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Molecular, morphological, and ecological niche differentiation of sympatric sister oak species, *Quercus virginiana* and *Q. geminata* (Fagaceae)[1]

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The genus *Quercus* (the oaks) is notorious for interspecific hybrization, generating questions about the mechanisms that permit coexistence of closely related species. Two sister oak species, *Quercus virginiana* and *Q. geminata*, occur in sympatry in Florida and throughout the southeastern United States. In 11 sites from northern and southeastern regions of Florida, we used a leaf-based morphological index to identify individuals to species. Eleven nuclear microsatellite markers significantly differentiated between the species with a high correspondence between molecular and morphological typing of specimens. Nevertheless, Bayesian clustering analysis indicates interspecific gene flow, and six of 109 individuals had mixed ancestry. The identity of several individuals also was mismatched using molecular markers and morphological characters. In a common environment, the two species performed differently in terms of photosynthetic performance and growth, corresponding to their divergent ecological niches with respect to soil moisture and other edaphic properties. Our data support earlier hypotheses that divergence in flowering time causes assortative mating, allowing these ecologically distinct sister species to occur in sympatry. Limited gene flow that permits ecological differentiation helps to explain the overdispersion of oak species in local communities.

Key words: Fagaceae; Florida; flowering time; habitat differentiation; morphological variation; nuclear microsatellites; *Quercus geminata*; *Q. virginiana*.

Ecological divergence often occurs among close relatives during adaptive radiation (Schluter, 2000a, b) but requires reproductive isolation. The mechanisms of reproductive isolation and the extent to which it occurs are important for the generation and maintenance of diversity and for the assembly of communities (Cavender-Bares et al., 2009). Studies of recently diverged lineages demonstrate that gene flow between populations can be critical for increasing genetic variation that promotes adaptive divergence (Grant and Grant, 2006, 2008). In animals, the boundaries between species often tend to be discrete (Coyne and Orr, 2004), but there is less clarity in plant species, particularly in lineages known to hybridize such as the oaks (*Quercus*). It is widely understood that hybridization and hybrid zones are common among oaks (Trelease, 1924; Palmer, 1948; Whittemore and Schaal, 1991; Spellenberg and Bacon, 1996; Dumolin-Lapegue et al., 1998; Petit et al., 2004; Cristofolini and Crema, 2005; Craft and Ashley, 2006). Gene exchange and hybridization within the genus has stimulated much debate on species concepts in the past, and it has been suggested that the biological species concept is inappropriate for oaks (Burger, 1975; Coyne, 1994). Oaks have also played a central role in questions about the importance of introgression in plant evolution (Anderson, 1949; Muller, 1952; Schaal et al., 1991; Rieseberg and Wendel, 1993; Gonzalez-Rodriguez et al., 2004), stimulated discussions on the role of ecological factors limiting hybridization (Muller, 1952), and served as a model in the development of species concepts that rely on ecological criteria (Stebbins, 1950; Muller, 1952; VanValen, 1976). In this study, we seek to determine the extent to which two sympatric sister species of live oaks (*Quercus virginiana* Miller and *Q. geminata* Small; *Quercus* section *Virentes* Nixon) are discrete taxonomic units based on genetic, morphological, and ecophysiological evidence and to investigate the extent to which gene flow occurs between them.

Quercus geminata and *Q. virginiana* (Fig. 1A) are widespread in the southeastern United States, occurring largely in sympatry (Fig. 1B) but in contrasting habitats (Fig. 1C) (Muller, 1961; Nixon and Muller, 1997; Cavender-Bares et al., 2004a). *Quercus virginiana* occurs in mesic to hydric soils and has a broad habitat distribution and large range, while *Q. geminata* occurs in drier soils (Fig. 1C) with lower nutrient availability and has both a narrower habitat distribution (Cavender-Bares et al., 2004a) and smaller range (Fig. 1B). The two species are believed to be sister taxa based on taxonomic characters (Nixon and Muller, 1997), and this interpretation is supported by chloroplast data (Manos et al., 1999) and nuclear ITS data (Cavender-Bares et al., 2004b). According to several floristic treatments, the two species are thought to have the ability to hybridize over most of their sympatric range while retaining their morphological and ecological identity (Kurz and Godfrey, 1962; Nixon, 1985). Nixon (1985) and Nixon and Muller (1997) hypothesized that flowering time divergence was a reproductive isolating mechanism that permitted the coexistence and distinct identities of *Q. virginiana* and *Q. geminata.* Their delineation as distinct species is not universally accepted, however, and they are occasionally treated as varieties rather than true species (e.g., *Q. virginiana* var. *geminata* Sargent (1918); Gilman and Watson, 1994). Genetic data that might address the question of discrete taxonomic identities have not previously been reported.

In this study, we sought to determine whether these sister oak species are distinct taxonomic units and whether their apparent

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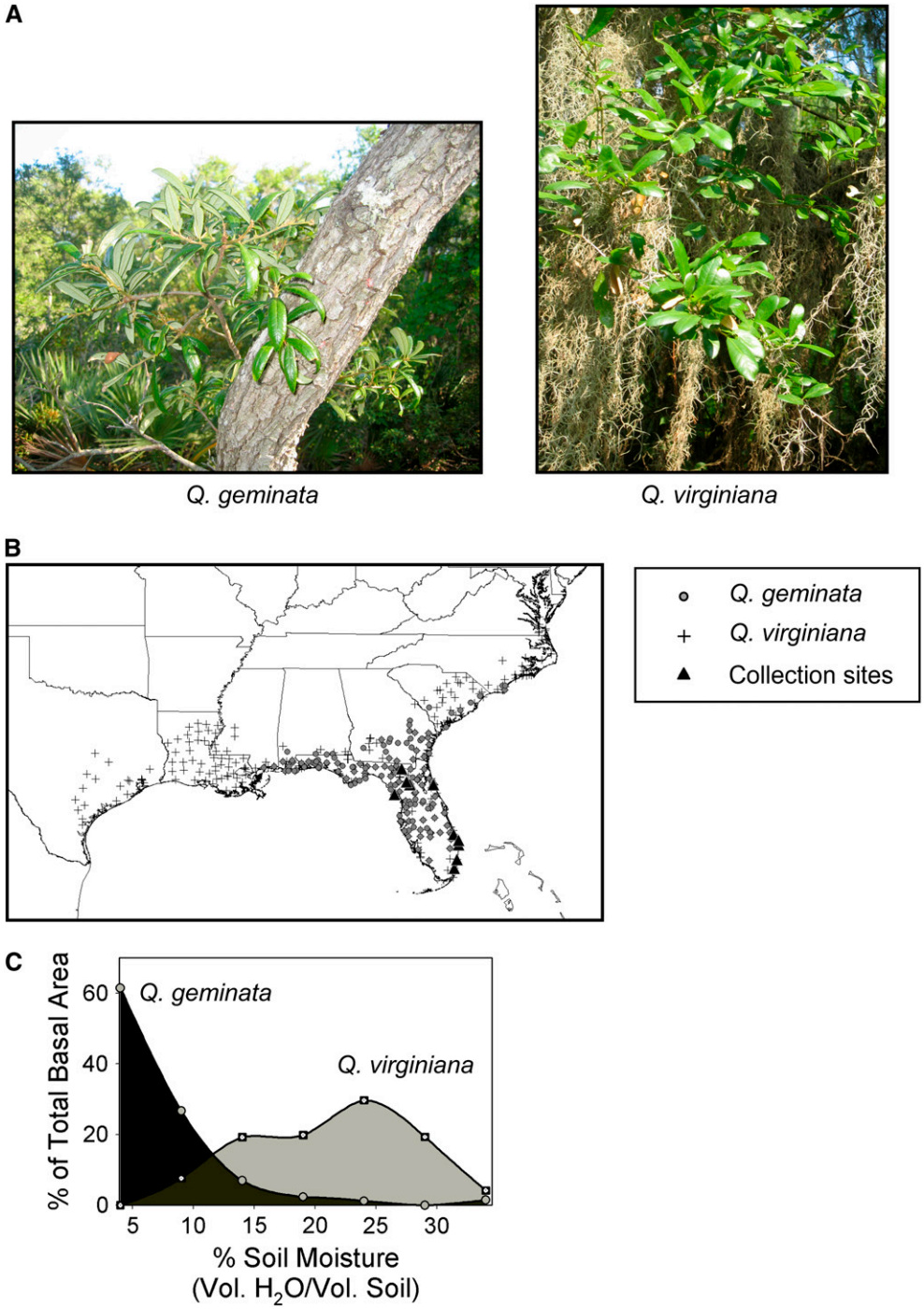

