACTCAT-3'
EcoRI adapter 1	5'-CTCGTAGACTGCGTACC-3'
EcoRI adapter 2	5'-ATTGGTACGCAGTCTAC-3'
MseI-c preselect	5'-GATGAGTCCTGAGTAAC-3'
EcoRI-a preselect	5'-GACTGCGTACCCAAATTCA-3'
MseI-cgag	5'-GATGAGTCCTGAGTAACGAG-3'
MseI-cctg	5'-GATGAGTCCTGAGTAACCTG-3'
EcoRI-aaca (FAM)	FAM 5'-GACTGCGTACCCAAATTCAACA-3'
EcoRI-aagt (HEX)	HEX 5'-GACTGCGTACCCAAATTCAAGT-3'
EcoRI-atat (NED)	TET 5'-GACTGCGTACCCAAATTCATAT-3'

Restriction/Ligation reaction

1. Construction of MseI and EcoRI adapters. For each adapter, single stranded complementary oligos are combined , denatured, and annealed by slowly lowering the temperature. For each sample, 1μL of each adapter is required. For the MseI adapter, the MseI adapter 1 and MseI adapter 2 oligos, both at 100μM, are combined in equal parts. Separately, for the EcoRI adapter, the EcoRI adapter 1 and EcoRI adapter 2 oligos, at 10μM, are combined in equal parts. Once the adapter oligos are combined, they are denatured at 95ºC for 1 min then allowed to cool to room temperature slowly.

2. The restriction enzyme digest and ligation of adapters are completed simultaneously. Total reaction volume of the restriction/ligation reaction is 12μL. Combine ~50ng sample genomic DNA; 1.1μL 0.5M NaCl; 0.55 μL BSA at 10mg/mL (Promega); 1μL MseI adapter (50μM); 1μL EcoRI adapter (5μM); 0.1μL MseI (New England Biolabs); 0.42μL EcoRI (New England Biolabs); 0.33μL T4 DNA ligase (New England Biolabs); 1.2μL T4 DNA ligase buffer (New England Biolabs); and 3.8μL ddH_2O. Incubate the reaction at 37ºC for 3h, followed by 15min at 72ºC.

3. Dilute the restriction/ligation product with 120μL of 10μM Tris-HCl pH 7.5.

Pre-selective PCR

1. Combine the following for each pre-selective PCR reaction: 2 μL 10x PCR buffer (Eppendorf); 0.5 μL dNTP's 10mM (Promega); 1 μL EcoRI-A preselect primer (10μM); 1 μL MseI-C preselect primer (10μM); 0.1 μL Taq (Eppendorf); 1 μL formamide (Applied Biosystems); 9.2 μL ddH_2O; 5 μL diluted restriction/ligation product. The reaction conditions are 2min at 72ºC; followed by 25 cycles of 20s at 94ºC, 30s at 56ºC, and 2min at 72ºC. The final extension time of 2min at 72ºC is followed by 30min at 60ºC.

2. Dilute the pre-selective PCR product with 40 μL Tris-HCl pH 7.5.

Selective PCR

1. Combine the following for each selective PCR reaction: 2 μL 10x PCR buffer (Eppendorf); 0.5 μL dNTP's 10mM (Promega); 0.5 μL EcoRI+4 selective labeled (HEX, NED, or FAM) primer (10μM); 1 μL MseI+4 selective primer (10μM); 0.1 μL Taq (Eppendorf); 1 μL formamide (Applied Biosystems); 11.9 μL ddH_2O; 3 μL diluted preselective PCR product. For *Schisandra glabra*, the primer combinations used were EcoRI-aaca(FAM)/MseI-cctg, EcoRI-aagt(HEX)/MseI-cgag, EcoRI-atat(NED)/MseI-cgag. The selective PCR program starts with 2 min at 94ºC followed by cycles 1-10: 20s at 94ºC, 30s at 66ºC (decrease 1ºC per cycle), 2min at 72ºC. Cycles 11-40 are 20s at 94ºC, 30s at 56ºC, 2min at 72ºC. The reaction is completed by a 30 min incubation at 60ºC.

