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MORPHOLOGICAL AND RAPD ANALYSIS OF HYBRIDIZATION BETWEEN *QUERCUS AFFINIS* AND *Q. LAURINA* (FAGACEAE), TWO MEXICAN RED OAKS[1]

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Quercus affinis and *Q. laurina* are two closely related Mexican red oaks with partially overlapping distributions. Within the area of overlap, there are localities where morphological intergradation occurs. A previous hypothesis explained this pattern as a result of secondary contact between the two species, followed by hybridization and introgression. This possibility was analyzed here by examining foliar and genetic variation in 16 localities situated along a macrogeographic gradient, which included morphologically representative populations of both species and populations from within the area of overlap. Maximum-likelihood hybrid index scores calculated from nine semi-diagnostic RAPD markers indicated a shift in the genetic composition of populations from one species to the other along the macrogeographic gradient, with genetically intermediate populations situated in the area of overlap. Foliar variation followed a partially congruent pattern, but *Q. laurina*-like morphology predominated in some of the genetically intermediate populations. There were several instances of correlated frequency changeovers of single RAPD markers and morphological characters along the macrogeographic gradient and a few cases of markedly parallel patterns between markers. The results were interpreted as consistent with a hypothesis of secondary contact between the two oak species that has resulted in some differential introgression among markers.

Key words: hybrid zones; hybridization; *Quercus affinis*; *Quercus laurina*; RAPD markers.

Hybridization and introgression are considered important influences in the adaptive evolution and diversification of many plant groups (Rieseberg and Ellstrand, 1993; Rieseberg and Wendel, 1993; Arnold, 1997). The genus *Quercus* (the oaks) has long been considered a group with an unusually high frequency of interspecific hybridization (Burger, 1975; Van Valen, 1976). Although morphological patterns of variation that support or are consistent with hybridization between many oak species pairs have been reported (Palmer, 1948; Cooperrider, 1957; Brophy and Parnell, 1974; Hardin, 1975; Jensen et al., 1993; Bacon and Spellenberg, 1996), detailed studies of the patterns and frequency of interspecific nuclear and cytoplasmic genetic exchange have focused on only a few species complexes, notably European and North American white oaks. The results of these studies include the demonstration of extensive local sharing of cytoplasmic haplotypes (i.e., cytoplasmic introgression) among sympatric white oak species (Whittemore and Schaal, 1991; Dumolin-Lapègue et al., 1999), low genetic differentiation between possibly hybridizing species as measured by allozymes and nuclear molecular markers (Hokanson et al., 1993; Kleinschmit et al., 1995; Samuel et al., 1995; Bodénès et al., 1997; Howard et al., 1997; Bruschi et al., 2000), direct evidence of considerable asymmetric pollination of *Q. robur* progenies by *Q. petraea* in a local stand (Bacilieri et al., 1996b), and the documentation of a mosaic hybrid zone between *Q. grisea* and *Q. gambelli* in New Mexico (Howard et al., 1997).

Red oaks are restricted to the New World and are in general thought to hybridize more extensively than white oaks (Guttman and Weigt, 1989; Jensen et al., 1993), but fewer detailed studies of hybridization processes have been conducted on this group (Jensen et al., 1993; Bacon and Spellenberg, 1996). Mexico is considered the center of diversity of *Quercus* in the Western Hemisphere, with an estimated total number of species between 135 and 150 and 86 endemics (Nixon, 1993a). Of these, 55 species are red oaks, with 41 of them endemic. Diverse topography, climate, and habitat probably exerted an important influence in the process of radiation and maintenance of oak species diversity in this region (Nixon, 1993a). Paleobotanical evidence suggests that the cooler, drier, and more variable climates that developed after the Eocene-Oligocene transition in North America encouraged the evolution and migration of *Quercus* (Daghlian and Crepet, 1983; Borgardt and Pigg, 1999). The history of oaks in Mexico was probably characterized by range shifts, expansions, and contractions, a product of climatic changes producing the opportunity for periods of differentiation followed by secondary contact between taxa (Bacon and Spellenberg, 1996). However, very few studies have been conducted on population genetics, hybridization processes, and speciation of Mexican oaks.

Recently, several red oak complexes have been identified in Mexico by specialists (Valencia, 1994; Bacon and Spellenberg, 1996). One of these complexes is formed by the closely related species *Quercus affinis* Scheidweiler and *Q. laurina* Humboldt