Fig. 1. (A) Photograph of *Quercus geminata* (left) and *Q. virginiana* (right) at Washington Oaks Gardens State Park in Florida. (B) Herbarium records showing approximate ranges for *Q. geminata* (gray circles) and *Q. virginiana* (crosses) from the University of North Carolina, University of South Carolina, Missouri Botanical Garden, the University of Florida, Louisiana State University, and Florida State University, as well as USDA observations records. Collection sites for this study are shown with black triangles. (C) Contrasting habitat affinities are shown for *Q. geminata* and *Q. virginiana* based on their distributions across a soil moisture gradient in Florida (adapted from Cavender-Bares et al., 2004a).

ecological and morphological differentiation has a genetic basis. Our specific objectives were to (1) determine the extent to which *Q. geminata* and *Q. virginiana* are functionally distinct in a common environment, (2) determine the extent to which the species are morphologically and genetically distinct throughout a large area of their distribution in Florida, (3) examine whether molecular typing of individuals is associated with taxonomically relevant leaf morphology, and (4) determine the degree of gene flow between the two putative species. Finally, at one site in northern central Florida, we examined whether flowering time of individuals of the two species differed enough to limit interspecific gene flow.

We examined functional traits of seedlings of the two species grown in a common garden, compared leaf morphological characters on field-collected specimens to molecular marker variation for the same specimens, and monitored phenology on trees in northern central Florida. These species were chosen because they represent an important case study for examining the extent to which closely related sympatric species can maintain ecological differentiation. Both species are ecologically important and relatively common (Putz et al., 1984; Menges and Kohfeldt, 1995; Cavender-Bares et al., 2004a; Spector and Putz, 2006).

MATERIALS AND METHODS

Study species and sampling—Leaf samples and herbarium specimens for genetic analysis were collected for *Quercus geminata* and *Q. virginiana* in state parks or preserves, where we could be relatively confident that trees were not planted. Samples were collected at six sites in north Florida and five sites in south Florida (Fig. 1B). A total of 53 individuals identified in the field as *Q. geminata*, and 58 individuals of *Q. virginiana* were sampled. It was possible to find both species at some sites but not all. A list of sites and samples sizes is shown in Table 1. The smaller sample size for *Q. geminata* in the south resulted from greater difficulty in finding individuals there, most likely due to smaller population sizes and a patchier distribution in the south relative to the north. The low abundance of *Q. geminata* in the south contrasts with *Q. virginiana*, which remained abundant both in coastal southern Florida as well as in the northern region. Representative specimens from each site are stored permanently at the University of Minnesota Herbarium within the Bell Museum of Natural History (Appendix 1).

Morphological trait analysis of field-collected specimens—Taxonomically relevant morphological traits identified by Kurz and Godfrey (1962) and Nixon and Muller (1997) were used to distinguish species during collection. We subsequently developed an index for leaf characters from Kurz and Godfrey (1962) based on the following descriptions: (1) *Q. geminata*—"The leaves are thick and leathery, rugose-veiny, the veins being deeply impressed on the upper surface. The lower surfaces are densely and tightly tomentose…" (p. 75); (2) *Q. virginiana*—"The leaf blades tend to be flat or flattish, the margin bony-opaque, scarcely revolute… The lower surfaces are tightly tomentose, the tomentose scarcely evident to the naked eye…" (p. 103). These characters are consistent with those described by Nixon and Muller (1997). The index was calculated by summing the following components, scored as follows:

1. Rugose venation (0 = no, 0.5 = moderate, 1 = extreme)
2. Revolute margins (0 = no, 0.5 = moderate, 1 = extreme)
3. Abaxial pubescence (0 = none or very sparse fused-stellate hairs, 0.5 = sparse to intermediate density, 1 = densely pubescent including stellate and additional felted or erect hairs)
4. Midvein thickness (mm) at 1/3 distance between base of petiole and tip of leaf relative to the leaf area.
5. Leaf mass per area (g/m^2) as a measure for "thick and leathery."

Each measurement was averaged for three leaves per individual sample. For traits 4 and 5, higher values are diagnostic for *Q. geminata* and lower values for *Q. virginiana*. These trait values were normalized to vary between 0 and 1. Traits 1–3 were scored so that values closer to 0 matched the taxonomic description of *Q. virginiana* and values closer to 1 matched the description for *Q. geminata.* Traits 1–4 were measured using a stereomicroscope at 60×. Leaf mass per area was determined by digitally scanning the dry leaves and measuring leaf area using the freeware ImageJ (Rasband 1997–2006). Leaves were then weighed on an analytical balance to calculate the ratio of leaf mass to leaf area. All traits were measured blind. In other words, samples were collected and assigned initial sample numbers and species identities in the field by J. Cavender-Bares, but A. Pahlich did all the morphometric measurements in the laboratory without knowledge of the preliminary species identification or results of the genetic analyses. Trait values were used for multivariate analysis to assign individuals to morphological species. To differentiate individuals into groups by morphology, we performed a hierarchical clustering analysis in the program Primer version 5 from a Bray–Curtis dissimilarity matrix calculated from all of the traits. We also ran a principal component analysis (PCA) and used the first axis loadings to examine morphological differentiation between species, sites, and regions with ANOVA and across latitude with ANCOVA. Finally, we calculated a morphological trait index. All traits were summed for each sample and divided by 5 to get an index with values between 0 and 1. The midpoint of the index (0.5) is intermediate between the two species. The advantage of the morphological index is that the values are meaningful at the level of individual specimens.

DNA extraction and microsatellite analysis—Genomic DNA was extracted from leaves using the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA) according to the manufacturer's protocol. Sample sizes varied between sites and species. For the genetic study, we used 11 previously published nuclear microsatellite primers: QpZAG 1/2, QpZAG 1/5, QpZAG 9, QpZAG 15, QpZAG 16, QpZAG 36, QpZAG 46, QpZAG 102, QpZAG 110 (Steinkeller et al., 1997); QrZAG 11, QrZAG 30 (Kampfer et al., 1998). Forward primers were labeled with a fluorescent tag (HEX, NED, 6-FAM). Polymerase chain reaction (PCR) was performed in a 15-μL reaction volume (15 mM $MgCl_2$, 200 μM dNTPs, 0.2 μM of each primer, 10 ng template DNA and 0.05 U *Taq* polymerase). To amplify the fragments, we used a PCR program consisting of an initial denaturation step of 3 min at 94°C; followed by 40 cycles of 1 min at 94°C denaturation, 1 min at 50°C annealing and 1 min at 72°C extension; and a final extension at 72°C for 10 min.

For fragment analysis, PCR products of one individual and three primers (one each fluorescent label) were pooled and were diluted 1:10–1:80 depending on the strength of the bands on the agarose test gel. GeneScan analysis was done at the Bio Medical Genomics Center, University of Minnesota (run on an Applied Biosystems 3130 × 1, using POP-4 polymer. ROX 4 HD was used as a size standard). Alleles were scored using the Genoprofiler software (You et al., 2007). Chloroplast sequences in the *trnT-trnD* region were also examined, but

TABLE 1. Collection sites for the two live oak species (*Quercus*), including park or preserve name, region (north "N" or south "S"), sample sizes for each species in each site, mean annual temperature (MAT), mean minimum temperature of the coldest month (Min), annual precipitation (Precip, mm), and mean coordinates.

