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Molecular differentiation and diversity among the California red oaks (Fagaceae; *Quercus* section Lobatae)

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Abstract A recent epidemic of *Phytopthora* (Sudden Oak Death) in coastal woodlands of California is causing severe mortality in some oak species belonging to the red oak (Lobatae) group. To predict the risks of spread of this disease, an understanding of the relationships among California's red oak species and of their population genetic structure is needed. We focus here on relationships among the four species of red oak. Whereas morphological distinction of *Quercus wislizeni* and *Quercus parvula* can pose problems, *Quercus kelloggii* and *Quercus agrifolia* in pure forms are easily distinguishable from one another and from *Q. wislizeni* and *Q. parvula* in the field. However, hybrids among all species combinations are known to occur in nature and these can confound data from ecological studies. Our results revealed greatest differentiation of the deciduous *Q. kelloggii*, with only weak AFLP fragment differentiation of the three remaining evergreen species. The molecular data suggest a closer affinity of *Q. agrifolia* with *Q. wislizeni* and *Q. parvula* contrary to earlier suggestions that its origins are likely to have been with northern deciduous oaks probably through a common ancestor with *Q. kelloggii*. Interior and coastal populations of *Q. wislizeni* separated in dendrograms based on phenetic and genetic distances suggesting probable isolation in different glacial refugia. The position of *Q. parvula* remains ambiguous, having a closer affinity with interior populations of *Q. wislizeni* and with *Q. agrifolia*, than with coastal populations of *Q. wislizeni*. Mean population differentiation in *Q. wislizeni* was 0.18, which is somewhat higher than the average for other oak species, suggesting that range fragmentation has occurred in the past, resulting in a metapopulation structure. Our results provide evidence that introgression among these species may be causing reticulation, further confounding species separation. Whereas *Phytopthora* has been reported on *Q. agrifolia, Q. parvula* and *Q. kelloggii*, it has not yet been detected in natural populations of *Q. wislizeni*. The species relationships that our molecular data show suggest that this is more likely a result of escape due to ecological tolerances than to genetic differences.

Keywords California red oaks · Species differentiation · Genetic diversity · AFLP

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Introduction

The genus *Quercus* is widely distributed across the Northern Hemisphere with about 500 species in North America, Europe and Asia (Nixon 1989). Oaks of Europe and Asia are represented by subgenus *Quercus*, section Quercus (white oaks) and subgenus *Cyclobalanopsis* (cycle cup oaks), whereas oaks of North America are represented by subgenus *Quercus*, which includes section Protobalanus (intermediate oaks, golden cup oaks), section Quercus and section Lobatae (red oaks) (Nixon 1993). Interfertility among almost all combinations within a section raises serious problems for phylogenetic analyses (Manos and Fairbrothers 1987; Nixon 2002) and has led to multispecies concepts in oaks (Van Valen 1976).

High levels of phenotypic plasticity (Tucker 1974; Jensen et al. 1984; Jensen 1988) and genetic variation (Manos and Fairbrothers 1987; Guttman and Weigt 1988; Kremer and Petit 1993; Samuel et al. 1995) probably contribute to the success of the genus. However, these traits confound attempts to understand the ecological genetics of oak taxa and may result in confusion over phylogenetic relationships among species. The importance of oaks in temperate deciduous forests and in woodlands in Mediterranean zones has resulted in a large number of studies of their ecology and more recently their population genetics and phylogeny. Most of the genetic work has been carried out on eastern North American

(Manos and Fairbrothers 1987; Guttman and Weigt 1988; Schnabel and Hamrick 1990) and European species (Kremer and Petit 1993; see articles in Kremer 2002). In general, the population genetics of oak species has followed expectations of highly outcrossing, wind-pollinated, long-lived woody species (Hamrick and Godt 1989; Hamrick et al. 1992). Despite the importance of oak woodlands in California, very little is known about the genetics of these species; however, adaptation to an ecologically diverse landscape and the frequent occurrence of hybrids is likely to have contributed to a high level of genetic complexity.