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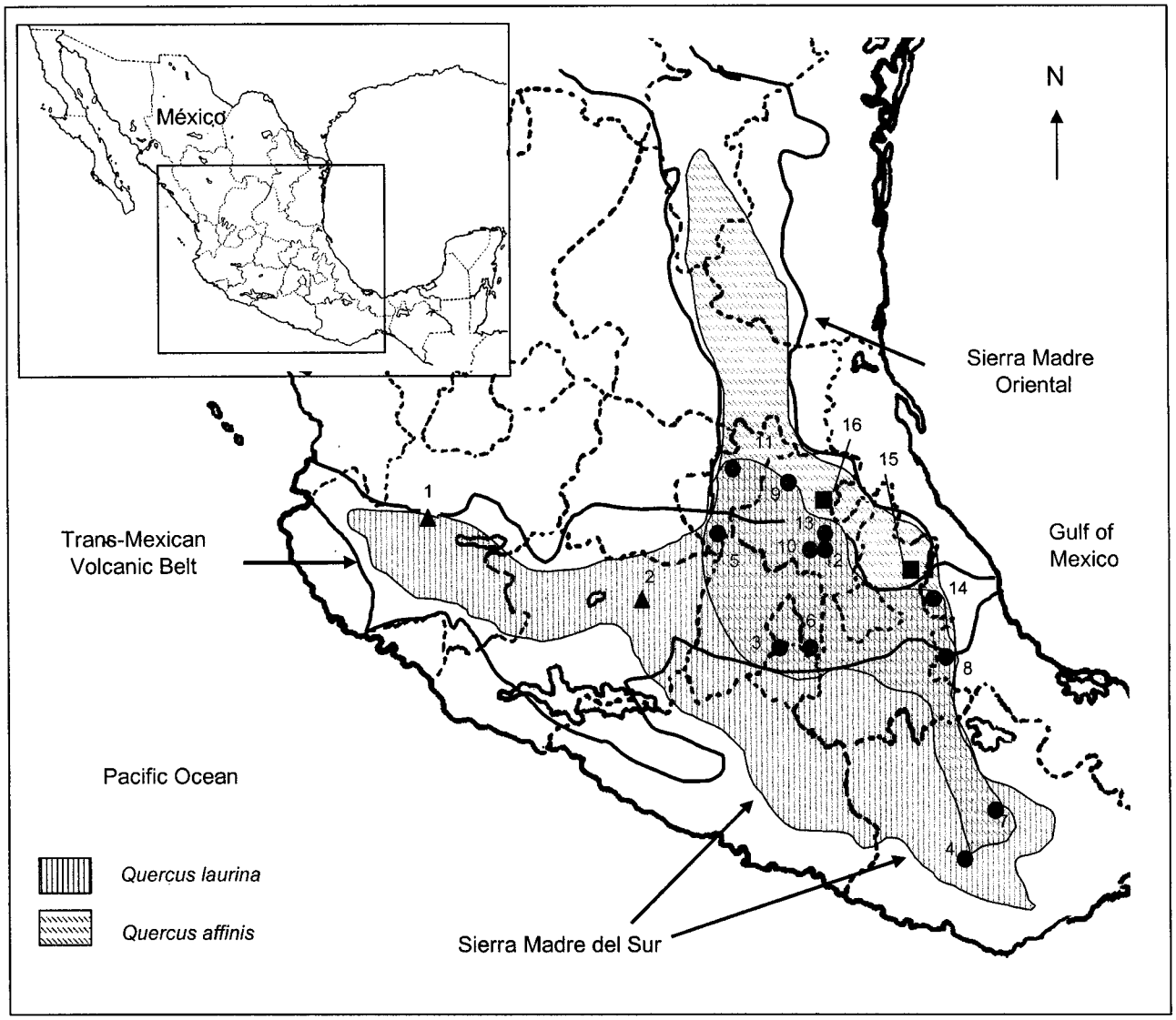

Fig. 1. Map of the geographical range of *Quercus affinis* and *Q. laurina* and the locations of the sampled populations. Squares symbolize morphologically representative populations of *Q. affinis*, triangles symbolize morphologically representative populations of *Q. laurina*, and circles symbolize populations in which morphological intergradation occurs. Numbers next to each symbol correspond to those given in Table 1.

et Bonpland (subgenus *Quercus*, section *Lobate*; Nixon, 1993b), which have partially overlapping distributions and show morphological intergradation within the region of overlap, but otherwise remain distinct in localities outside of this area. The objective of this study was to gain insight into the origin and structure of this hybrid zone.

The two species in this complex show a wide range of morphological variation and are difficult to delimit taxonomically. *Quercus laurina* probably also hybridizes with at least four other red oak species besides *Q. affinis* (*Q. crassifolia*, *Q. crassipes*, *Q. mexicana*, and *Q. rubramenta*). According to a recent systematic study, 25 taxa, including species and varieties, are synonymous with *Q. laurina* and nine with *Q. affinis* (Valencia, 1994). An analysis of phenology, wood anatomy, foliar architecture, and pollen ultrastructure revealed that few characters are consistently differentiated between both species across the whole range of their geographical distribution (Valencia, 1994). Morphological intergradation occurs in localities situated in the eastern portion of the Trans-Mexican Volcanic Belt and northern Oaxaca, while individuals from populations outside of these areas usually can be unambiguously determined (Valencia, 1994). Morphologically representative populations of *Q. laurina* are distributed along elevations of the Sierra Madre del Sur and the western region of the volcanic belt, at altitudes that vary between 2440 and 3065 m, and morphologically representative populations of *Q. affinis* are along the Sierra Madre Oriental, with an altitudinal range of 1600–2800 m (Fig. 1). From this pattern of morphological geographic variation, *Q. affinis* and *Q. laurina* were hypothesized to be two closely related species that may have diverged in isolation (*Q. affinis* in the Sierra Madre Oriental and *Q. laurina* in the Sierra Madre del Sur) probably during the mid- or late Pliocene and entered into secondary contact in the volcanic belt after a period of range expansion favored by climatic conditions at the beginning of the Pleistocene. The climatic pulsations of the Pleistocene probably determined recurrent

periods of secondary contact and periods of range contraction and divergence (Valencia, 1994). According to this hypothesis, as a result of frequent hybridization and introgression during periods of secondary contact, the morphological differences between the two species have been obscured in some localities (Valencia, 1994).