		N		Temperature (°C)				
Site	Region	*Q. geminata*	*Q. virginiana*	MAT	Min	Precip (mm)	Latitude	Longitude
Big Shoals	N	13	8	19.9	4.4	1342	30.34889374	–82.71039845
Cedar Key	N	9	3	20.7	6.1	1178	29.17881174	–83.01038862
Morningside Nature Center	N	2	1	20.5	6.0	1307	29.65379331	–82.27852441
San Felasco Hammock	N	12	8	20.3	5.4	1333	29.73114543	–82.44961106
Paynes Prairie	N	0	7	20.6	6.0	1322	29.60810155	–82.29854578
Washington Oaks	N	7	8	21.0	7.7	1269	29.61953201	–81.19491159
Jonathan Dickinson	S	8	0	23.3	12.5	1421	27.00998138	–80.11368954
MacArthur Beach	S	1	8	23.5	12.9	1469	26.82717469	–80.04404588
Savannas	S	1	2	23.0	11.7	1347	27.31027558	–80.27375671
Barnacle	S	0	2	24.2	15.8	1273	25.72624994	–80.24401766
Hugh Taylor Birch	S	0	9	24.1	14.3	1514	26.14213729	–80.10465591
Total samples		53	56					

these data did not vary within or between species and, therefore, are not reported.

Common garden experiment—Data from a multispecies common garden experiment previously described (Cavender-Bares et al., 2004a) was reanalyzed to examine differentiation of physiological and functional traits between *Q. geminata* and *Q. virginiana*. Briefly, acorns were collected from natural stands of *Q. geminata* and *Q. virginiana* in Alachua Co. Florida at three state parks. Approximately 50 seedlings of each species were grown in a low nutrient mixture of sand, peat, and vermiculite in greenhouse facilities at Harvard University and subsampled randomly in their second or third year of growth. Plants were watered to field capacity each week, and 18 g of slow release fertilizer was applied prior to the start of each growing season, providing minimal fertility similar to sites intermediate between the native habitat affinities of the two species in the field. Measured traits included total biomass (g), plant height (m), total leaf number, total branch length (m), total leaf mass (g), aboveground mass, root mass, stem diameter (cm), root diameter, leaf size (m^2), leaf mass per area (g/m^2), number of leaf flushes (periodic initiation of new growth, identified by leaf scars on the main stem), photosynthetic rate (A, $mmol \cdot m^{-2} \cdot s^{-1}$), conductance rate ($g_s$, $mmol \cdot m^{-2} \cdot s^{-1}$), transpiration rate ($E$, $mmol \cdot m^{-2} \cdot s^{-1}$), water use efficiency (WUE, A/g_s), leaf lifespan.

Flowering times and habitat affinities—Although several studies have examined ecophysiological variation among cooccurring oak species (Cavender-Bares and Holbrook, 2001; Cavender-Bares et al., 2004a), flowering dates were not previously reported. Flowering for seven trees of each species was recorded on a weekly basis between January and May 1999 in San Felasco Hammock State Preserve. The distribution of species in 74 randomly established plots in five conservation areas in northern central Florida was reported in Cavender-Bares et al., 2004a. Species abundances across a soil moisture gradient (Fig. 1C) and other nutrient gradients (not shown) were determined based on the basal area of species within soil moisture or nutrient bins as previously described.

Data analyses—*Statistics*—The genetic structure of the populations was analyzed by both Wright's F-statistic based on differences in allele frequencies (Weir, 1996) and by R_{ST}-statistics based on differences in the allele size (Slatkin, 1995). R_{ST} assumes a stepwise mutation model in which more similar allele sizes diverged more recently (Zhu et al., 2000; Balloux and Lugon-Moulin, 2002; Hardy et al., 2003). Standard genetic diversity parameters were determined for each population using the programs Arlequin (Excoffier et al., 2005) and GenAlEx (Peakall and Smouse, 2006).

For the interspecific comparison between the two species, the individuals for which we successfully extracted and amplified DNA of each species were pooled across sites (53 *Q. geminata* individuals collected at seven sites; 56 *Q. virginiana* individuals collected at 10 sites). To test for differentiation due to geographic distance rather then species identity, we pooled all 78 individuals collected in north Florida and the 31 in south Florida, independently of species. We also calculated the pairwise F_{ST} or R_{ST} between species and sites (this included only sites with more than five individuals). We used PCA in GenAlEx to plot the relationship between genetic distance matrix elements on the first two principal coordinates. Clustering of individuals indicates similarity in alleles and allele frequencies.

To test for underlying genetic structure and admixture among the populations and to determine how well the genetic structure in the molecular data corresponds to phenotypic characterization of species, we used a Bayesian clustering algorithm implemented in the program Structure (Pritchard et al., 2000). The program assigns individuals to admixtures of ancestral populations, where the number of populations is unknown. We used a burn-in period of 50 000 with 1 000 000 Markov chain Monte Carlo (MCMC) replicates applying an admixture ancestry model. We set k (the number of ancestral populations) to range from 1 to 10 and ran three iterations of each. To test for isolation by distance, Mantel tests were performed using GenAlEx (Peakall and Smouse 2006) and the program Zt (Bonnet and Peer, 2002). The null hypothesis is that there is no significant relationship, in which case, the observed value should fall within the distribution generated by randomly shuffling the data. If there is significant relationship between the two data sets, the observed correlation will be more extreme (closer to +1 or –1) than the values generated by random permutation, at least 95% of the time. The program Zt gave the same results as GenAlEx, and we used Zt for partial Mantel tests in which the residuals of two matrices are compared to a third. The partial Mantel enabled us to use species identity as a third matrix to control for a potential species effect.

RESULTS

Morphological analysis—Hierarchical clustering analysis for field-collected specimens divided the individuals into two main groups, one corresponding to *Q. geminata* and the other to *Q. virginiana*, indicating clear morphological differentiation between them regardless of collection location (Fig. 2A). The division into two distinct groups corresponded to the bimodal distribution found for the morphological index values (Fig. 2B) and to the significant differentiation in the first axis of the morphological principal component analysis (Table 2). We note that the individual leaf characters were unimodally distributed rather than clearly separated between the species, indicating that no particular leaf character distinguishes them definitively; rather, the integration of a series of characters is apparently critical for clear differentiation. The morphological index values for the first group, corresponding to *Q. virginiana* samples, ranged between 0.1 and 0.45 with the highest frequency at 0.2. The distribution of the second morphological group, corresponding to *Q. geminata* samples, ranged from 0.55 to 0.9 with the highest frequency at 0.8.

In almost all cases, our original taxonomic assignments made at the time of field collection were maintained after applying the index, except for three individuals from Big Shoals and one individual from Morning Side Nature Center. Maintaining the original taxon assignments did not change the overall results in any significant way. Dividing the samples into north and south did not alter the range of index values for each species. Therefore, the index separates the two species equally well in both northern and southern locations (data not shown). The clustering analysis shows individuals from Big Shoals outside the main clusters for *Q. geminata*, suggesting that these might be hybrids. Other authors have shown that morphological characters in hybrid oaks do not necessarily match genetic markers (Ishida et al., 2003; Gonzalez-Rodriguez et al., 2004).

The first axis of the principal component analysis (PCA; not shown) explained 62% of the total variation and separated individuals into two distinct groups. ANOVA results for the loadings of the first axis of the morphological PCA showed a highly significant effect of species and site but no regional effect when northern and southern populations were grouped separately (Table 2). When latitudes of collection sites were used as a covariate, the species effect was highly significant but latitude was not. Combined, these results provide strong statistical support for using leaf morphology as a means of differentiating individuals into two morphological species.

Allelic diversity and heterozygosity—From the analysis of the 11 microsatellites, we found a total of 195 alleles for the 109 samples for both species combined but only 167 for *Q. geminata* and 157 for *Q. virginiana*. The number of alleles per locus for both species combined ranged from nine (*Zag110*) to 25 (*Zag11*) (Appendix S1, see Supplemental Data with the online version of this article), with a mean value of 14.1 ± 1.6. The mean number of alleles per species was 14.545 ± 1.88 and 13.7 ± 1.32 for *Q. geminata* and *Q. virginiana*, respectively. The mean expected heterozygosity across all loci and both species was 0.79. There were private alleles for each species at every locus (Appendix S1). The average number of private alleles per locus was 3.1 ± 0.76 for *Q. geminata* and 2.273 ± 0.79 for *Q. virginiana*. These data indicate that the two species differ not just in distributions of allele frequencies, but also in allele sizes.

