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HOW DISCRETE ARE OAK SPECIES? INSIGHTS FROM A HYBRID ZONE BETWEEN *QUERCUS GRISEA* AND *QUERCUS GAMBELII*

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Abstract.—The white oaks *Quercus gambelii* and *Q. grisea* overlap in distribution in New Mexico and Arizona. Within the region of overlap, there are numerous instances of contact between the two taxa. In some areas of contact morphologically, intermediate trees are common, whereas in others, morphologically intermediate trees are rare or absent. We describe a set of RAPD markers that distinguish between the two species and use these markers to examine patterns of gene exchange in an area of contact in the San Mateo Mountains of New Mexico. The markers are highly coincident with morphology and confirm that hybridization between the two species takes place. Despite the occurrence of hybrids, both species remain distinct, even in areas of sympatry, and marker exchange appears to be limited.

Key words.—Hybridization, introgression, mosaic hybrid zone, oak, *Quercus gambelii*, *Quercus grisea*, species.

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Over the past 20 years, the study of hybrid zones has grown from a backwater of systematics to one of the most dynamic areas in evolutionary biology and ecology. The growth can be attributed to demonstrations via molecular genetic surveys of natural populations that hybrid zones are a common phenomenon (Feder 1979; Patton et al. 1979; Harrison 1986; Howard 1986; DePamphilis and Wyatt 1990; Rieseberg et al. 1991; Arnold 1997). Also, there is a heightened awareness among evolutionists that hybrid zones can provide insight into the role of natural selection in the speciation process (Butlin 1989; Howard 1993), the genetic architecture of reproductive isolation (Harrison 1990; Barton and Gale 1993), and the properties of the species boundary (Harrison and Rand 1989; Harrison 1993). Ecologists have also begun to study hybrid zones, spurred on by interests in the effect of hybridization on the ecological and evolutionary dynamics of associated organisms (Whitham 1989; Boecklen and Spellenberg 1990; Floate and Whitham 1993, 1994; Gaylord et al. 1996), the effect of hybridization on the demographic success of field populations (Stucky 1985), the potential role of hybrid zones as centers of biodiversity (Martinsen and Whitham 1994; Whitham et al. 1994), and the importance of hybridization in the origin and conservation of rare taxa (Rieseberg et al. 1989).

It is widely understood that hybridization and hybrid zones are common among oaks (Trelease 1924; Palmer 1948; Muller 1952; Tucker 1961; Cottam et al. 1982; Spellenberg 1995); and oaks have played a central role in the debate over the importance of introgression in plant evolution (Anderson 1949; Whittemore and Schaal 1991; Rieseberg and Wendel 1993), stimulated thinking on the role of ecological factors in limiting hybridization (Stebbins et al. 1947; Muller 1952; Rushton 1993), and served as model organisms in the development of species concepts that rely on ecological criteria (Van Valen 1976). Despite the perception that hybrid zones are a well-documented phenomenon among oaks, few genetic analyses of oak hybrid zones exist. The presence of hybridization typically is inferred on the basis of morphological characters (Rushton 1993), attributes that may be quite plastic and easily misinterpreted (Jones 1959; Rieseberg 1995). Indeed, estimates of the level of hybridization between two well-studied European oaks *Quercus robur* and *Q. petraea*, based on morphology, vary widely (Aas 1993).

Quercus gambelii Nutt. and *Q. grisea* Liebm. are members of different subsections of the white oaks (Nixon 1993). In general, *Q. gambelii* has a more northerly distribution than *Q. grisea*, ranging from Utah and Colorado into northern Mexico (Little 1976), and is typically found at higher elevations, often associated with ponderosa pines. *Quercus grisea* reaches the northern limits of its range in Arizona and New Mexico (Little 1976), and is a codominant in the pinyon-juniper zone. The two taxa are sympatric at various sites, particularly in New Mexico. Where they occur together, they exhibit varying levels of hybridization, according to morphological classification of trees (Tucker 1961; R. Spellenberg, pers. comm.). In general, hybrids appear to be more common on xeric mountain slopes and much less common in more mesic valleys. A lessening of the quantity and quality of male gametes produced by *Q. gambelii* on xeric hillsides may account for this pattern (Williams et al., unpubl. data).

To better understand patterns of gene exchange between *Q. grisea* and *Q. gambelii*, as well as the structure of local hybrid zones, we undertook the development of randomly amplified polymorphic DNA (RAPD) markers that could differentiate between the two taxa. In this paper, we describe the markers and their patterns of inheritance, examine the multivariate association between the RAPD markers and a suite of morphological characters, document the structure (clinal or mosaic) of a zone of overlap and hybridization in the San Mateo Mountains of New Mexico, and assess the effect of hybridization on the genetic distinctness of the two species.