However, morphological intergradation may arise from secondary contact between differentiated populations or be primary in origin (Durrett et al., 2000). In the second case, morphological and genetic intermediacy represents the ancestral state, and apparent parents are the result of divergence from a common gene pool (Beckstrom-Sternberg et al., 1991). Although sometimes it may be difficult to discriminate between either scenario (Endler, 1977), features of hybrid zones such as parallel patterns in the changeovers from the character states of one parental population to those of the other for several presumably independent characters across the area of intergradation and nonrandom associations among markers derived from the same parental population in the center of the zone (i.e., linkage disequilibrium) are generally considered strong indications of an origin via secondary contact (Durrett et al., 2000). Molecular markers can provide a large number of neutral and independent characters that are extremely useful in the genetic analysis of hybrid zones (Rieseberg and Ellstrand, 1993). Compared to allozymes, markers such as AFLP or RAPD are more appropriate for the study of oak hybrid zones, because they have usually provided better discrimination between closely related, hybridizing oak species (Bodénès et al., 1997; Coart et al., 2002). RAPD markers have already been used in the study of oak hybrid zones (Howard et al., 1997).

In this study we measured foliar attributes to characterize phenotypic differentiation between isolated populations of *Q. affinis* and *Q. laurina*, identified several RAPD markers that showed substantial frequency variation between these populations, and then used these traits to assess the structure of morphological and genetic variation at a macrogeographic level, which included the distribution area of both species and the intergradation zone. The particular goals were (a) to determine if the hypothesis of a secondary hybrid zone between *Q. affinis* and *Q. laurina* is supported, (b) to assess the degree of congruence between morphological and molecular variation, and (c) to gain insight into the macrogeographic spatial structure of the intergradation zone.

MATERIALS AND METHODS

Selection of localities and sampling procedure—Examination of 431 specimens from 154 different localities deposited at the National Herbarium of Mexico (MEXU) aided in identifying populations morphologically representative of *Q. affinis* and *Q. laurina*, as well as potential sites of morphological intergradation between them. Two populations considered morphologically representative of each species were sampled: *Q. laurina* from localities in the states of Jalisco (population 1, Tequila) and Michoacán (population 2, Mil Cumbres) in the western portion of the volcanic belt and *Q. affinis* from Hidalgo (population 16, Zacualtipán) and Puebla (population 15, Zacapoaxtla) in the southern region of the Sierra Madre Oriental morphotectonic province (Ferrusquía-Villafranca, 1993). Fourteen other populations situated along the volcanic belt (most of them in the eastern region) and northern Oaxaca with various degrees of morphological intergradation were also chosen for sampling (Table 1, Fig. 1). The populations were numbered according to a geographic gradient that reflects their position relative to the distribution areas of the two species and the intergradation zone. This was done by jointly considering localities' latitude and longitude. In this way populations follow and order from more western–southern populations to more eastern–northern populations.

TABLE 1. Locality name, state, geographic coordinates, and sample size for the collecting sites.

Locality number and name	State	Latitude, N/ longitude, W	Sample size
Q. laurina sites			
1 Tequila	Jalisco	20°50′/103°48′	22
2 Mil Cumbres	Michoacán	19°40′/100°55′	20
Mixed sites			
3 Cuernavaca	Morelos	19°05′/99°15′	32
4 Santa Inés	Oaxaca	17°03′/96°55′	26
5 Amealco	Querétaro	20°10′/100°20′	21
6 Ozumba	México	19°05′/98°42′	31
7 Llano de Flores	Oaxaca	17°30′/96°30′	22
8 Puerto Aire	Veracruz	18°45′/97°30′	37
9 Jacala	Hidalgo	20°50′/99°05′	31
10 El Chico	Hidalgo	20°05′/98°40′	35
11 Pinal de Amoles	Querétaro	21°01′/99°40′	30
12 Cerro Navajas	Hidalgo	20°12′/98°30′	40
13 El Zembo	Hidalgo	20°15′/98°32′	45
14 Jalacingo	Veracruz	19°30′/97°15′	34
Q. affinis sites			
15 Zacapoaxtla	Puebla	19°50′/97°40′	34
16 Zacualtipán	Hidalgo	20°39′/98°40′	35

In each locality, 3–5 young intact leaves for molecular analysis and two randomly chosen branches for morphological analyses were collected from each individual. Sampling was performed by randomly choosing an adult tree every 100 or 200 m (depending on the specific locality) along a transect. Twenty to 45 trees were sampled per locality. Leaves for molecular analyses were immediately frozen in liquid nitrogen and the branches were pressed as herbarium specimens. In total, 495 individuals were sampled.

RAPD markers—Markers that could differentiate between the two species were developed through randomly amplified polymorphic DNA (RAPD). DNA of individuals from a morphologically representative population of each species (Zacualtipán [16] and Tequila [1], respectively; Table 1) was amplified using 131 10-bp random oligonucleotides (Series A–L; Operon Technologies, Alameda, California, USA). Satisfactory amplifications were obtained with 94 of these primers, from which 79 gave reproducible results, according to a second round of assays. In total, 711 fragments were consistently amplified in both series of assays. From these 711 bands nine, produced by seven primers, had substantial differences in frequency between the two species (Table 2). The reproducibility of these nine markers was corroborated in a third round of assays, and their presence/absence was later scored in all individuals.