Four species of red oak are native to the Pacific coastal forests north of Mexico. These include *Quercus kelloggii* Newb. (California black oak), *Quercus agrifolia* Née (coast live oak), *Quercus wislizeni* A.DC. (interior live oak) and *Quercus parvula* E. Greene. The latter species includes an island shrub variety and a mainland tree variety referred to as Shreve oak [*Q. parvula* var. *shrevei* (Muller) Nixon & Muller]. This latter variety is considered in the present work. Whereas *Q. agrifolia* and *Q. parvula* are strictly coastal species, *Q. kelloggii* and *Q. wislizeni* have broader ranges in the Coastal Ranges and the Sierra Nevada/Cascade Ranges. *Q. agrifolia* extends from northern California to northern Baja California, Mexico, and *Q. parvula* is best described from central coastal California from south of Monterey to Santa Cruz, but because of hybridization its northern limit is obscure. *Q. kelloggii* extends from the MacKenzie river in western Oregon to San Diego County in coastal California and to the southern Sierra Nevada. *Q. wislizeni* extends from north-western California through the Coast Ranges to northern Baja California and through the Cascade ranges from Shasta County, California to the Tehachapi Mountains of southern California. In the southern range interior live oak is probably only found as var. *frutescens*.

Recently, a pathogen (*Phytopthora ramorum*) has resulted in serious mortality of *Q. kelloggii, Q. agrifolia* and *Q. parvula*. Inoculation studies have shown that *Q. wislizeni* is also susceptible, but may show a lower level of response (Dodd, unpublished data). The rapid spread of this disease has highlighted the need to understand more of the genetic structure of these species and their populations. Population variation in *Q. agrifolia, Q. wislizeni* and *Q. parvula* has been studied using several biochemical markers (Dodd et al. 1993a, b, 1997, 2002), suggesting marked differentiation of coastal and Sierran populations of *Q. wislizeni*, differentiation of central coastal populations of *Q. agrifolia* from more southern and more northern population groups and substantial introgression among *Q. agrifolia, Q. wislizeni* and *Q. parvula* in north coastal California. In the present work, we report the first study of molecular genetic variation within and among these species in an attempt to better understand their phylogenetic relationships and their population genetic structure.

Materials and methods

Experimental material

Foliage was collected from 104 individuals from 11 populations (six Sierra Nevada and five coastal) of *Q. wislizeni* var. *wislizeni*, 30 individuals from four populations of *Q. parvula* var. *shrevei*, 20 individuals from four populations of *Q. agrifolia* (two populations allopatric to *Q. wislizeni* and *Q. parvula* and two populations sympatric with *Q. parvula*) and 33 individuals from nine populations of *Q. kelloggii* (including two populations allopatric to *Q. agrifolia* and *Q. parvula*) (Fig. 1). Within each population, foliage was collected from mature trees separated by at least 50 meters. Leaves were kept on ice and subsequently at 4 °C until extraction.

AFLP analyses

Genomic DNA was extracted following a simplified Doyle and Doyle method (Cullings 1992). The AFLP method of Vos et al. (1995) was performed following the protocol of Life Technologies (Rockville, Md., USA). DNA was restricted with *Eco*RI and *Mse*I (1.25 U/μl of each) in a reaction buffer [10 mM tris-HCl (pH 7.5), 10 mM Mg-acetate and 50 mM K-acetate] in a total volume of 12.5 μl. The restriction reaction was carried out at 37 °C for 2 h followed by denaturation of restriction enzymes at 65 °C for 10 min. Adapter ligation was conducted in the ligation buffer using T4 DNA ligase for 2 h at 20 °C. Subsequently, the ligation product was diluted 1:10 for primary amplification.

Primary amplification was carried out in a standard polymerase chain reaction (PCR) cocktail containing 1.5 mM of $MgCl_2$ and 0.5 mM of each of the primary amplification primers. For the primers, the *Eco*RI primer sequence was identical to the adapter

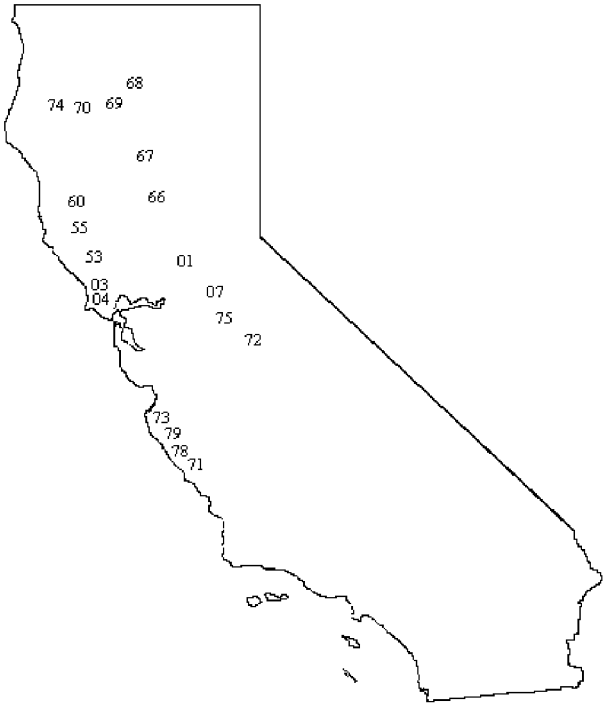

Fig. 1 Map of sampling sites of the California red oaks. *Numbers* on the map refer to the following population codes, where different species that were sampled in sympatry carry the same numeric code: *Q. wislizeni* W72, W75, W07, W66, W67, W68, W69, W70, W60, W55, W53; *Q. parvula* P71, P78, P79, P73; *Q. agrifolia* AA78, A79, A04, A03; *Q. kelloggii* K01, K66, K68, K69, K70, K74, K60, K55, K53