Fragment sizing

All fragments are sized on an ABI 3100 automated sequencer with a 35cm capillary. For analysis, each well contains 0.5 μL of each selective labeled primer combination (e.g. one of each "color" – HEX, FAM, and NED), 0.4 μL of ROX-500 size standard (Applied Biosystems), and 13 μL of formamide (Applied Biosystems).

Appendix 2.

Study site descriptions.

1. Panther Creek, Stephens County, Georgia. 2.4 km south of Yonah Dam on Panther Creek Rd. Large *Schisandra glabra* population on southeast side of Panther Creek at the base of slope as it meet the floodplain of the creek, continuing up the slope ~100 meters. Many large vines >3cm in diameter with both pistillate and staminate flowers abundant. Large stolons >3cm also on ground under leaf litter. Mature seeds rare in August 2005 with 1-4 berries per fruit. Associated plants include *Cornus florida, Fagus grandifolia, Quercus rubra, Acer rubrum, Magnolia frasieri, Panax quinquefolius, Rhododendron minus, Kalmia latifolia, Stewartia ovata, Tilia heterophylla, Magnolia acuminata, Tsuga canadensis, Carpinus caroliniana, Liriodendron tulipifera, Acer saccharum, Asimina triloba, Actaea pachypoda, Sanguinea canadensis, Cercis canadensis,* and *Acer rubrum.* Elevation: 251 m. 34.6733°N, 83.3533°W. Voucher: *Valente 311* (TENN).

2. Sope Creek, Cobb County, Georgia. A few (<15) *Schisandra glabra* plants on the ground on the north facing slope 0.4 km up a small tributary of Sope Creek in the Chattahoochee National Recreation Area. This tributary enters Sope Creek from the west, directly across from large ruins of old factory buildings. No flowers or significant vines (only ground patches) observed in June 2005. Elevation: 274 m. 33.9369°N, 84.4362°W. Voucher: *M. Valente 313* (TENN).

3. Hard Labor Creek State Park, Morgan County, Georgia. *Schisandra glabra* scattered along the NW facing slope along Mountain Hill Branch, 0.4 km NE of its confluence with Hard Labor Creek. Many *Schisandra* vines climbing over *Kalmia latifolia* and *Cornus florida* in the understory, and a few large vines into the canopy. Both staminate and pistillate flowers were observed in June 2005, however no mature fruits were observed in August 2005. Elevation: 181 m. 33.6645°N, 83.5909°W. Voucher: *M. Valente 314* (TENN).

4. Crowley's Ridge, Lee County, Arkansas. *Schisandra glabra* relatively common in deep loess on slopes and terrace of an intermittent stream, just N of Lee County Rd 221, 12 miles S of Marianna, Arkansas. Staminate and carpellate flowers observed in June 2005, however no mature fruits were observed in August 2006. Dominant trees include *Fagus grandifolia, Asimina triloba, Fraxinus pennsylvatica,* and *Magnolia grandiflora.* Elevation: 74 m. 34.6941°N, 90.6797°W. Voucher: *Valente 315* (TENN).

5. Wolfpen Creek, McCreary County, Kentucky. *Schisandra glabra* population on north facing slope from intermittent creek up to bluff line in the upper part of Wolfpen Creek's canyon covering relatively large area, but mostly on the ground. A few vines were climbing over understory shrubs, but none >2 cm in diameter were observed. No flowers were observed in June 2006. Other species at the site included *Fagus grandifolia, Hamamelis virginiana, Magnolia macrophylla, Acer rubrum, Asimina triloba, Ilex opaca, Ostrya virginiana, Kalmia latifolia, Rhododendron catawbiense, Quercus rubra,* and

Tsuga canadensis. Elevation: 356 m. 36.68549ºN, 84.60166ºW. Voucher: *M. Valente 318* (TENN).

6. Meeman-Shelby Forest, Shelby County, Tennessee. *Schisandra glabra* not uncommon on the slopes surrounding an intermittent creek in deep loess. Several immature fruits were observed in August 2006, with 8-10 berries per fruit. Common trees included *Fagus grandifolia, Aesculus pavia, Liriodendron tulipifera, Cladrastic kentukea, Quercus alba, Liquidambar styraciflua,* and *Magnolia acuminata.* Elevation: 88 m. 35.3426°N, 90.0438°W. Voucher: *M. Valente 320* (TENN).