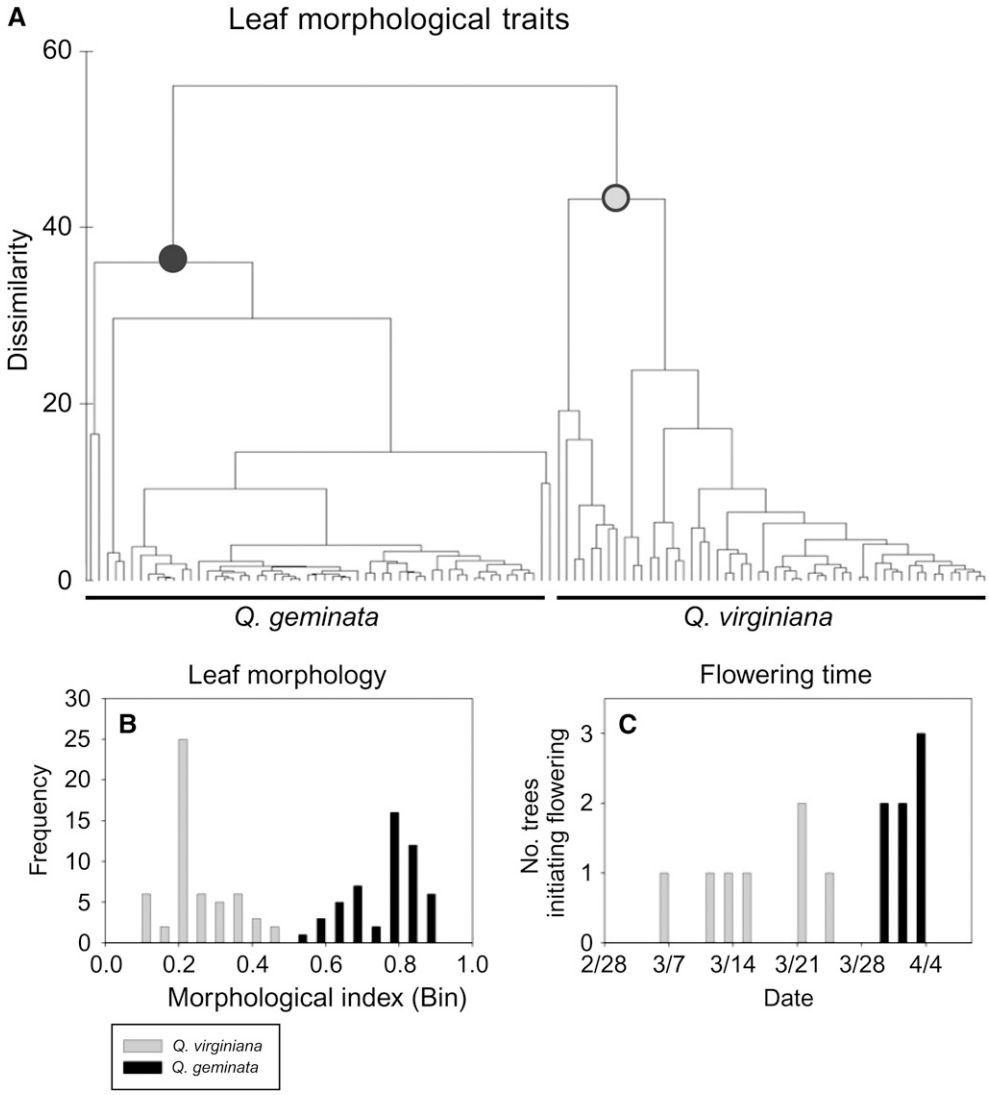

Fig. 2. (A) Hierarchical clustering analysis using Bray–Curtis dissimilarity for five morphological characters of *Quercus* leaves measured on all collected samples for which the nuclear microsatellite markers were analyzed. Leaf characters included rugosity of venation, revoluteness of leaf margins, abaxial pubescence, midvein thickness at 1/3 distance between base of petiole and leaf tip divided by leaf area, and leaf mass per area, following floristic treatments of the two species by Kurz and Godfrey (1962) and Nixon and Muller (1997) as explained in the text. Individuals were assigned to morphological species based on the uppermost hierarchical grouping, as indicated. (B) Morphological trait index showing a bimodal distribution of index frequencies that correspond to the groups in (A). (C) Flowering times of *Q. virginiana* and *Q. geminata* at San Felasco Hammock State Preserve in northern central Florida, monitored for seven trees of each species. White and gray bars show the number of trees initiating male flowering at each date for *Q. virginiana* and *Q. geminata*, respectively. The average timing of flowering initiation is approximately 2 weeks earlier in *Q. virginiana* than in *Q. geminata*.

Global differentiation between species and regions*—R_{ST} *and* F_{ST}**—Globally, there was a significant genetic differentiation between *Q. virginiana* and *Q. geminata* populations pooled for each species across all sites in Florida (R_{ST} 0.179; F_{ST} 0.086***). Over all loci, the estimate of R_{ST} was more than twice the value of F_{ST}. R_{ST} is expected to be higher than F_{ST} when stepwise-like mutations contribute to population differentiation (Ross et al., 1997; Hardy et al., 2003). There was also significant genetic differentiation between the northern and the southern region when all samples were pooled within regions, regardless of species. However, the R_{ST} and F_{ST} values were much lower (R_{ST} 0.038***; F_{ST} 0.011***) than for the between-species comparison. These results indicate that the gene flow between species is much lower than the gene flow between regions within a species. We also analyzed the data locus by locus to determine whether all loci contributed equally to the neutral genetic variation between species. Seven of 11 markers showed significant differentiation (Table 3)., and two loci had particularly high F_{ST} values (0.375, *Zag36* and 0.436, *Zag46*).

***Pairwise differences between the two species in northern and southern Florida*—**Within each geographic region (northern vs. southern Florida), there was significant differentiation between the two species, as in the global analysis (Table 4). The genetic differentiation between the two species in the south was higher than the differentiation in the north. Within each species, there was also significant differentiation between populations in northern and southern Florida. The differentiation between

TABLE 2. ANOVA and ANCOVA for the effects of species (*Quercus geminata* vs. *Q. virginiana*) and location on variation in morphological traits of leaves collected in the field. The loadings of the first principal component analysis, which explained 62% of the variation, were used as an integrative measure of morphological variation. (A) ANOVA with species and site treated as main fixed factors. Trait values were highly significantly differentiated by species and by site. (B) ANOVA with species and region (northern vs. southern Florida) treated as main fixed factors. Morphological traits were significantly differentiated among species but not between regions. (C) ANCOVA with species treated as a main effect and latitude treated as a covariate. There was no effect of latitude and no interaction. In all analyses, the first principal component axis of the morphological analysis was the dependent variable. Very similar results were obtained using the morphological index as the dependent variable. Significant factors ($P < 0.05$ are shown in boldface.

Source	df	SS	*F* ratio	*P*
A)				
Species	**1**	**219.133**	**548.563**	**<0.0001**
Site	**11**	**15.394**	**3.503**	**0.000**
Error	96	38.349		
Total	108	432.486		
B)				
Species	**1**	**275.576**	**539.250**	**<0.0001**
Region	1	0.083	0.162	0.689
Region × Species	1	0.012	0.024	0.878
Error	105	53.659		
Total	108	432.486		
C)				
Latitude	1	0.017	0.034	0.854
Species	**1**	**350.622**	**687.011**	**<0.0001**
Latitude × Species	1	0.088	0.172	0.679
Error	105	53.659		
Total	108	432.486		

regions within *Q. virginiana* was greater than within *Q. geminata*. This pattern might be due to the smaller sample size of *Q. geminata* in the south. Pairwise R_{ST} and F_{ST} values for the two species across all of the collection sites show greater differentiation between species within and across sites than differentiation among populations of the same species across sites (Table 5).