DNA was isolated using the procedure proposed by Lefort and Douglas (1999) with only minor modifications. Isolated DNA was stored in deionized water at −20°C. The concentration of DNA in solution was determined with a DNA fluorometer (Hoefer Pharmacia Biotech, San Francisco, California, USA), following procedures supplied by the manufacturer. Amplification reactions were carried out in a 25-μL mix containing 10 ng template DNA, 50 mM KCl, 10 mM Tris-HCl; pH 9, 0.1% Triton x-100, 2 mM $MgCl_2$, 0.1 mM each dNTP, 0.2 μM of a single 10-mer primer, and 1 unit *Taq* polymerase (Gibco/Invitrogen, San Diego, California, USA). The thermal cycling program was run on a MJ Research (Waterton, Massachusetts, USA) thermal cycler. The program consisted of 45 cycles, each at 94°C for 1 min, annealing at 36°C for 1 min, and extension at 72°C for 2 min. A final extension at 72°C for 15 min was included.

The amplification products were electrophoresed in 1.5% (m/v) agarose gels with TBE buffer at 200 V for 2 h and stained with ethidium bromide. Gels with amplification fragments were visualized and photographed under UV light. Molecular mass of the RAPD bands was estimated by reference to a 123-bp ladder (Gibco/Invitrogen, San Diego, California, USA), with aid of

TABLE 2. Frequency of nine semidiagnostic RAPD markers in morphologically representative populations of *Q. affinis* and *Q. laurina*.

Population	Marker								
	A5-7	A7-9	A8-1	A10-3	B17-4	B17-6	C9-3	C9-5	I7-2
Q. affinis									
Zacualtipán	0.750	0.188	1.000	1.000	0.844	0.750	0.781	0.000	0.531
Q. laurina									
Tequila	0.048	0.619	0.500	0.550	0.000	0.300	0.150	0.500	0.150

the Alpha Ease Version 4 program (Alpha Innotech, San Leandro, California, USA).

Morphological analyses—The following morphological variables were quantified in 10 randomly chosen leaves from each individual: total length (TL), lamina length (LL), petiole length (PL), maximal width (MW), distance from the base of the leaf to the point of maximal width (PMW), and teeth number (TN). Additionally, the ratios of PL/TL, MW/LL, and PMW/LL were calculated. Individual tree means were obtained for each variable and used in further analysis.

Data analysis—Because none of the markers identified as useful was completely diagnostic, a maximum likelihood estimate of hybrid index scores was used, instead of the conventional arithmetic index employed when completely diagnostic markers are available. The algorithm used was the one developed by Hardig et al. (2000) specifically for RAPD markers (M. Morgan, Washington State University, personal communication). The program standardizes the resulting scores to range between zero and one. In the program, populations Tequila and Zacualtipán represented *Q. laurina* and *Q. affinis*, respectively. The frequency of the nine RAPD markers in these two populations thus constituted the end points for calculating hybrid index scores of all plants. These two populations constitute geographical extremes, are morphologically representative of their respective species, and had the largest frequency differences in the nine RAPD markers.

Discriminant function analysis (Tabachnick and Fidell, 1989) was used to assess multivariate morphological differentiation between representative populations of *Q. affinis* and *Q. laurina*. Individuals from populations Tequila and Zacualtipán were first analyzed to obtain a canonical discriminant function. Discriminant scores calculated with this function were then obtained for trees from all populations.

To identify geographical patterns of variation in the genetic and morphological composition of populations, product-moment correlations (Sokal and Rohlf, 1995) were calculated for the populations' hybrid index scores and morphological discriminant scores with each localities' latitude and longitude.

We calculated pairwise correlations among the frequencies of the RAPD markers on a population-by-population basis, as well as among frequencies of RAPD markers and mean values of morphological characters to determine possible associations in the patterns of change of these presumably independent characters across the area of intergradation that may indicate an origin for this area from secondary contact between the two oak species. To better visualize the patterns, plots for the frequency of markers that had high correlation coefficients were constructed, with populations following their order in the macrogeographic gradient. To ease the comparison between RAPD marker frequencies and morphological characters in these plots, values of morphological characters were transformed to range between zero and unity, with values close to zero representing *Q. laurina* and values closer to one representing *Q. affinis*.

To test for linear associations between RAPD markers and morphological variables at the level of individual trees, a multiple regression analysis was performed between maximum likelihood hybrid index scores and the set of morphological variables. Additionally, each morphological variable was regressed against the set of RAPD markers. These analyses were performed on the total set of individuals over all populations and then separately within each population.

RESULTS

Histograms of the maximum likelihood hybrid index scores for each population are presented in Fig. 2A. Individuals from the Tequila population had scores that ranged from 0.00 to 0.50, with a mean of 0.24 (SD 0.153). Three individuals from this population had hybrid index scores that deviated towards the hybrid condition by more than one standard deviation from the population mean. Two of these individuals lacked the two markers (A7–9 and C9–5) semi-diagnostic of *Q. laurina* and at the same time possessed three markers semi-diagnostic of *Q. affinis*. The hybrid index score of the third individual was 0.50; this individual possessed markers A7–9 and C9–5 and also harbored six of the seven markers semi-diagnostic of *Q. affinis*. Individual hybrid index scores in population Zacualtipán ranged from 0.69 to 1.00, with a mean of 0.88 (SD 0.098). Five individuals from this population had scores that deviated more than one standard deviation below the mean. These individuals lacked three or four of the semi-diagnostic markers of *Q. affinis*, but did not possess any semi-diagnostic marker of *Q. laurina*.