7. Tunica Hills, West Feliciana Parish, Louisiana. Large Schisandra glabra population on NE facing ravine ~20 m W an unnamed tributary of Tunica Bayou in deep loess, otherwise rare in the area. Many large vines >3cm in diameter climbing ~20m into the canopy. No fruits observed in August 2006. Dominant trees were *Fagus grandifolia* and *Magnolia grandiflora*. Elevation: 47 m. 30.9424°N, 91.5173°W. Voucher: *Valente 321* (TENN).

8. Aspalaga Landing, Gadsden County, Florida. Many *Schisandra glabra* individuals climbing small trees and shrubs on the bluffs east of the Apalachicola River. Bluffs just east of terminus of Aspalaga Road at the Apalachicola River south of I-10. No large ground patches were present and no fruits were seen in August 2006. Associated plant species included *Magnolia grandiflora, Dirca palustris, Adiantum capillus-veneris, Epidendrum conopseum, Acer rubrum, Fagus grandifolia, Ilex opaca, Parthenocissus quinquefolia, Quercus muhlenbergii, Rhapidophyllum hystrix, Ulmus alata, and Magnolia asheii*. Elevation: 29 m. 30.6171°N, 84.9077°W. Voucher: *Valente 322* (TENN).

9. Pratt Creek, Bibb County, Alabama. 20-30 *Schisandra glabra* plants on stream terraces on both sides of Pratt Creek within 1 km upstream and downstream of Oak Trace. A few large vines climbing into the canopy at the base of a Ketona Dolomite outcrop W of Pratt Creek. No evidence of flowers or fruit seen in September 2006. Other species at the site included *Acer leucoderme, Frangula caroliniana, Magnolia acuminata, Quercus alba,* and *Rhapidophyllum hystrix*. Elevation: 83 m. 33.0475°N, 87.0917°W. Voucher: *Valente 323* (TENN).

10. Zacualtipan, Hidalgo, Mexico. Very large *Schisandra glabra* population in cloudforest on N facing slope along unmarked trail 3.4 km N of Zacualtipan on the road to Tianguistengo. >50 large vines climbing into the canopy and over understory trees and shrubs. Abundant large red, globose (1.5 cm in diameter),sweet smelling and fermenting fruits, very noticeable alcohol smell (very wine-like odor), in October 2006. Associated plants included *Pinus* sp., *Cornus* sp., *Liquidambar styraciflua, Ostrya* sp., *Quercus* sp., *Clethra mexicana, Ulmus mexicana, Magnolia* sp., *Rubus* sp., *Peperomia* sp., *Mitchella repens, Conostegia* sp., *Hoffmania* sp., *Begonia* sp., *Pinguicula* sp., *Solanum* sp., *Pilea* sp., *Rondolettia* sp., *and Lophosoria* sp. Elevation: 1992 m. 20.6896°N, 98.6756°W. Voucher: *A. Campos 6107* (MEXU, TENN).

VITA

Matthew Joseph Valente was born in Florence, Alabama, on August 31, 1982. Matthew attended elementary school at St. Joseph Regional Catholic School and high school at Coffee High School, where he graduated with honors in 2000. Following high school, Matthew enrolled at Auburn University, Auburn, Alabama, and graduated with a B.S. in Botany with a concentration in Ecology and Evolution in 2004. At Auburn, Matthew participated in research on torus lignification in hardwoods with Roland Dute, which was published in the International Association of Wood Anatomists Journal in 2004. In the same year, Matthew was awarded the Young Botanist of the Year Award from the Botanical Society of America and began graduate school at the University of Tennessee in the Department of Botany, which soon merged with the Department of Ecology and Evolutionary Biology. While taking classes and conducting research in Joe Williams' lab, Matthew was a graduate teaching assistant instructing labs and lectures for Introduction to Botany courses. Upon completion of his M.S. in Ecology and Evolutionary Biology, Matthew will pursue a Ph.D. in the Department of Geography at the University of Tennessee, advised by Sally Horn.