Bayesian analysis of population structure—Structure (Pritchard et al., 2000) assigned the highest posterior probability to a population structure with two ancestral populations (For $k = 1$ ln|P| = −5086.4 ± 9.24, for $k = 2$, ln|P| = −4830.03 ± 16.14, for $k = 3$, ln|P| = −4911.7 ±23.62, $k = 4$, ln|P|= −4907.23 ± 28.78. Individuals were assigned to one of these two populations, which appear to cluster into the two species groups. A bar graph (Fig. 3) shows individuals from *Q. geminata* (based on the morphological index) from the northern and southern region assigned largely to the first population or admixtures of both populations, while individuals of *Q. virginiana* were assigned largely to the second population. There were no well-defined clusters separating the northern populations from the southern populations even when the simulation for four populations was examined.

PCA—The principal coordinate analysis, which groups individuals based on genetic similarity, also showed an obvious separation between the two species (Fig. 4). Two *Q. virginiana* morphotypes from northern Florida appear within the *Q. geminata* cluster. These are from Big Shoals, the northernmost site.

TABLE 3. Locus by locus analysis of pairwise F_{ST} values for the two species (*Q. geminata* and *Q. virginiana*) for 11 microsatellites loci. Pairwise F_{ST} values were calculated pooling sites and regions. *P* values are based on permutation tests and indicate whether F_{ST} values are different than 0; F_{ST} values are in boldface if $P < 0.05$.

Locus	Pairwise F_{ST}	*P*
ZAG36	**0.375**	**0.000**
ZAG11	**0.019**	**0.000**
ZAG1/5	**0.138**	**0.000**
ZAG1/2	0.009	0.072
ZAG102	0.004	0.180
ZAG09	0.007	0.099
ZAG15	**0.079**	**0.000**
ZAG30	**0.026**	**0.000**
ZAG46	**0.436**	**0.000**
ZAG16	0.004	0.207
ZAG110	**0.088**	**0.000**

There are also three outliers along the second axis of variation, along which there is no differentiation between the two species. The individuals with + in the symbol are those that were shown as admixtures in the Bayesian analysis. These show no obvious grouping and are not outliers.

Linkage disequilibrium between loci—We conducted likelihood ratio tests for linkage disequilibrium between pairs of loci (Slatkin and Excoffier, 1996) to determine whether there was evidence for independent sorting of alleles (Appendix S2, see Supplemental Data with the online version of this article). Eight of 11 loci showed significant linkage disequilibrium with at least one other locus. Three loci (*Zag102*, *Zag30*, and *Zag46*) showed associations with four other loci. Note that this test assumes Hardy–Weinberg proportions of genotypes, and departures from HW (found for *Zag11*, *Zag102*, and *Zag15*) may cause significant rejection of the test, possibly accounting for some of these associations. Associations between two pairs of loci (*Zag35* and *Zag46*, *Zag102* and *Zag15*) might be expected due to physical linkage, according to genetic linkage maps for *Quercus robur* and *Q. petreae* (Barreneche et al., 2004). Overall, only 11 of 55 possible pairs of loci showed significant association, indicating that there is a considerable degree of independent sorting of alleles.

Geographic distance—A simple Mantel test was conducted comparing the genetic distance matrix to the log of the geographic distance to determine whether isolation by distance could explain the genetic variation. The two matrices were significantly correlated ($r = 0.52$, $P < 0.0001$). The correlation may have resulted, in part, from differences in species among sites. Therefore, a partial Mantel test was conducted controlling for species differences. The partial correlation coefficient (0.39) in this case was lower, although the genetic and geographic matrices were still highly significantly correlated ($P < 0.001$) based on a null distribution in which the residuals of the regression of the genetic distance matrix on the geographic matrix were permuted 999 times. This indicates that geographic distance significantly explains the genetic distance, even when differences in species are controlled for. A Mantel test was also conducted comparing the genetic distance matrix and the morphological character matrix ($r = 0.47$, $P < 0.0001$). The partial Mantel test controlling for species differences remained significant ($r = 0.19$, $P < 0.001$), although the correlation coefficient was much

TABLE 4. Matrix of pairwise F_{ST} and R_{ST} values between species within regions and between regions within species. Arlequin was used to compare all samples grouped within regions for each species (four groups total). F_{ST} values are shown on the upper diagonal; R_{ST} values are shown on the lower diagonal. Asterisks indicate that values are significantly different from zero based on 1000 permutations: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

		Florida North		Florida South	
Region	Species	*Q. geminata*	*Q. virginiana*	*Q. geminata*	*Q. virginiana*
Florida North	*Q. geminata*	—	0.079***	0.009	0.097***
	Q. virginiana	0.156***	—	0.095***	0.011**
Florida South	*Q. geminata*	0.054*	0.185***	—	0.119***
	Q. virginiana	0.226***	0.037*	0.286***	—

lower. This result indicates that the correspondence between the matrices extends below the species level.

Associations between genetic variation and leaf morphology—We also used a Mantel test to compare the multivariate morphological variation to genetic variation and found a highly significant association ($r = 0.40$, $P < 0.0001$). For each individual trait and locus, tests of association were also conducted (online Appendix S2). Many loci were associated with multiple traits, and all loci except *Zag16* were significantly associated with at least one trait. Five loci (*Zag46*, *Zag15*, *Zag1/5*, *Zag11*, *Zag36*) were associated with three or more traits. All traits were significantly associated with at least one locus. Rugosity of venation, revoluteness of leaf margins, and abaxial pubescence were associated with nine, eight, and seven loci, respectively. The widespread associations between traits and loci indicate that differentiation in traits or in alleles between the two populations is not the result of linkage between individual traits and loci but from the significant population structure that exists between *Q. geminata* and *Q. virginiana*.

Phenotypic trait variation—*Common garden*—Under a common environment, the two species had significant differentiation with respect to total biomass and suites of traits related to patterns of allocation, as well as leaf mass per area and maximum photosynthetic rate (Table 6). *Quercus virginiana* had greater total biomass, greater total leaf area, lower leaf mass per area (LMA), and greater allocation to shoots relative to roots compared to *Q. geminata*. Photosynthetic rates on an area basis (A_{max_area}) were similar. However, *Q. geminata* has more mass per unit area of leaves (higher LMA). Therefore, CO_2 assimilation per unit leaf mass (A_{max_mass}) was significantly higher in *Q. virginiana*. Conductance and transpiration calculated on an area basis, as well as instantaneous water use efficiency (A/g_s) also did not differ between the two species. However, given the lower LMA of *Q. virginiana*, the greater total leaf area produced, and the higher leaf area relative to total biomass, the water loss per unit biomass is greater in *Q. virginiana* than *Q. geminata*.

Phenology—Male flowering was initiated significantly earlier (ca. 2 weeks) in *Q. virginiana* than in *Q. geminata*, based on our initial data for one year at one site in northern central Florida (Table 6B).

DISCUSSION

Genetic and morphological differentiation between Q. geminata and Q. virginiana—There is strong evidence for the discreteness of the sympatric sister species, *Q. geminata* and *Q. virginiana,* based on neutral molecular variation throughout a large area of their distribution in Florida. We demonstrate good correspondence between taxonomically relevant morphological variation and genetic variation and show evidence for ecophysiological differentiation of the two species in a common garden environment (Table 6), corresponding to their contrasting ecological distributions (Fig. 1C). Despite genetic, morphological, and ecological niche differentiation, there is nevertheless clear evidence for gene flow between the species, indicating incomplete reproductive isolation. Five percent of individuals showed mixed ancestry (40–60% admixture) based on Bayesian clustering analysis of the nuclear microsatellite data (Fig. 3). Both the clustering analysis and principal coordinate analysis also show several individuals (~3%) that were morphologically typed as *Q. virginiana* but were typed by molecular markers as *Q. geminata* (Figs. 3, 4). Mismatches between morphology and genotypes could result from high intraspecific variation, including phenotypic plasticity, given that leaf characters were examined on field-collected specimens, but may also indicate hybridization. The morphological assignments were consistent with genotypes in all sites except for Big Shoals and Morning Side, which could indicate either that introgression occurs more frequently at these sites or that other factors there have led to atypical morphology for *Q. geminata* specimens such that they appear more like *Q. virginiana*. The presence of an admixed individual (Fig. 3) at the Big Shoals site in addition to four mismatches supports hybridization as the likely cause.