sequence, whereas the *Mse*I primer had an extra cytosine ("C") as a selective nucleotide (Table 1). The PCR reaction was performed in a Teachne Genius thermocycler for 20 cycles using the following cycling parameters: 30 s at 94 °C, 60 s at 56 °C and 60 s at 72 °C. The primary amplification product was then diluted 1:50 for selective amplification. Selective amplification was carried out in a standard PCR reaction cocktail containing 2.5 mM of $MgCl_2$ and 0.5 μM of the selective amplification primers. The PCR program comprised the following cycles: 13 cycles of 30 s at 94 °C, 30 s at 65 °C (annealing temperature was lowered 0.7 °C at each cycle) and 60 s at 72 °C, followed by 23 cycles of 30 s at 94 °C, 30 s at 56 °C and 60 s at 72 °C. Four sets of selective primers were adopted from a wide range of sets of primers that were tested in pilot runs (Table 1). Selective amplification products were resolved on 6% polyacrylamide native gels. Gels were stained with Gelstar (FMS Bioproducts, Rockland, me., USA.) following manufacturer's protocols and documented with a Kodak DC 120 digital camera. Bands were scored as present/absent using Gelcompar II software (Applied Maths, Kortrijk, Belgium).

Statistical analysis

The AFLP bands were assumed to be single amplified products representing a locus with two allelic states; one in which amplification occurred and the null allele in which base substitutions resulted in lack of enzyme cutting or amplification at the selected site. The banding patterns were analyzed as phenetic data and as genetic data for analyzing relationships among species and extracting information on genetic diversity and genetic structure. As genetic data, Mendelian segregation of the alleles was assumed and allele frequencies were estimated assuming no inbreeding; oaks are commonly considered to be highly outcrossing species (Bacilieri et al. 1996). Frequencies of the recessive allele were calculated using the Taylor expansion (Lynch and Milligan 1994).

Genetic diversities were estimated from the number of polymorphic loci (95% criterion) and from Nei's (1978) unbiased expected heterozygosity averaged over all loci (Yeh et al. 1999). Fragments that were present in all individuals of a species were considered as fixed fragments. Fragments that occurred in 10%, or fewer of all individuals were treated as rare fragments and their proportions for each species were calculated. Fragments that were unique to a species were treated as private fragments. Estimates for population differentiation were obtained using the Tools for Population Genetics Analysis (TFPGA) Version 1.3 program made available as freeware by Miller (1996). Weir and Cockerham's (1984) θ was used as a small population-size estimate of Wright's (1978) F_{ST} and 95% confidence intervals were obtained by bootstrapping 1,000 replicates over loci.

For phenetic analyses, partitioning of genetic variation among species, and among and within populations, was estimated by hierarchical AMOVA using WINAMOVA Version 1.55 (Excoffier et al. 1992). Input files of dominant AFLP markers were prepared using AMOVA-PREP (Miller 1998). The Huff distance metric, based on the proportion of markers that differ between any pair of individuals was used. Probabilities of variance components were estimated from distributions generated from 1,000 random permutations.

A UPGMA cluster analysis and a principal coordinates analysis were carried out on a matrix of Jaccard's similarities among all individuals. This coefficient gives the ratio of the number of positive matches to the total number of characters minus the number of negative matches, therefore omitting consideration of negative matches. Sequencing of AFLP bands has shown that shared bands are homologous (Parsons and Shaw 2001), but the homology of shared null bands is not known. Principal coordinates analysis and cluster analysis were carried out using NTSYS-pc, version 1.8 (Rohlf 1993). To compare the phenetic partitioning of populations with a genetic-based partition, an unrooted phylogenetic tree was constructed from a matrix of Nei's (1978) unbiased genetic distances using the UPGMA procedure in TFPGA software. The reliability of this and all other trees was tested by bootstrap analysis with 1,000 replications.