MATERIALS AND METHODS

Study Sites and Organisms

We collected *Q. gambelii* leaves from three sites at which *Q. grisea* did not occur (henceforth called "isolated sites"):

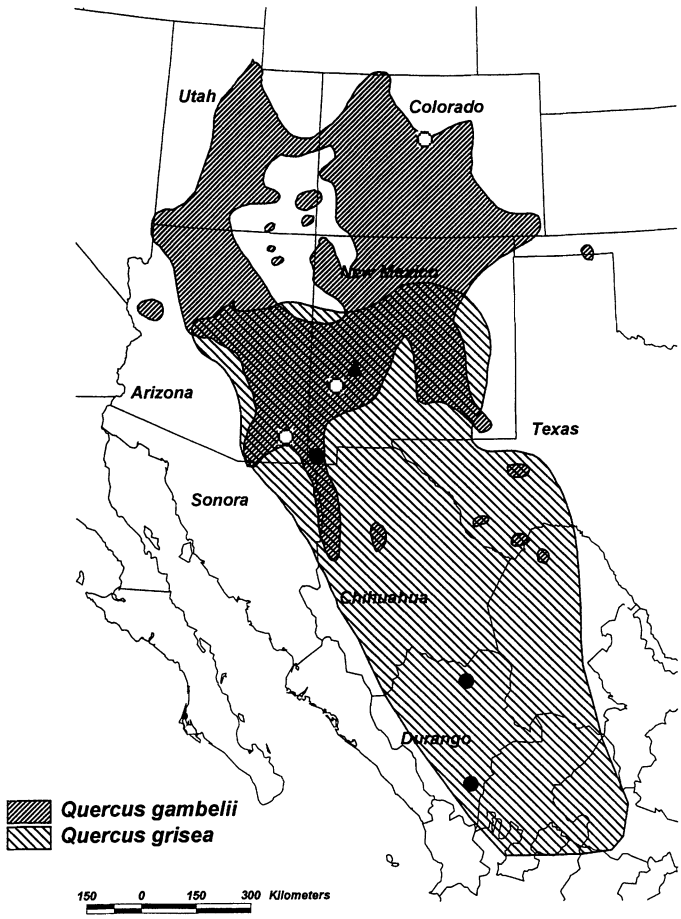

FIG. 1. Map of the southwestern United States and Northern Mexico showing the ranges of *Quercus grisea* and *Q. gambelii* as well as the location of sampling sites. Closed circles represent isolated populations of *Q. grisea*, open circles represent isolated populations of *Q. gambelii*, and the triangle represents the San Mateo Mountains.

a site near Boulder, Colorado, far north of the range of *Q. grisea*, a site in the Black Range Mountains of New Mexico, and a high elevation site in the Chiricahua Mountains of Arizona. We collected *Q. grisea* from three isolated sites, as well: two sites in Mexico far south of the range of *Q. gambelii* and a site in the Peloncillos Mountains of New Mexico. Figure 1 shows the locations of the collecting sites and Table 1 provides additional information about collections, including sample sizes.

To document levels of hybridization and patterns of gene exchange in an area of sympatry, we collected from five populations situated in the Mount Withington area of the San Mateo Mountains of central New Mexico. All sites are located near Forest Road 549 and distances between sites given below represent the distances along the road. From the first site (low site), we collected leaves from trees that were classified as *Q. grisea* on the basis of morphology. The low site is a dry and exposed, relatively flat area. The nearest *Q. gambelii* occurred approximately 400 m away at the bottom of a small runoff-fed valley. The second site (Gaylord's site) is 15.5 km west of the low site. Gaylord's site occupies an east-facing slope of Mount Withington at an elevation of 2460 m. The area is dominated by *Q. grisea*, although a few morphologically intermediate trees occur as well. The third site (mixed site) occurs 2.0 km farther along the road, as one ascends the mountain, at an elevation of about 2590 m. The area, which crosses a ridge top, is dry and exposed and contains both parental species and a broad array of intermediates. Trees were not sampled randomly; instead, we attempted to obtain several representatives of each morphological type. The fourth site (Monica Saddle) is 2.2 km from the mixed site. The Monica Saddle site is on a north-facing slope at 2760 m in the mixed conifer zone. All oaks at this site are morphologically *Q. gambelii*. The fifth and highest site was Grassy Lookout, which is 11.6 km from Monica Saddle. Grassy Lookout is on a west-facing ridge at 2895 m in the spruce-fir zone. All oaks at this site are *Q. gambelii* in appearance. All sampled leaves were stored at −80°C prior to isolation of DNA.

TABLE 1. Oak collecting sites.

Site designation	Geographic coordinates (lat. and long.)	Species composition	n[1]
Isolated sites			
Boulder CO[2]	40°00′54″N, 105°16′12″W	*Q. gambelii*	18
Black Range NM[3]	33°22′40″N, 108°13′35″W	*Q. gambelii*	11
Chiricahuas AZ[4]	31°58′20″N, 109°03′00″W	*Q. gambelii*	12
Peloncillos NM	32°12′00″N, 109°00′30″W	*Q. grisea*	11
Las Nieves MEX[5]	26°22′30″N, 105°19′30″W	*Q. grisea*	4
South Durango MEX	23°50′00″N, 104°45′30″W	*Q. grisea*	5
Mount Withington, NM sites			
Low site	34°58′00″N, 107°27′30″W	*Q. grisea*	20
Gaylord's site	34°57′30″N, 107°30′35″W	*Q. grisea*	10
Mixed site	34°56′30″N, 107°30′35″W	Mixed	88
Monica Saddle	33°53′59″N, 107°30′35″W	*Q. gambelii*	18
Grassy Lookout	33°52′00″N, 107°28′30″W	*Q. gambelii*	17

[1] Sample size.
[2] Colorado, U.S.
[3] New Mexico, U.S.
[4] Arizona, U.S.
[5] Mexico.

TABLE 2. Summary of scoring procedure for the calculation of character index scores. Each tree was characterized for each primer. A character state typical of *Q. grisea* was assigned a score of −1. A character state typical of *Q. gambelii* was assigned a score of +1. The score of a tree could range from −6 to +8.