The relatively clear genetic and morphological differentiation between the two oak species in most sites contrasts documented patterns of high gene flow between other sympatric and closely related oak species (Whittemore and Schaal, 1991; Bacilieri et al., 1996; Tomlinson et al., 2000; Williams et al., 2001; González-Rodríguez et al., 2004, b; Craft and Ashley, 2006; Lexer et al., 2006). The *Q. virginiana–Q. geminata* example corroborates previous studies demonstrating that sympatric oak species appear to maintain distinct morphology (e.g., Camus, 1936–1954; Kurz and Godfrey, 1962; Nixon and Muller, 1997; Manos et al., 1999; Craft et al., 2002; Ishida et al., 2003) and recent phylogenetic work on oaks using multiple low copy nuclear genes that shows surprisingly good resolution for the genus (Oh and Manos, 2008). It also underscores Muller's (1952) assertion that hybridization is much less frequent than commonly assumed and Nixon and Muller's (1997) observation that the two species are easily separable. At the same time, gene flow does occur between *Q. virginiana* and *Q. geminata* at a level that may serve to increase genetic variation within each population, potentially promoting adaptive divergence (Grant and Grant, 2008). While most of the genetic differentiation is found between species, significant population structure also results from isolation by distance, indicating that genetic drift contributes to the observed genetic variation.

TABLE 5. Matrix of pairwise F_{ST} and R_{ST} values between species within sites and between sites within species. Comparisons were carried out in Arlequin for samples grouped within sites for each species (11 groups total). Only sites with more than five samples were included. F_{ST} values are shown on the upper diagonal; R_{ST} values are shown on the lower diagonal. Asterisks indicate that values are significantly different from zero based on 1000 permutations: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

	Big Shoals		San Felasco		Paynes Prairie	Cedar Key	Washington Oaks		Jonathon Dickinison	Taylor Birch	MacArthur
Sites and species	*Q. geminata*	*Q. virginiana*	*Q. geminata*	*Q. virginiana*	*Q. virginiana*	*Q. geminata*	*Q. geminata*	*Q. virginiana*	*Q. geminata*	*Q. virginiana*	*Q. virginiana*
Big Shoals											
Q. geminata	—	0.071***	0.017*	0.097***	0.151***	0.019	0.011	0.129***	0.012	0.130***	0.141***
Q. virginiana	0.178***	—	0.041**	0.017	0.064***	0.077***	0.033*	0.053***	0.055**	0.067***	0.075***
San Felasco Hammock											
Q. geminata	0.059*	0.052	—	0.076***	0.130***	0.019	0.022*	0.096***	0.026*	0.105***	0.097***
Q. virginiana	0.212***	0.025	0.151***	—	0.012	0.093***	0.058***	0.001	0.102***	–0.003	0.03*
Paynes Prairie											
Q. virginiana	0.285***	0.017	0.213***	0.020	—	0.152***	0.111***	–0.006	0.176***	0.032*	0.059***
Cedar Key											
Q. geminata	–0.031	0.135**	0.035	0.193**	0.252***	—	0.038*	0.124***	0.029*	0.135***	0.134***
Washington Oaks											
Q. geminata	0.057	0.073	0.037	0.072	0.165**	0.064	—	0.094***	0.028*	0.092***	0.116***
Q. virginiana	0.213***	0.106*	0.197***	–0.004	0.109*	0.199**	0.121*	—	0.161***	0.017	0.030*
Jonathan Dickinson											
Q. geminata	0.024	0.158**	0.052	0.246***	0.274***	0.021	0.140**	0.310***	—	0.157***	0.122***
Hugh Taylor Birch											
Q. virginiana	0.283***	0.109*	0.198***	0.026	0.156*	0.253***	0.152**	–0.055	0.328***	—	0.052**
MacArthur Beach											
Q. virginiana	0.327***	0.190***	0.329***	0.086*	0.118*	0.317***	0.211**	0.058	0.413***	0.164**	—

Locus-by-locus variation—The 11 microsatellites differed in how informative they were in distinguishing the two species (Table 3). Only seven of the 11 markers were able to differentiate individuals from each species. It is possible that the microsatellites with the high F_{ST} values differentiating the two species are located in genomic regions that are under divergent selection (Clark et al., 2007). Consequently, the markers with low F_{ST} values might be located in regions with repressed recombination. The widespread association between individual traits and loci, as well as the strong association between the multivariate trait dissimilarity matrix and the genetic distance matrix, reinforces the conclusion that mating occurs predominantly within each population causing differentiation in both alleles and in phenotypic traits.

Ecological niche differentiation—*Quercus virginiana* occurs on moister and richer soils than *Q. geminata* based on ecological studies in Florida (Myers and Ewel, 1990; Cavender-Bares et al., 2004a) as well as taxonomic treatments of the species (Kurz and Godfrey, 1962; Nixon and Muller, 1997). Cavender-Bares et al. (2004a) found significant niche differentiation across soil types between the two species based on average soil moisture to 1 m depth (Fig. 1C), pH, calcium content, exchangeable NH_4 and NO_3, and exchangeable P, with *Q. virginiana* occurring on moister, more nutrient rich, and higher pH sites than *Q. geminata*. *Quercus virginiana* has a broader distribution across the range of variation in all of the edaphic factors relative to *Q. geminata* with an overall Levins β habitat breadth (a measure that ranges between 0 and 1; Levins, 1968) of 0.58 ± 0.06 compared to 0.36 ± 0.05 for *Q. geminata*. Corresponding to its more resource rich native habitat, *Q. virginiana* has faster growth and higher photosynthetic rates per gram of leaf tissue than *Q. geminata* when grown in a common environment (Table 5), providing evidence of adaptive differentiation. The resource allocation patterns of *Q. virginiana* support this faster growth strategy. It has thinner leaves (lower LMA) and allocates more to leaf area relative to total plant biomass, thus maximizing light capture and total plant photosynthetic capacity. *Quercus virginiana* also allocates less to root mass than shoot mass, even when examined allometrically (not shown) accounting for differences in plant size (cf. Reich, 2002). In contrast, the slower growth strategy of *Q. geminata* is accompanied by a greater investment in roots relative to shoots and lower allocation to leaf area per unit biomass. Lower evaporative surface area and greater proportional belowground biomass permits *Q. geminata* to conserve water. This conservative strategy matches the lower water availability in their native habitat. Significant functional differentiation that corresponds to habitat differentiation supports the hypothesis that the two species have experienced disruptive selection causing niche differentiation. Ecological divergence in close relatives that occur in sympatry may be expected (Schluter, 2000a, b). Here we show that niche differentiation, maintained in part by limited gene flow likely due to differences in flowering time, helps explain previously observed patterns of phylogenetic overdispersion (close relatives co-occur less than expected) in oak-dominated communities in this region (Cavender-Bares et al., 2004b).