The frequency distributions of hybrid index scores in all other populations were to some degree intermediate between those of populations Tequila and Zacualtipán. A transition can be observed in Fig. 2A from frequency distributions situated towards the left of the graphs that characterized populations collected in the western region of the volcanic belt or southern Oaxaca to the more intermediate distributions in populations collected from the intergradation zone to frequency distributions shifted to the right side of the graphs characteristic of populations from the eastern region of the volcanic belt or southern Sierra Madre Oriental. A significant but very weak product-moment correlation between hybrid index scores and latitude was detected ($r = 0.106$; $P = 0.030$). This correlation was clearer for longitude ($r = -0.346$; $P < 0.0001$).

The first canonical function derived from discriminant analysis on the morphological traits explained 100% of the variation and provided highly significant discrimination (Wilks' lambda = 0.105; df = 18; $P < 0.001$) between *Q. laurina* individuals from population Tequila and *Q. affinis* individuals from population Zacualtipán. The standardized canonical discriminant function coefficient of each morphological variable is given in Table 3. The variable with the highest coefficient was petiole length. Scores for trees from population Tequila calculated with this canonical discriminant function ranged from −5.911 to −2.320, while *Q. affinis* trees from population Zacualtipán had scores that varied between 0.108 and 4.945. Figure 2B shows the frequency distributions of morphological discriminant scores in all populations. Individuals with scores similar to those observed in morphologically representative populations of *Q. laurina* were preponderant in more western and/or southern populations, with an increasing proportion of

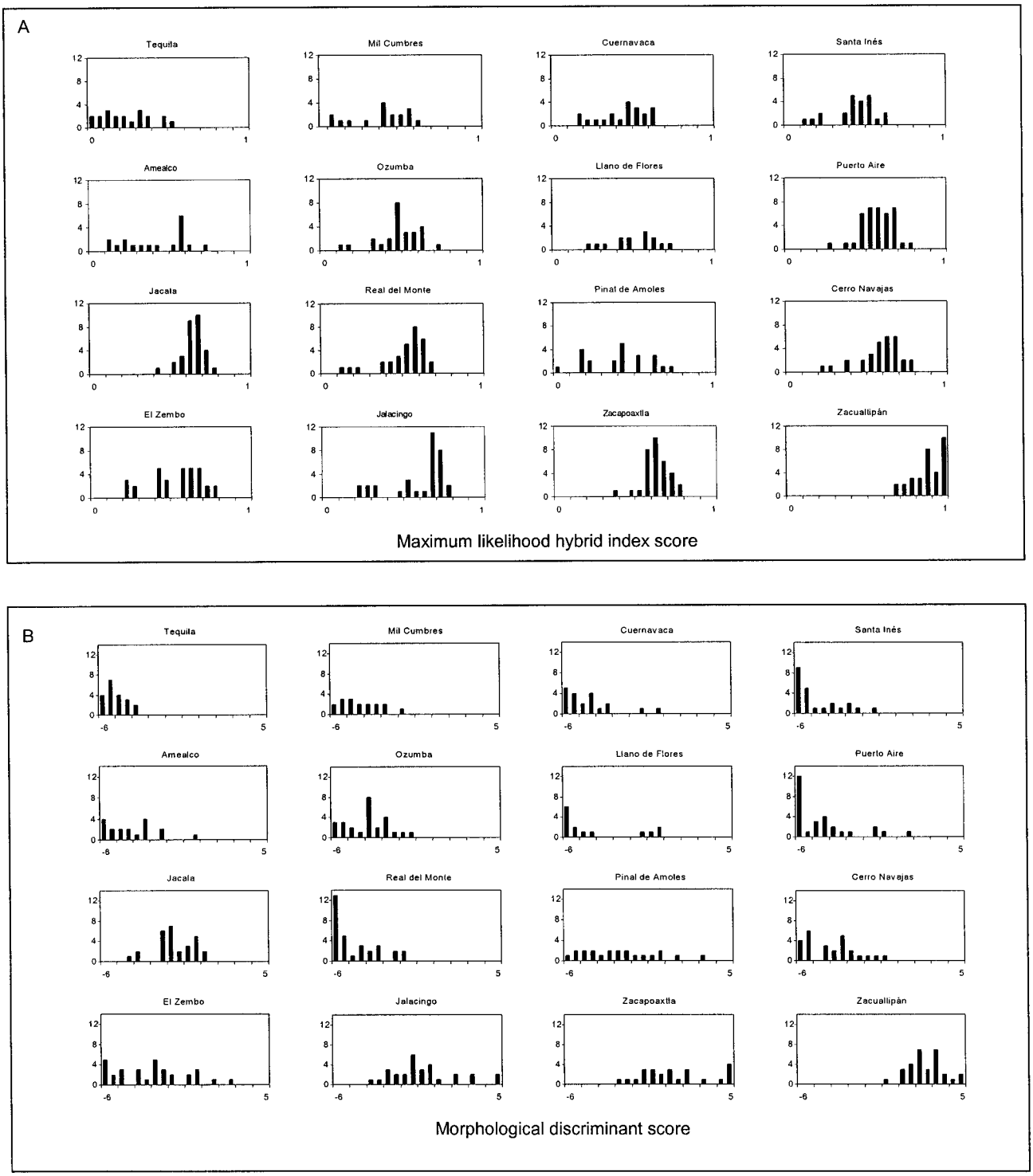

Fig. 2. Frequency distribution in each population of *Q. affinis–Q. laurina* of (A) maximum-likelihood hybrid index scores and (B) morphological canonical discriminant function scores. The *y*-axis represents the number of individuals.

intermediate individuals towards the intergradation zone and a majority of individuals with *Q. affinis* morphology in northeastern populations.