Primer	Sequence	Fragment size	Character state	Score
111	AGT AGA CGG G	712 bp	Absent	+1
		871 bp	Present	+1
			Absent	−1
285	GGG CGC CTA G	636 bp	Present	−1
			Absent	+1
290	CCG CGA GCA C	906 bp	Present	+1
			Absent	−1
438	AGA CGG CCG G	1066 bp	Present	−1
			Absent	+1
		1193 bp	Present	+1
471	CCG ACC GGA A	517 bp	Present	+1
			Absent	−1
540	CGG ACC GCG T	926 bp	Present	+1
			Absent	−1

To better understand the geographic structure of the zone of sympatry in the San Mateo Mountains, we identified trees as *Q. grisea*, *Q. gambelii*, or hybrid on the basis of morphology along a 6.9-km transect stretching down the side of Mount Withington from Monica Saddle to the mouth of Monica Canyon. To sample, we followed Forest road 549, stopping every 0.3 km to visually inspect all accessible oaks that were within 50 m of the stopping point. The trees were categorized as *Q. gambelii*, *Q. grisea*, or hybrid based on a suite of morphological characters described by Aguilar and Boecklen (1992). Morphological descriptions of both species can be found in the Appendix.

RAPD Analysis

To identify diagnostic or species-specific RAPD markers we surveyed individuals of *Q. grisea* and *Q. gambelii* from isolated populations. "Diagnostic markers" are markers that occur in all individuals of one species and no individuals of a second species. "Species-specific markers" are markers that are unique to one species but not necessarily found in all individuals. Individuals were characterized with 700 10-bp primers. Six primers provided a total of 14 markers that appeared to be specific (or almost specific) to one species or the other. The markers were used to construct character index scores for individuals from sites of allopatry and from sites within the zone of overlap in the San Mateos.

Character index scores provide a good way to visualize the genotypic composition of a population, and hence the level of isolation and gene exchange between two taxa (Howard and Waring 1991). To construct character index scores for the trees characterized via RAPD analysis, a marker specific to *Q. grisea* (the presence or absence of a particular fragment) was assigned a score of −1. A marker specific to *Q. gambelii* was assigned a score of +1. The character index score of an individual represented the sum of its scores over all six primers (a summary of the scoring procedure can be found in Table 2).

For the RAPD assays, standard DNA extraction procedures for plants were used (Whittemore and Schaal 1991). Genomic DNA was stored in TE solution at −20°C. Amplification reactions were performed in volumes of 25 μL containing 5 ng of genomic DNA; 10.4 μL of sterile water; 2.5 μL of 10X amplification buffer; 10.0 μL of a mixture of dNTPs with equal volumes of 1 mM dATP, dCTP, dGTP, and dTTP; 1.0 μL of a 10-bp primer (15 ng/μL); and 0.1 μL of DNA taq polymerase (5 units/μL). The thermal cycler was programmed for one cycle of 3 min at 92°C (denaturation), 1 min at 36°C (annealing), and 2 min at 72°C (extension), followed by 45 cycles of 1 min at 94°C, 1 min at 36°C, and 2 min at 72°C. Amplification products were separated by electrophoresis in a 1.4% agarose gel and were detected by staining with ethidium bromide. Bands containing as little as 1 ng of DNA can be detected by direct examination of such a gel in ultraviolet light. The sizes of amplification products were estimated using methods described in Cain and Murray (1994).

Inheritance Patterns of RAPD Markers

We investigated the pattern of inheritance of markers by amplifying the DNA of parental and F_1 individuals from two hybrid crosses. The first cross was carried out at Monica Canyon, a site between Gaylord's site and the low area. The second cross was performed at the mixed site. We began by stripping male buds from branches of *Q. gambelii* and bagging the branches with number 6 white paper bags before female flowers became receptive. Pollen from *Q. grisea* was gathered by picking catkins from a tree and allowing the catkins to dry and dehisce in paper bags for up to 24 hours. To pollinate female flowers, we temporarily removed bags and touched wet stigmatic surfaces with a pollen dipped plasti-filamented touch-up paint brush. The bags were then replaced on the branches until natural pollination was no longer a possibility.

Mature acorns were allowed to fall into protective screens placed around each branch. Acorns were planted in a greenhouse in a 5:1 mixture of standard potting soil (Sunshine Mix; Fison's Horticulture Inc., Bellevue, WA) and native soil. Pots were watered every four days, and temperature was held to a daily maximum of 25.5°C. Leaves from seedlings with more than two leaves were sampled four months after planting. Samples were placed on ice for several hours and then frozen at −80°C.

Morphological Analysis

We quantified patterns of leaf morphology of trees from the mixed site by haphazardly sampling 10 leaves from each of the 88 trees and measuring perimeter, area, length, width, petiole length, number of lobes, and depth of lobes. We then subsampled three leaves and measured the densities of upper-surface and lower-surface hairs, the number of rays per lower-surface hair, and the number of rays per upper-surface hair. Finally, we calculated means for each tree, and natural-log transformed the means.

We used canonical correlation analysis (Dixon et al. 1990) to test for a linear association between our RAPD markers and the set of morphological variables described above. Ca-

TABLE 3. Frequencies of RAPD fragments in isolated populations of *Q. grisea* and *Q. gambelii*.