Isolating mechanisms—Several mechanisms have been proposed to prevent sympatric oak species from fusing, including strong selection against maladapted hybrids (e.g., Muller, 1952), genetic incompatibilities (Muller, 1952), or isolation by

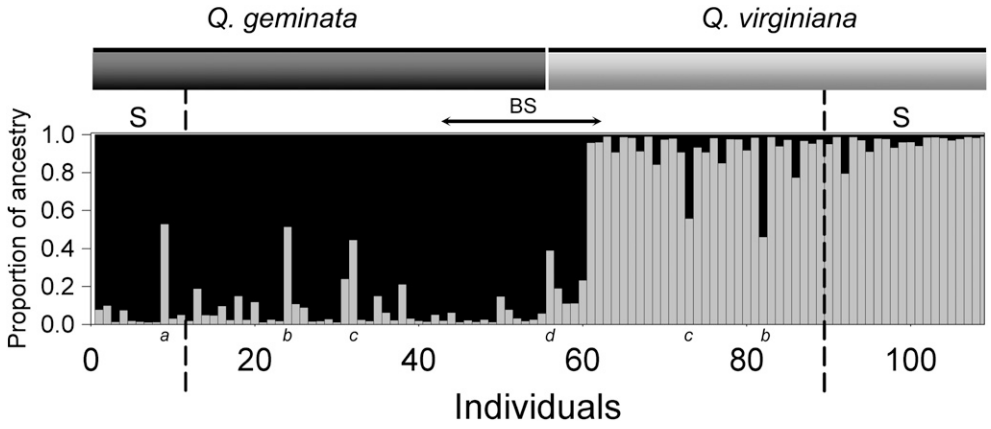

Fig. 3. Results of Bayesian analysis using the program Structure (Pritchard et al., 2000), which assesses the most likely number of populations based allele frequency for 11 nuclear microsatellites using the log-likelihood probability. Population assignments are made for each individual. Black vertical bars represent individuals assigned to *Q. geminata* ancestral group. Gray vertical bars represent individuals assigned to the *Q. virginiana* ancestral group. The black and gray horizontal bars at the top of the figure divide the two species based on morphology. Bars 1–56 are assigned to the *Q. geminata* morphotype; bars 57–109 are assigned to the *Q. virginiana* morphotype. Individuals left of the first hashed line or right of the second hashed line are from southern sites (S), including. Individuals between the hashed lines are from northern sites. Several individuals showed mixed ancestry, indicated by letters below the x-axis were from the following sites: a = MacArthur, b = Washington Oaks, c = San Felasco, d = Big Shoals. Mismatches between genotypes and morphotypes were apparent at the Big Shoals site (BS) where three individuals (58–60) were morphotyped as *Q. virginiana* but assigned to the *Q. geminata* ancestral group based on nuclear microsatellites. Individuals are arranged by site from south to north in the *Q. geminata* group and from north to south in the *Q. virginiana* group, such that the BS site forms a contiguous block in the center, indicated by the two-way arrow above the graph.

time due to phenological variation (Nixon and Muller, 1997). In *Quercus gambelii* Nutt. and *Q. grisea* Liebm., two ecologically differentiated sympatric oak species in western North America, early postfertilization processes associated with pollen performance have been inferred to play a strong role in species fidelity (Williams et al., 2001). The distinct identities of the European sympatric sister species *Q. robur* L. and *Q. petraea* (Mattuschka) Liebl. (e.g., Muir et al., 2000; Coart et al., 2002; but see Gomory and Schmidtova, 2007), have been hypothesized to result from preferential backcrosses that allow pure species populations to develop from hybrid populations (Bacilieri et al., 1996; Petit et al., 2004).

Phenological data from northern central Florida (Fig. 2C) suggests that *Q. virginiana* flowers several weeks before *Q. geminata* in the spring. While these data are from only one year and in one region, this difference in flowering time has been observed by previous investigators in other regions. Sargent (1918) observed that in the vicinity of Biloxi, Mississippi, *Q. virginiana* flowered well before what he called *Q. virginiana* var. *geminata* (equivalent to *Q. geminata*; Nixon, 1985). Nixon (1985) conducted an exhaustive survey across a 3-mile transect from Biloxi to Gulfport, Mississippi in one day, documenting phenological stages for individuals from both species. He found that *Q. virginiana* flowers were further developed than *Q. geminata* flowers, with little overlap in phenological stage between the two species. Nixon (1985) and Nixon and Muller (1997) further reported that herbarium records from throughout the range also showed consistently that *Q. virginiana* flowers earlier than *Q. geminata*, although this difference was more pronounced in southern Florida than in North Carolina.

It is not clear whether differentiation in flowering time is genetically based or the result of phenotypic plasticity, or both. The occurrence of *Q. geminata* on drier soils may cause or reinforce delayed flowering. In the southeastern United States in general, and in Florida, in particular, rainfall is lowest during the late winter months before the onset of spring (Chen and Gerber, 1990). Water availability may pose limits to flower production because flowering requires significant hydraulic support to develop and sustain desiccation-sensitive tissues (Chapotin et al., 2003). Drier soils may thus prevent early flowering and may also select against individuals that are genetically programmed to flower early, providing positive feedback between flowering time and habitat differentiation.

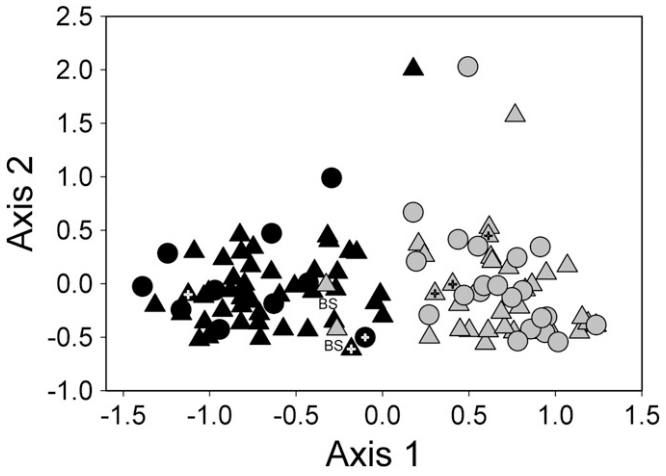

Fig. 4. Principal coordinate analysis showing the first and second axes using 11 microsatellites and mean genetic distances between sites and species in the program GenAlEx (Peakall and Smouse, 2006). Colors represent morphological species groups (*Q. geminata* = black symbols; *Q. virginiana* = gray symbols*)*. Circles are individuals from the northern collection region; triangles are from the southern region. Two individuals from the Big Shoals site (BS) are marked with BS. Symbols with plus sign (+) in the middle are those that are indicated as admixtures in the Bayesian analysis (Fig. 3).

TABLE 6. Functional traits and phenology of *Q. geminata* and *Q. virginiana*. (A) Functional trait, including traits related to growth and gas exchange, of the two live oak species grown in a common garden and (B) mean date of the initiation of male flowering (in Julian days; means shown as a calendar date) for seven trees of each species in northern Central Florida. For each trait, species means, ±SE, the number of individuals (*N*), the *F* ratio and *P* values for ANOVA are given; the species was treated as a main fixed effect. Traits that show significant differentiation are shown in boldface ($P < 0.05$).

	Q. geminata			*Q. virginiana*				
Variable	*N*	Mean	±SE	*N*	Mean	±SE	*F* ratio	*P*
A)								
Morphological trait								
Total biomass (g)	35	16	1.71	36	39.1	4.16	29.26	<0.0001
Plant height (cm)	33	52.3	3.09	33	80.6	5.21	31.93	<0.0001
Total number of leaves	35	55.5	6.22	35	161	18.63	31.64	<0.0001
Total branch length (cm)	33	30	5.86	32	166.4	21.74	37.25	<0.0001
Total leaf mass (g)	35	5.1	0.55	36	10.7	1.15	20.93	<0.0001
Aboveground mass (g)	35	8.6	1.14	36	23.6	2.92	25.74	<0.0001
Root mass (g)	35	7.4	0.72	36	15.5	1.53	25.23	<0.0001
Stem diameter (cm)	31	0.5	0.05	32	0.8	0.04	20.08	<0.0001
Root diameter (cm)	35	1.1	0.09	36	1.5	0.07	10.89	0.0016
A_{max_mass} (mmol/s)	31	0.31	0.012	41	0.37	0.013	10.68	0.0017
Leaf mass per area (g/m^2)	34	154.8	27.4	33	108.3	3.39	9.01	0.0039
Leaf size (cm^2)	33	32.7	1.73	33	41.4	2.4	8.91	0.0042
Root to shoot ratio	35	1.13	0.13	36	0.78	0.05	6.63	0.012
Number of flushes	33	5.4	0.17	32	6	0.18	6.58	0.013
A_{max_area} (A, mmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	31	16.3	0.65	41	17.7	0.64	2.21	0.1419
Conductance (g_s, mmol $H_2O \cdot m^{-2} \cdot s^{1}$)	31	0.436	0.656	41	0.363	0.636	1.11	0.296
WUE (A/g_s)	31	52.2	4.64	41	59	3.93	0.128	0.2626
Leaf lifespan (weeks)	28	45.9	2.52	37	48.3	1.73	0.63	0.4296
Transpiration (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$)	31	5.06	0.274	41	5.21	0.223	0.186	0.6681
B)								
Male flowering date (d)	7	16-Mar	2.43	7	1-Apr	0.68	37.265	<0.0001

Notes: A_{max_area} = CO_2 assimilation per unit area; A_{max_mass} = CO_2 assimilation per unit leaf mass; WUE = water use efficiency.