However, the frequency distributions of maximum likelihood hybrid index scores and morphological discriminant scores were only weakly congruent. A regression analysis indicated a low but significant global linear relationship between both variables ($R^2 = 0.179$; $F_{1,493} = 85.29$; $P < 0.0001$). In most populations, the proportion of morphologically intermediate individuals was smaller than the proportion of genetically intermediate individuals, even in the intergradation zone. *Quercus laurina*-like morphology seems to predominate in

TABLE 3. Standardized canonical discriminant function coefficients.

Morphological variable	Coefficient
Total length (TL)	−1.065
Lamina length (LL)	−0.441
Petiole length (PL)	3.045
Maximal width (MW)	−0.183
Distance from base to point of maximal width (PMW)	−0.667
Teeth number (TN)	0.791
Petiole length/total length (PL/TL)	−2.573
Maximal width/lamina length (MW/LL)	−0.591
Distance from base to point of maximal width/lamina length (PMW/LL)	0.199

these cases. Morphological discriminant scores were significantly correlated to latitude ($r = 0.182$; $P = 0.0001$) but not to longitude ($r = -0.004$; $P = 0.93$).

Pairwise correlations of the frequency of RAPD markers across populations are presented in Table 4. All correlations were of the expected sign, that is, negative frequency correlations among semidiagnostic markers of the two species and positive correlations among markers of the same species. Eleven of 36 (31%) of these comparisons were significant. As expected from these correlations, the patterns of frequency changeover on a population-by-population basis among several RAPD fragments were relatively parallel (Fig. 3). Significant associations were also found between the frequency of individual RAPD markers and population mean values of morphological characters (Table 5). Marked patterns of parallel change were found between the frequency of marker A5–7 and mean teeth number and between marker B17–4 and mean petiole length (Fig. 3).

Over all populations, the regression analysis indicated a significant linear association between the maximum likelihood hybrid index scores and the set of morphological variables, although the correlation coefficient was low (multivariate $R^2 = 0.251$; $F_{9,485} = 14.17$; $P < 0.0001$). All morphological variables also had significant relationships with the set of RAPD markers (Table 6). Correlations within populations were in general nonsignificant. In populations Amealco [5], Cerro Navajas [12], Jacala [9], and Puerto Aire [8], one morphological variable (different in each case) and four in the case of population Jalacingo [14], were significantly correlated to RAPD variation.

DISCUSSION

The results of this study support the occurrence of morphological and genetic intergradation between *Q. affinis* and *Q. laurina*. There is also evidence of correlated frequency changeovers among RAPD markers and among RAPD markers and morphological characters on a population-by-population basis along the macrogeographic gradient represented in this study. These patterns seem consistent with a secondary contact hypothesis for the origin of the intergradation area between these two oak species. In primary zones, parallel frequency changes are expected for traits responding similarly to selection gradients, but not for neutral markers (Durrett et al., 2000). Leaf morphology might certainly experience natural selection by the environment, but under a primary origin scenario, to explain the correlated changeover patterns observed here would require physical linkage of most RAPD markers with loci controlling foliar traits, which seems improbable a priori. Although congruent in general, the frequency changeovers of only a few marker pairs had high correlation coefficients and were patently parallel. This could indicate that some differential introgression between markers has occurred after secondary contact. Several studies have shown that differential introgression of neutral markers in secondary zones can occur and depends on linkage to loci under selection and time since contact (Durrett et al., 2000). Localized and parallel introgression of all species-specific markers is expected in very recent contact zones (Rieseberg and Ellstrand, 1993; Durrett et al., 2000). In taxa with a longer history of interaction, chromosome segments containing genes with advantageous or neutral effects in heterospecific backgrounds may have a more widespread introgression, while negatively selected ones will be impeded from crossing species' boundaries and will remain localized. This may create nonconcordant patterns of variation among different markers (Martinsen et al., 2001). In the original hypothesis of a secondary contact between *Q. affinis* and *Q. laurina*, and based on biogeographic considerations and paleobotanical evidence, Valencia (1994) postulated that genetic interactions between these two species probably date from the beginning of the Pleistocene. Even if this estimation

TABLE 4. Correlation coefficients between frequencies of pairs of RAPD markers across populations. Asterisks indicate significant values ($P < 0.05$).