	Q. grisea			*Q. gambelii*		
Primer	South Durango	North Durango	Pelen-cillos Mts.	Col.[1]	Chir.[2]	Black Range
111						
n	5	4	11	18	12	11
712	1.00	1.00	1.00	0.16	0.25	0.27
871	—	—	—	0.94	1.00	1.00
285						
n	5	4	11	18	12	11
636	1.00	1.00	1.00	0.06	—	—
290						
n	5	4	11	18	12	11
906	—	—	0.09	0.94	1.00	0.91
438						
n	5	4	11	18	12	11
1066	0.80	1.00	1.00	0.11	—	—
1193	—	—	—	0.61	0.67	0.73
471						
n	5	4	11	18	12	11
517	—	—	0.27	1.00	1.00	1.00
540						
n	5	4	11	18	12	11
926	0.40	—	—	1.00	1.00	1.00

[1] Colorado.
[2] Chiricahua Mountains.

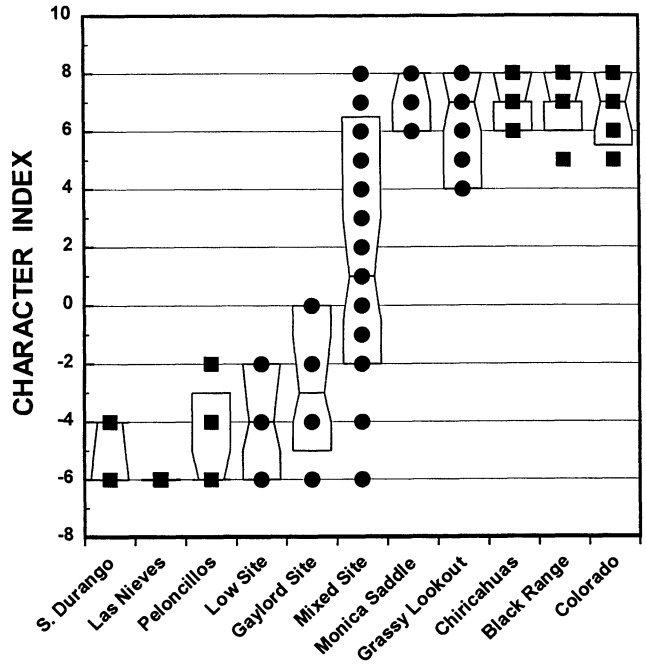

FIG. 2. Box plots of the distribution of character index scores found in isolated and sympatric populations. The upper and lower borders of the boxes represent the 90th and 10th percentiles, respectively. The constricted sections of the boxes span the 25th to 75th percentiles. Medians are given by horizontal bars. The closed circles and closed squares give the range of scores observed in the populations. The closed squares are used for isolated populations and the closed circles are used for populations from the zone of sympatry in the San Mateo Mountains. Pure *Quercus grisea* have character index scores at or near −6 and pure *Q. gambelii* have scores at or near +8.

nonical correlation analysis is an extension of linear regression and correlation analysis and is a common multivariate technique used to examine patterns of covariation between two sets of variables.

RESULTS

The 10-bp primers 111, 285, 290, 438, 471, and 540 from the University of British Columbia (Biotechnology Laboratory, Vancouver) provided character states (the presence or absence of particular fragments) specific to either *Q. grisea* or *Q. gambelii*. The DNA fragments and their frequency of occurrence in isolated populations of the two species are shown in Table 3. To understand how species-specific markers were identified, consider primer 111. Primer 111 provided three markers: (1) an 871-bp fragment unique to isolated *Q. gambelii*; (2) the absence of this fragment, which was considered unique to isolated populations of *Q. grisea* (although one *Q. gambelii* individual from Colorado lacked the fragment); and (3) the absence of a 712-bp fragment, a character state found only in isolated populations of *Q. gambelii*.

Although some of the so-called species-specific markers were not unique to one species or the other, in most cases the presence of a marker in the "wrong" species was limited to a single population. For example, we considered the 926-bp fragment amplified by primer 540 to be a marker of *Q. gambelii*, even though it occurred in *Q. grisea*, because its occurrence was limited to a single population quite distant from the area of sympatry. On the other hand, we did not consider the 712-bp fragment, amplified by primer 111, a *Q. grisea* marker because it occurred in all three isolated populations of *Q. gambelii* at moderate frequencies.

From the 14 markers provided by the six primers we constructed character index profiles of each population. The character index score of an individual tree represented the sum of its scores over all six primers. Because there were more *Q. gambelii* (+1) markers than *Q. grisea* (−1) markers the score of a tree could range from −6 to +8 (Fig. 2). Scores of trees from the three isolated populations of *Q. gambelii* ranged from +5 to +8. Scores of trees from the three isolated populations of *Q. grisea* ranged from −6 to −2. The scores of trees from the mixed population, in which we sampled the full range of morphological types, ranged from −6 to +8, with the majority of scores clustered from −2 to +3. Thus, the character index scores support the contention that extensive hybridization occurs at this site. Because we did not sample randomly at the mixed site, we cannot provide an estimate of the percentage of hybrids in the mixed population. However, 89% of the trees sampled harbored markers of both species and hence showed evidence of mixed ancestry. Table 4 shows the frequency of RAPD fragments in trees sampled at the mixed site, as well as in other populations near or within the zone of sympatry.

Quercus grisea from Gaylord's site, which is 2 km from the mixed site and contains some morphologically intermediate trees, also displayed evidence of mixed ancestry. All

TABLE 4. Frequencies of RAPD fragments in populations from a zone of sympatry between *Q. grisea* and *Q. gambelii*.