As discussed, the association of genetically based ecophysiological and performance traits with habitat (Table 6, Fig. 1C) points to specialization and niche differentiation. Therefore, hybrids may be expected to have a fitness disadvantage (e.g., Muller, 1952) except in intermediate habitats. Traits under disruptive selection for contrasting soil types may be genetically correlated with flowering time causing assortative mating and providing a gene flow barrier between the two species that reinforces ecological differentiation. This mechanism would also permit low-level gene flow, particularly under certain environmental conditions, because it does not involve incompatibility.

Assortative mating facilitated by flowering time separation was concluded to be a mechanism causing sympatric speciation in the palms on Lord Howe Island (Savolainen et al., 2006). In the case of the live oaks, however, we argue that sympatric speciation is not necessary to explain the origins or current overlapping distributions of *Q. virginiana* and *Q. geminata*. Historical changes in sea level (Müller et al., 2008) since Florida's emergence above sea level 20 million years ago (Ma) would have provided ample opportunities for allopatric speciation due to island formation and geographic vicariance. Florida underwent fluctuations in sea level as high as 25 m above current levels throughout the Pleistocene with multiple high sea level episodes in the Pliocene and throughout the Miocene. Allopatric speciation is suggested by differences between the two species in allele sizes, not just in the distribution of allele frequencies. Such shifts are likely to have occurred over longer divergence times than changes in frequencies (Slatkin, 1995), suggesting historically low levels of introgression. The current range overlap may be relatively recent resulting from Holocene range expansion during periods of intermediate or low sea level. Once in sympatry, contrasting soil moisture regimes could have promoted flowering time divergence, and habitat associated environmental factors (such as soil moisture and fire regime (Myers, 1985; Cavender-Bares et al., 2004a)) could have selected against hybrids, reinforcing the species boundary.

We hypothesize that *Q. geminata* and *Q. virginiana* are a good example of adaptive speciation (sensu Vences and Wake, 2007) whose ecological niche differentiation is maintained, in part, through non-overlapping flowering times (isolation by time sensu Hendry and Day [2005]). This isolation barrier should be leaky enough to permit limited gene flow between the species under certain conditions but is nevertheless sufficient to maintain ecological differentiation, which may be reinforced by selection against maladapted hybrids.

The isolation barrier may be most leaky farther north, given that five of six examples of mixed ancestry and all genotype–-morphotype mismatches were in the northern region. Nixon (1985) and Nixon and Muller (1997) hypothesized that gene flow should increase with latitude because flowering times of the two species might overlap to a greater extent due to slower warming in the spring. We are currently investigating this hypothesis throughout the entire range of the species through the long-term Live Oak Phenology network (LOPnet). However, greater introgression in the north could also be explained by the fact that there are more sites in the north where both species present and that there is a greater population density of *Q. geminata* in the north. Both factors increase the likelihood of interspecific gene flow.

In conclusion, we demonstrated that *Q. virginiana* and *Q. geminata* are genetically well differentiated and ecologically divergent, indicating nonrandom breeding between the populations. Ecological divergence, which requires limited gene flow between these sympatric sister species, helps explain patterns of phylogenetic overdispersion in oak communities in this region (Cavender-Bares et al., 2004b). Despite discrete species boundaries, interspecific hybridization and introgression occurs with a frequency on the order of 5%, which is sufficient to increase genetic variation in both populations that could promote local adaptation and differentiation (Grant and Grant, 2008). Flowering time divergence is likely to be important in explaining population structure of these sympatric oaks and warrants further study in this and other systems. Of particular interest would be examination of the timing of reproduction in hybrids relative to individuals that can be clearly assigned to a species and to determine how genotypic and flowering time differentiation varies with latitude. Investigation of selection against hybrids would also be important in understanding the mechanisms of isolation between these two species. Variation in fitness among genotypes across habitats could be tested for by reciprocally transplanting seedlings of each species, as well as hybrids from controlled crosses, into habitats associated with each species. Future work in this area will help elucidate mechanisms involved in the generation and maintenance of diversity and contribute to an understanding of the evolutionary processes involved in community assembly.

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APPENDIX 1. Collection information for representative live oak specimens from each population within each species; vouchers are held at the University of Minnesota Herbarium in the Bell Museum of Natural History.

ID	Species	Collection no.	Latitude	Longitude	Collection information (locality; year)
FL-SF53	*Quercus geminata* Laut. et Schleet.	JCB127	29.72745208	–82.44612610	San Felasco Hammock Preserve State Park, FL; 2004
FL-WO9	*Quercus geminata* Laut. et Schleet.	JCB130	29.62607791	–81.20329823	Washington Oaks Gardens State Park, FL; 2004
FL-BS98	*Quercus geminata* Laut. et Schleet.	JCB104	30.33469473	–82.71021866	Big Shoals State Park, 2004
FL-CK26	*Quercus geminata* Laut. et Schleet.	JCB107	29.16308044	–83.02817568	Cedar Key Scrub State Reserve, FL; 2004
FL-JD164	*Quercus geminata* Laut. et Schleet.	JCB112	27.01129627	–80.12140349	Jonathan Dickinson State Park, FL; 2004
FL-MA144	*Quercus geminata* Laut. et Schleet.	JCB116	26.82720152	–80.04515181	John D. MacArthur Beach State Park, FL; 2004
FL-MO81	*Quercus geminata* Laut. et Schleet.	JCB118	29.65688257	–82.27469640	Morningside Nature Center, FL; 2004
FL-SA187	*Quercus geminata* Laut. et Schleet.	JCB123	27.30867606	–80.27379395	Savannas Preserve State Park, FL; 2004
FL-SF60	*Quercus virginiana* Laut. et Schleet.	JCB128	29.77475870	–82.47631889	San Felasco Hammock Preserve State Park, FL; 2004
FL-TB133	*Quercus virginiana* Laut. et Schleet.	JCB129	26.14189619	–80.10444037	Hugh Taylor Birch State Park, FL; 2004
FL-WO15	*Quercus virginiana* Laut. et Schleet.	JCB131	29.70228793	–81.22679153	Washington Oaks Gardens State Park, FL; 2004
FL-BA139	*Quercus virginiana* Laut. et Schleet.	JCB103	25.72624994	–80.24401766	Barnacle Historic State Park, FL; 2004
FL-BS96	*Quercus virginiana* Laut. et Schleet.	JCB105	30.35601628	–82.68791232	Big Shoals State Park, FL; 2004
FL-CK22	*Quercus virginiana* Laut. et Schleet.	JCB106	29.13506191	–83.03378016	Cedar Key Scrub State Reserve, FL; 2004
FL-MA146	*Quercus virginiana* Laut. et Schleet.	JCB115	26.82659240	–80.04504100	John D. MacArthur Beach State Park, FL; 2004
FL-MO83	*Quercus virginiana* Laut. et Schleet.	JCB119	29.65388261	–82.27818268	Morningside Nature Center, FL, 2004
FL-PP32	*Quercus virginiana* Laut. et Schleet.	JCB120	29.61059553	–82.30490612	Paynes Prairie Preserve State Park, FL; 2004
FL-SA189	*Quercus virginiana* Laut. et Schleet.	JCB122	27.31129909	–80.27363336	Savannas Preserve State Park, FL; 2004