	A05-7	A07-9	A08-1	A10-3	B17-4	B17-6	C09-3	C09-5	I07-2
A05-7	—	−0.056	0.531*	0.588*	0.657*	0.180	0.264	−0.395	0.674*
A07-9		—	−0.159	−0.403	−0.324	−0.283	−0.310	0.146	−0.393
A08-1			—	0.871*	0.712*	0.409	0.238	−0.571*	0.322
A10-3				—	0.728*	0.486	0.165	−0.448	0.483
B17-4					—	0.576*	0.213	−0.260	0.502*
B17-6						—	0.201	−0.226	0.536*
C09-3							—	−0.120	0.386
C09-5								—	−0.353
I07-2									—

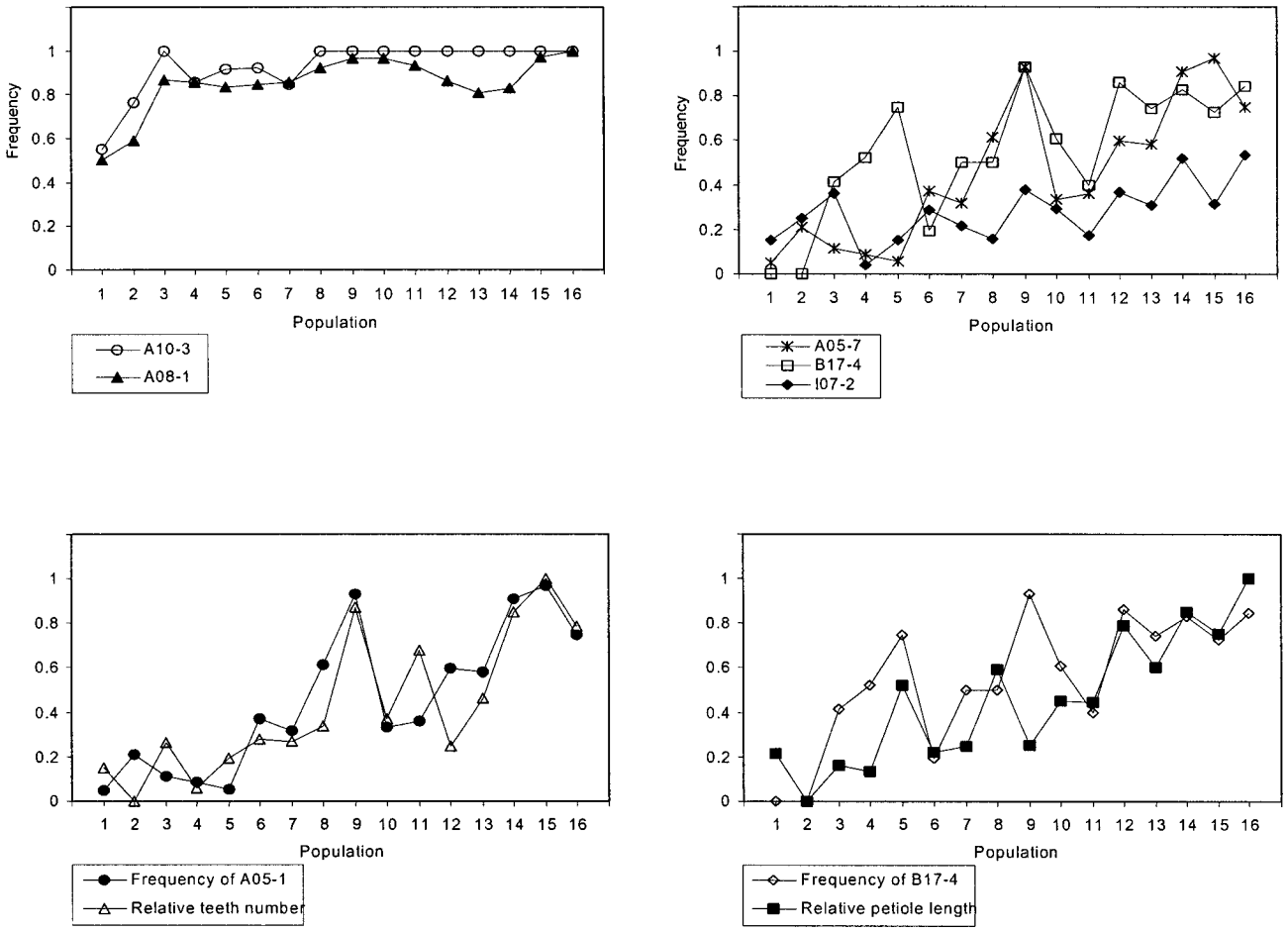

Fig. 3. Plots of the frequency of RAPD markers and RAPD markers and mean value of morphological characters with similar patterns of changeover on a population-by-population basis along the macrogeographic gradient.

is not very accurate, it nevertheless seems like the hybrid zone between these two species is not very recent. It is thus probable that enough time has elapsed for some differential introgression to occur.

In this study, we observed significant global multivariate correlations between phenotypic and molecular variation. However, these correlation coefficients were quite low, and for most populations, the degree of genetic and morphological intermediacy indicated by frequency distributions of hybrid index scores and morphological discriminant scores (Fig. 2A and B) were only partially congruent. In general, the number of morphologically intermediate individuals is smaller than the number of genetically intermediate individuals and, in fact, clear incongruence is observed in populations Cerro Navajas [12], Puerto Aire [8], and El Chico [10], in which nearly all individuals appeared genetically intermediate or even closer to *Q. affinis*, but were morphologically more similar to isolated *Q. laurina*. Other studies of hybridization between oak species have also found incongruence or only partial congruence between morphological and molecular variation. For example, although nuclear and cytoplasmic markers support extensive genetic exchange between the two species in the *Q. petraea-*

TABLE 5. Correlation coefficients between populations' mean values of morphological variables (MV) and RAPD marker frequencies. Asterisks indicate significant values ($P < 0.05$). See Table 3 for explanations of abbreviations.