Primer	Populations				
	Low site	Gaylord's site	Mixed site	Monica Saddle	Grassy Lookout
111					
n	20	10	88	18	17
712	1.00	1.00	0.68	0.28	0.35
871	0.65	0.10	0.81	1.00	1.00
285					
n	20	10	88	18	17
636	1.00	0.90	0.82	0.11	0.12
290					
n	20	10	88	18	17
906	0.25	0.60	0.93	1.00	1.00
438					
n	20	10	88	18	17
1066	0.95	1.00	0.83	—	0.12
1193	—	—	0.15	0.94	0.59
471					
n	20	10	88	18	17
517	0.05	0.30	0.65	1.00	1.00
540					
n	20	10	88	18	17
926	0.15	0.60	0.69	1.00	1.00

TABLE 5. Fragment patterns of parents and F_1 individuals in two crosses between *Q. grisea* and *Q. gambelii*. The four trees used in the crosses were from the zone of sympatry in the San Mateo Mountains. *Quercus grisea* was the pollen donor in both crosses. Numbers in parentheses under the F_1 column give the number of offspring displaying a particular fragment pattern.

Cross	Primer	Parents		F_1
		gris	*gam*	
1	111	712 871	712 871	712/871(10), 712(1), 871(2)
	285	636	—	636(3), —(10)
	290	—	906	906(13)
	438	1066	—	1066(11), —(2)*
	471	—	517	517(12)
	540	—	926	926(13)
2	111	712	871	712/871(6), 712(8)
	285	636	636	636(14)
	290	906	906	906(14)
	438	1066	1066 1193	1066/1193(12), 1066(2)**
	471	—	517	517(14)
	540	926	926	926(14)

* Statistically significant deviation ($P < 0.05$) from random segregation expectations.

** Statistically significant deviation ($P < 0.01$) from random segregation expectations.

but one tree from this site contained markers of both species. *Quercus grisea* farther from the mixed site also manifested evidence of introgression. At the low site, 80% of the trees had genotypes indicative of mixed ancestry. Despite the evidence of introgression at both sites, the preponderance of markers in most trees were typical of *Q. grisea*. Indeed, most of the trees from the two sites had character index scores that fell within the range found in isolated populations of *Q. grisea* (Fig. 2). This reflects the fact that isolated trees sometimes lacked one or more species-specific markers and occasionally harbored a marker considered typical of the other species.

Quercus gambelii from Monica Saddle and Grassy Lookout showed less evidence of introgression. Eleven percent of the trees sampled from Monica Saddle and 24% of the trees from Grassy Lookout harbored markers of both species and were considered to have mixed ancestry. The character index profiles of these populations closely resembled the character index profiles of isolated *Q. gambelii*, with only two trees (both from Grassy Lookout) exhibiting scores outside the range seen in isolated populations (Fig. 2). Thus, gene flow between the two species appears to be asymmetrical. Genomic elements of *Q. gambelii* occur more frequently in trees that are morphologically *Q. grisea* than vice versa.

Table 5 displays the phenotypes of the parents and offspring of two crosses between *Q. gambelii* and *Q. grisea*. In general, the inheritance patterns of the species-specific RAPD markers of *Q. gambelii* and *Q. grisea* could be accommodated within a Mendelian framework that assumes amplified fragments are dominant character states and each fragment represents a different locus. For example, consider primer 285. In the second cross, both parents displayed the 636-bp fragment, as did all 14 offspring. These patterns are consistent with the idea that one or both parents were homozygous for this fragment. In the first cross, only the *Q. grisea* parent displayed the 636-bp fragment and the fragment occurred in three of 13 offspring. These observations are consistent with the idea that the *Q. grisea* parent was heterozygous for the 636-bp fragment (Chi-square = 3.769, df = 1, $P > 0.05$).

The patterns of inheritance that could not be accommodated within this framework were those involving primer 438. In the second cross, the female parent exhibited the 1066-bp fragment and the 1193-bp fragment, whereas the male parent displayed only the 1066-bp fragment. All of the offspring exhibited the 1066-bp fragment, and 12 of 14 displayed the 1193-bp fragment. The first result is consistent with the hypothesis that one or both parents were homozygous for the 1066-bp fragment. However, the second result deviates significantly from a model that assumes the female parent was heterozygous for the 1193-bp fragment (Chi-square = 7.14, df = 1, $P < 0.01$). The patterns of inheritance in the first cross, in which the female parent exhibited neither fragment and the male parent displayed the 1066-bp fragment, suggests that the male parent was heterozygous for the fragment. But again, a greater than expected number of offspring displayed the fragment (Chi-square = 6.230, df = 1, $P < 0.05$).

The irregular segregation reported here for the products of primer 438 does not preclude the use of this primer in the analysis of the *Q. grisea–Q. gambelii* hybrid zone. Despite the segregation distortion, all of the phenotypes displayed by the offspring could be inferred to have occurred in the parents. In other words, the banding patterns associated with the primer do not appear to represent artifacts.

Canonical correlation analysis of trees from the mixed site indicated a highly significant linear association between the presence or absence of RAPD markers and the set of morphological variables (Table 6). A single pair of canonical variables was significant (Chi-square = 187.5, df = 88, $P < 0.001$) and exhibited a canonical correlation of 0.89 (Fig. 3).

TABLE 6. Canonical variable (CV) loadings for the set of RAPD markers and for the set of morphological variables. RAPD markers are indicated by primer number and fragment size. Variable loadings are correlations between the canonical variables and the original variables.