	RAPD markers								
MV	A05-7	A07-9	A08-1	A10-3	B17-4	B17-6	C09-3	C09-5	I07-2
TL	−0.563*	0.376	−0.565*	−0.419	−0.661*	−0.278	−0.586*	0.320	−0.408
LL	0.526*	0.341	−0.563*	−0.379	−0.623*	−0.253	−0.550*	0.369	−0.363
PL	−0.664*	0.459	−0.481	−0.549*	−0.699*	−0.324	−0.594*	0.111	−0.577
MW	−0.345	0.198	−0.204	−0.082	−0.186	−0.001	−0.443	0.579*	−0.381
PMW	−0.675*	0.189	−0.480	−0.306	−0.530*	0.045	−0.483	0.456	−0.399
TN	0.858*	−0.159	0.599*	0.567*	0.610*	0.097	0.259	−0.596*	0.618*
PL/TL	−0.011	−0.037	0.152	0.175	0.239	0.156	−0.096	0.465	−0.187
MW/LL	−0.594*	0.383	−0.283	−0.449	−0.545*	−0.215	−0.532*	−0.120	−0.547*
PMW/LL	−0.603*	−0.032	−0.215	−0.141	−0.248	0.297	−0.274	0.378	−0.349

TABLE 6. Squared multiple correlations between single morphological variables and the set of RAPD markers. See Table 3 for explanations of abbreviations.

Morphological variable	R^2	$F_{9,485}$	P
TL	0.107	4.99	<0.001
LL	0.100	4.69	<0.001
PL	0.128	5.92	<0.001
MW	0.089	4.25	<0.001
PMW	0.123	5.17	<0.001
TN	0.269	13.27	<0.001
PL/TL	0.120	5.55	<0.001
MW/LL	0.044	2.54	0.008
PMW/LL	0.095	4.51	<0.001

Q. robur complex, several studies have found that morphologically intermediate individuals are very infrequent (Bacilieri et al., 1996a; Kremer et al., 2002). Possible explanations for this phenomenon include maternal effects (i.e., hybrids are more similar to the species of their maternal parent), as well as selection against intermediate forms (Bacilieri et al., 1996a; Kremer et al., 2002). In a mixed stand of *Q. lobata* and *Q. douglasii*, Craft et al. (2002) found phenotypically intermediate trees that had little evidence of mixed ancestry according to microsatellites. On the other hand, only one of four trees with the highest probability of hybrid ancestry was intermediate in appearance. In contrast, a remarkably high correspondence between morphological variables and genetic markers was found in a hybrid zone between *Q. gambelli* and *Q. grisea* in New Mexico (Howard et al., 1997). It is possible that the result was obtained because a highly optimized set of discriminant markers was used and the data were subjected to a canonical correlation analysis, which resulted in a high correlation between both first canonical variates on markers and morphological traits.

Incongruence between morphology and molecular markers has been observed many times in plant hybrid zones (Rieseberg and Ellstrand, 1993), and in general, introgression of morphological characters is more restricted than introgression of molecular markers (Rieseberg and Wendel, 1993). It is thought that recombination of adaptively relevant morphological or physiological characters with a polygenic basis may result in individuals with unfit phenotypes, while this is not expected for individuals that combine neutral markers from different species (Shoemaker et al., 1996). Several times it has been asserted that hybridizing oak species are capable of remaining morphologically or ecologically different in the face of considerable introgression (Whittemore and Schaal, 1991; Howard et al., 1997) and this may be also occurring in the case of *Q. affinis* and *Q. laurina*, despite a probably ancient event of secondary contact between them. It is possible that species distinctness in hybridizing oaks is maintained because natural selection operates against the exchange of genes that constitute the basis of functional divergence (i.e., differential adaptation) between species, while considerable gene flow can occur at the rest of the genome, as suggested by Wu (2001) in the genic view of the process of speciation. Which ecological factors, as well as which traits and genes may account for the functional divergence between the two oaks studied here merits considerable future attention.

Another major theme in the literature on hybrid zones concerns the organization of such areas as simple clines or geographically more complex mosaics (Rand and Harrison, 1989). In the first case, a gradual transition is observed between the character states typical of each parental population (Barton and Hewitt, 1989). In our case, frequency distributions of hybrid index scores and morphological discriminant scores were significantly correlated to geographic coordinates and change (although with weak concordance, as discussed before) from isolated populations of *Q. laurina* to isolated populations of *Q. affinis* with a series of more or less intermediate populations in between. This would argue in favor of a clinal structure for this hybrid zone. However, the frequencies of single RAPD markers as well as the values of morphological variables seem to follow a more complex pattern of change across localities than what would be expected for a clinal hybrid zone. In general, mosaic zones can be characterized as patches of pure species populations and mixed populations scattered across a zone of overlap (Howard et al., 1997). At this moment, we cannot firmly argue in favor of such structure for the hybrid zone between *Q. affinis* and *Q. laurina*, because we primarily focused on populations that previously showed some morphological evidence of intergradation. The sampled populations are in fact scattered among populations that were judged to be pure according to the appearance of herbarium specimens, but it would nevertheless be necessary to include a sample of such populations in a larger survey using molecular markers to better understand the structure of this hybrid zone.

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