RAPD markers (primer: fragment)	CV1	Morphological variables	CV1
285: 636 bp	0.784	Perimeter	−0.953
290: 906 bp	−0.225	Area	−0.890
471: 517 bp	−0.682	Length	−0.813
438: 1066 bp	0.662	Width	−0.898
438: 1193 bp	−0.354	Petiole length	−0.709
111: 871 bp	−0.318	Number of lobes	−0.591
111: 712 bp	0.685	Lobe depth	−0.903
540: 926 bp	−0.452	Density of hairs (lower surface)	−0.591
		Rays per hair (lower surface)	0.867
		Density of hairs (upper surface)	−0.406
		Rays per hair (upper surface)	0.903

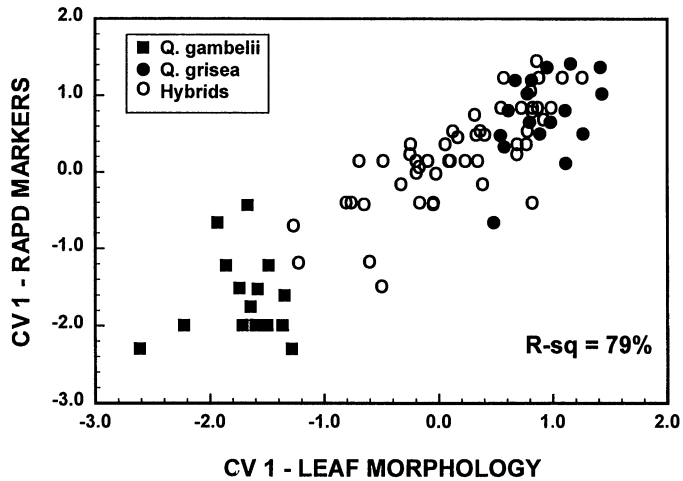

FIG. 3. Multivariate association between the set of RAPD markers and the set of morphological variables as described by the first pair of canonical variables (CV1). The relationship is highly significant ($P < 0.001$) and exhibits a squared multiple correlation coefficient of 0.789. The characterization of individual trees as *Quercus gambelii*, *Q. grisea*, or hybrid is based on the morphological classification scheme of Aguilar and Boecklen (1992).

The first canonical variable representing leaf morphology chiefly corresponded to decreasing leaf size and increasing numbers of rays per leaf hair, while the first canonical variable representing the RAPD markers largely corresponded to markers generated by four primers: 285 (636-bp fragment); 438 (1066-bp fragment); 111 (712-bp fragment); and 471 (absence of 517-bp fragment) (Table 6). The canonical variable generally ordinated individuals from those resembling *Q. gambelii* (lower left in Fig. 3) to those resembling *Q. grisea* (upper right in Fig. 3).

The set of RAPD markers faithfully represented variation in the set of morphological variables, as all squared multiple correlation coefficients between the morphological variables and the set of RAPD markers were significant (Table 7). The RAPD markers explained over 50% of the variation in leaf area, perimeter, length, width, petiole length, lobe depth, and rays per leaf hair (see R^2 column, Table 7). The morphological variables with the weakest associations with RAPD markers were numbers of lobes, density of lower surface hairs, and density of upper surface hairs. RAPD markers explained approximately 35% of the variation in the first two variables and approximately 19% of the variation in the last variable (Table 7).

The set of morphological variables significantly predicted the presence or absence of some RAPD markers, but not others (Table 7). For example, the set of morphological variables explained approximately 39–53% ($P < 0.001$) of the variation in the presence of the 636-bp fragment amplified by primer 285, the 517-bp fragment amplified by primer 471, and the 712-bp fragment amplified by primer 111. However, the set of morphological variables explained less than 16% ($P > 0.32$) of the variation in the presence of the 906-bp fragment amplified by primer 290.

The fact that RAPDs and morphology generally agree with regard to the identification of *Q. gambelii*, *Q. grisea*, and hybrids means that it is reasonable to rely on morphology to assess the structure of the zone of contact between the two taxa on the slopes of Mount Withington. Table 8 shows the taxonomic composition of oaks at sites along a 6.9-km transect stretching from Monica Saddle near the top of the mountain to Monica Canyon at the base of the mountain. *Quercus gambelii* clearly predominates at sites near the top and *Q. grisea* clearly predominates at sites near the base. In between, the two species form a mosaic with *Q. grisea* predominating on open, south-facing slopes, and *Q. gambelii* predominating

TABLE 7. Squared multiple correlations between each morphological variable and the set of RAPD markers, and between each RAPD marker and the set of morphological variables.

Morphological variable	R^2	$F_{8,74}$	P
Perimeter	0.724	24.27	< 0.001
Area	0.655	17.55	< 0.001
Length	0.556	11.57	< 0.001
Width	0.666	18.47	< 0.001
Petiole length	0.513	9.75	< 0.001
Number of lobes	0.350	4.96	< 0.001
Lobe depth	0.663	18.16	< 0.001
Density of hairs (lower surface)	0.346	4.89	< 0.001
Rays per hair (lower surface)	0.618	14.98	< 0.001
Density of hairs (upper surface)	0.190	2.17	0.040
Rays per hair (upper surface)	0.651	17.25	< 0.001
RAPD marker (primer: fragment)	**R^2**	**$F_{11,71}$**	**P**
285: 636 bp	0.533	7.38	< 0.001
290: 906 bp	0.154	1.17	0.328
471: 517 bp	0.482	6.01	< 0.001
438: 1066 bp	0.385	4.04	< 0.001
438: 1193 bp	0.221	1.83	0.085
111: 871 bp	0.226	1.88	0.078
111: 712 bp	0.456	5.40	< 0.001
540: 926 bp	0.236	1.99	0.060

TABLE 8. The percentage of *Q. grisea, Q. gambelii,* and hybrids present at a series of sites extending from Monica Saddle near the top of Mount Withington to the base of the mountain. Sites are separated by 0.3 km. All accessible trees (to a maximum of 20) within 50 meters of the stopping point were examined and categorized on the basis of a suite of morphological characters.

Site	*n*	*%gam*	*%gris*	%hybrids	Description of site
Monica	20	100	—	—	North-facing slope
0.3 km	20	100	—	—	East by northeast–facing slope
0.6 km	20	100	—	—	East-facing slope
0.9 km	20	100	—	—	North side of draw
1.2 km	20	55	10	35	East-facing slope
1.5 km	20	15	60	25	East by southeast–facing slope
1.8 km	0	—	—	—	East by southeast–facing slope
2.1 km	20	90	—	10	Northeast-facing slope (within 100 m of southern border of mixed site)
2.4 km	20	30	20	50	East by northeast–facing slope (on northern border of mixed site)
2.7 km	20	75	—	25	North-facing slope
3.0 km	20	90	—	10	North to south–facing outcrop
3.3 km	1	100	—	—	Southeast-facing slope
3.6 km	1	—	100	—	Southeast-facing slope
3.9 km	20	100	—	—	North by northeast–facing slope
4.2 km	0	—	—	—	Southeast-facing ridge
4.5 km	20	100	—	—	North-facing slope
4.8 km	6	—	100	—	Southeast-facing slope
5.1 km	9	—	78	22	South-facing slope
5.4 km	9	—	89	11	Northeast by east–facing slope
5.7 km	0	—	—	—	Southeast-facing, almost flat
6.0 km	0	—	—	—	Almost flat
6.3 km	0	—	—	—	Almost flat
6.6 km	0	—	—	—	Almost flat
6.9 km	11	—	73	27	Almost flat

on north-facing slopes and in draws. Wherever the two species occur together, intermediates also occur, but hybrids do not appear to outnumber morphologically "pure" individuals at any of the sites surveyed.

DISCUSSION

Quercus grisea and *Q. gambelii* are clearly quite similar genetically. In our survey of 700 10-bp primers, only six provided character states that were consistently associated with one species or the other. We hasten to note that many primers failed to amplify any DNA fragments and others amplified poorly and inconsistently. Moreover, we ignored some potentially diagnostic markers because the intensity of banding was weak. Therefore, six of 700 should not be interpreted as a genetic distance measure. Nevertheless, the two species were similar genetically and this similarity contrasts rather sharply with their morphological distinctness and their placement in separate subsections of the white oaks. The small number of species-specific markers tends to support the inference of Stebbins (1950) that the number of genes that differ between species of oaks is considerably smaller than is the case with most other plant species. Stebbins based this inference on the finding, unusual among plants, that trees resembling parentals could be recovered from the progeny of hybrid oaks, even when the number of progeny was quite small. Electrophoretic data also support Stebbins—genetic distances between species of oaks tend to be relatively small (Guttman and Weigt 1989; Kim et al. 1993).

Although the number of markers that separate *Q. gambelii* and *Q. grisea* is relatively small, the fact that these markers demonstrated geographic consistency indicates that each species is distinct and each has some genetic cohesion. Perhaps the most compelling evidence for the cohesion of the species emerges from a consideration of their genetic interactions in the San Mateo Mountains. At the mixed site, on the north side of Mount Withington, both parentals as well as a full suite of intermediate individuals occur, according to the genetic as well as morphological evidence (Figs. 2, 3). Moreover, other morphologically intermediate trees occur at numerous sites along a transect stretching from Monica Saddle to Monica Canyon (Table 8). Despite the hybridization, trees that appear to be "pure" on the basis of morphology predominate at most sampling sites on Mount Withington. Moreover, the character index scores of these trees based on RAPD genotypes, with only a few exceptions, fall within the range of scores found in isolated populations of *Q. grisea* and *Q. gambelii* (Fig. 2). As suggested by this finding, the correspondence between morphological variables and genetic markers is quite high among trees from the mixed site (canonical correlation of 0.89), and the degree of intermediacy in morphological characters agrees well with the degree of intermediacy in RAPD markers (Fig. 3).

Although both *Q. grisea* and *Q. gambelii* remain distinct in areas of sympatry, evidence of introgression exists. By this we mean that individuals that appear to be pure species on the basis of morphology harbor RAPD markers characteristic of the other species. This is particularly true of *Q. grisea.* At Gaylord's site, which is near the mixed site and at which some morphologically intermediate trees occur, all but one *Q. grisea* harbored at least one RAPD marker typical of *Q. gambelii.* The markers present were more than a small subset of Q. *gambelii* markers. Every marker considered unique to *Q. gambelii* occurred in at least one individual, with the exception of the 1193-bp fragment amplified by primer 438 (Table 4).

Even at a site lacking intermediate individuals (the low site), the majority of *Q. grisea* harbored one or more markers associated with *Q. gambelii.* However, the array of introgressant markers was smaller than at Gaylord's site. Absent, or largely absent, was the 1193-bp fragment amplified by primer 438, as well as *Q. gambelii* character states associated with primers 285 and 471 (Table 4). These results indicate that some *Q. gambelii* RAPD markers do not flow across the *Q. grisea* species boundary with the facility of others.

Introgression appears to be more limited in *Q. gambelii.* Eleven percent of individuals sampled from Monica Saddle and 24% of individuals from Grassy Lookout harbored markers of *Q. grisea.* The introgressant markers were limited to two primers, 285 and 438 (Table 4). It is of interest that these were two of the three primers that gave little evidence of

gene flow in the opposite direction. The asymmetry in gene flow between the two species may reflect the remnants of past hybridization as *Q. grisea* moved up the slopes of Mount Withington with the gradual drying out of the southwestern United States (Van Devender and Spaulding 1979). Alternatively, *Q. grisea* markers or genes closely linked to them, may be more strongly selected against in *Q. gambelii*, than are *Q. gambelii* markers in *Q. grisea*.

Lack of evidence for extensive marker exchange between *Q. gambelii* and *Q. grisea* is consistent with studies of nuclear gene markers in other oak species (Guttman and Weigt 1989; Ducousso et al. 1993) and stands in contrast to patterns of variation in chloroplast DNA in white oaks from the eastern United States. Whittemore and Schaal (1991) reported that a cladogram derived from chloroplast DNA polymorphisms among five white oak species was concordant not with species boundaries, but with the geographical locations of the populations. They noted that the same species were well differentiated morphologically, isozymically, and with regard to a ribosomal DNA marker. The latter results, as well as the results reported here, suggest that, among white oaks, nuclear DNA is not exchanged as freely as organellar DNA.

The abrupt genetic and morphological discontinuity between *Q. gambelii* and *Q. grisea*, despite areas of hybridization, indicates that selection acts to maintain coadapted complexes of alleles in the two species. Whether these alleles are spread throughout the genome, or localized in a few regions, is not clear. But it should be noted that the genetic similarity of the two taxa may reflect the long-term effects of introgression and may be an indication that the co-adapted alleles are quite localized.

Although hybrid zones are often interpreted as clines (Barton and Hewitt 1985), an ever-increasing number of empirical studies describe zones in which patterns of variation do not resemble simple monotonic clines (Harrison 1986; Howard 1986; Rand and Harrison 1989; Howard and Waring 1991; Shoemaker et al. 1994). Instead, these mosaic zones are characterized by patches of pure species populations and mixed populations scattered across a zone of overlap. This broken pattern of distribution can be considered *prima facie* evidence for the importance of environmental gradients in determining the position, width, and maintenance of the zone (Barton and Hewitt 1985).

The zone of overlap between *Q. grisea* and *Q. gambelii* in the San Mateo Mountains of New Mexico can be added to the list of mosaic hybrid zones. There is not a gradual transition from *Q. gambelii* to *Q. grisea* as one moves down the northeastern slope of Mount Withington. Rather the distribution of the two taxa and hybrids is decidedly patchy, with sites where *Q. grisea* predominate sometimes occurring above sites where *Q. gambelli* predominate (Table 8). In general, *Q. grisea* appear to be associated with drier sites facing the southeast and *Q. gambelii* appear to be associated with more mesic sites facing the northeast (Table 8). This pattern is consistent with the habitat association patterns of the two taxa outside areas of overlap and suggests that mosaicism within the zone of sympatry reflects mosaicism in the environment.

At virtually all sites where *Q. grisea* and *Q. gambelii* coexist in the San Mateo Mountains, morphologically intermediate individuals occur as well. At most sites, hybrids represent a minority of the population, but occasionally they predominate (e.g., at the mixed site in this study). Despite the varying levels of hybridization, the two taxa tend to remain distinct both within and outside areas of sympatry. The factors responsible for this genetic isolation remain unclear. Selection may play a role, but other factors, such as conspecific pollen precedence (Williams, unpubl. data), may also be important.

The lines between oak species are often presumed to be quite fuzzy by the biological community, but our results suggest that oak species that hybridize in nature can remain distinct over at least part of their genomes. These results are in accord with other genetic studies of oaks (Bacilieri et al. 1993) and indicate that oaks may not represent a greater challenge to traditional concepts of species than many other plant and animal taxa that form hybrid zones with close relatives.

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APPENDIX

Quercus gambelii is a tree composed of multiple ramets that are 1.8–5.0 m tall. Leaves are winter deciduous, green, 6.0–11.0 cm long, 3.0–6.0 cm wide, obovate, heavily lobed, shiny and minutely stellate on the upper surface, and dull green and scarcely stellate on the lower surface. *Quercus grisea* is a shrub composed of multiple ramets that are 0.3–1.5 m tall. Leaves are evergreen (or under extreme conditions deciduous), leathery, pale green, 2.0–4.0 cm long, 1.0–1.5 cm wide, lanceolate or oblong, entire, sometimes wavy, dull and moderately stellate on the upper surface, and densely covered with stellate pubescence on the lower surface. The F_1 hybrid *Q. undulata* Torr. is a shrub composed of multiple ramets that are 1.0–2.5 m tall. Leaves, which wither and brown in the winter but are retained for varying amounts of time, are dull green or pale green, 3.5–5.5 cm long, 1.5–2.5 cm wide, obovate to oblong, slightly lobed to serrate, sublustrous to dull and scarcely to moderately stellate on the upper surface, and dull green and moderately stellate to pale green and densely covered with stellate pubescence on the lower surface.