Results

A total of 311 band classes were detected from four primer sets, with approximately equal numbers from each primer combination (Table 1). Approximately equal numbers of bands were detected in *Q. agrifolia* and *Q. kelloggii*, more bands were detected in *Q. parvula*, and the greatest number was found in *Q. wislizeni*. In the last species, more fragments were found in interior, than in coastal, populations.

Estimates of gene diversity were slightly lower in *Q. agrifolia* and *Q. kelloggii*, although differences in H_e were not significantly different among species (Table 2). Proportions of polymorphic fragments were high in all species; 3.5% of fragments were fixed in *Q. kelloggii*, 1.6% were fixed in *Q. agrifolia*, whereas only one fragment (0.4%) was fixed in *Q. parvula* and no fragments were fixed in *Q. wislizeni*. Numbers of rare fragments were greatest in *Q. wislizeni*, and higher in interior populations, presumably reflecting the greater numbers of fragments detected among these individuals. Numbers of private fragments were also greatest in interior populations of *Q. wislizeni*.

The first three vectors of the principal coordinates analysis accounted for only 14% of the total variance. However, ordination of these vectors revealed a marked

Table 1 Primer combinations and numbers of AFLP band-classes identified among species of California red oaks

Restriction enzyme	Selective primer combinations				Total
*Eco*RI	AC	TC	GTA	TAC	
*Mse*I	CAA	GTG	GTG	TC	
Number of bands					
Q. agrifolia	70	56	63	67	256
Q. kelloggi	60	65	67	63	255
Q. parvula	66	67	71	69	273
Q. wislizeni	74	76	78	76	304
Coastal	70	70	75	68	283
Interior	73	72	75	74	294
Total	76	78	79	78	311

Table 2 Genetic diversity among species of California red oaks. Percent polymorphic loci (*P*%), numbers of fixed fragments (N_F), numbers of rare fragments (N_R), numbers of private fragments (N_{Pr}), expected heterozygosity (H_e). Standard deviations of the estimates shown in parentheses

Species	*P*%	N_F	N_R	N_{Pr}	H_e
Q. agrifolia	81.0	4	22	3	0.20 (0.17)
Q. kelloggii	79.1	9	19	2	0.20 (0.17)
Q. parvula	87.5	1	21	1	0.21 (0.17)
Q. wislizeni	97.8	0	43	10	0.21 (0.16)
Coastal	88.1	0	29	3	0.19 (0.17)
Interior	94.2	0	36	5	0.22 (0.16)

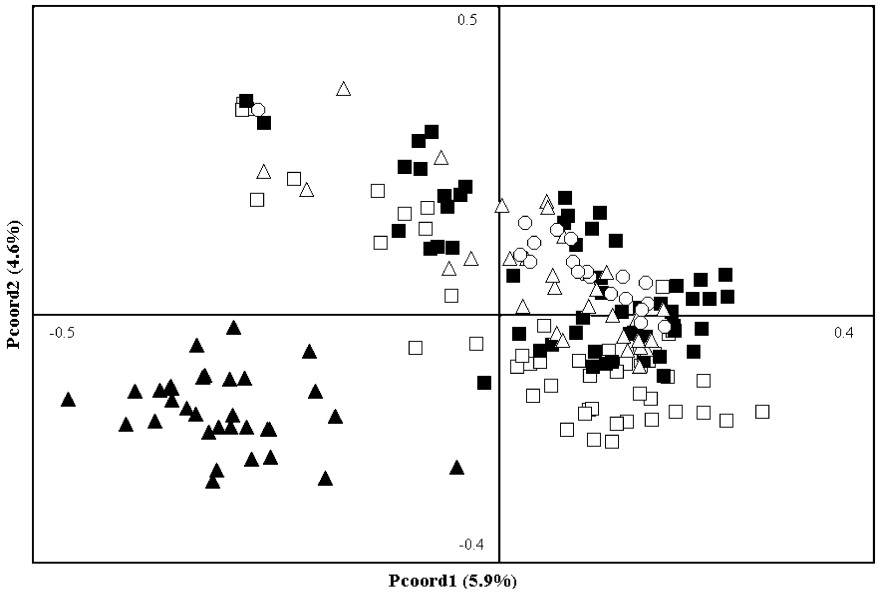

Fig. 2 Ordination of the first two vectors of principal coordinates analysis on AFLP molecular banding patterns among four California red oaks. Interior populations of *Q. wislizeni* shown by *filled rectangle*, coastal populations of *Q. wislizeni* shown by *open rectangle*, *Q. parvula* shown by *open triangle*, *Q. agrifolia* shown by *open circle*, *Q. kelloggii* shown by *closed triangle*

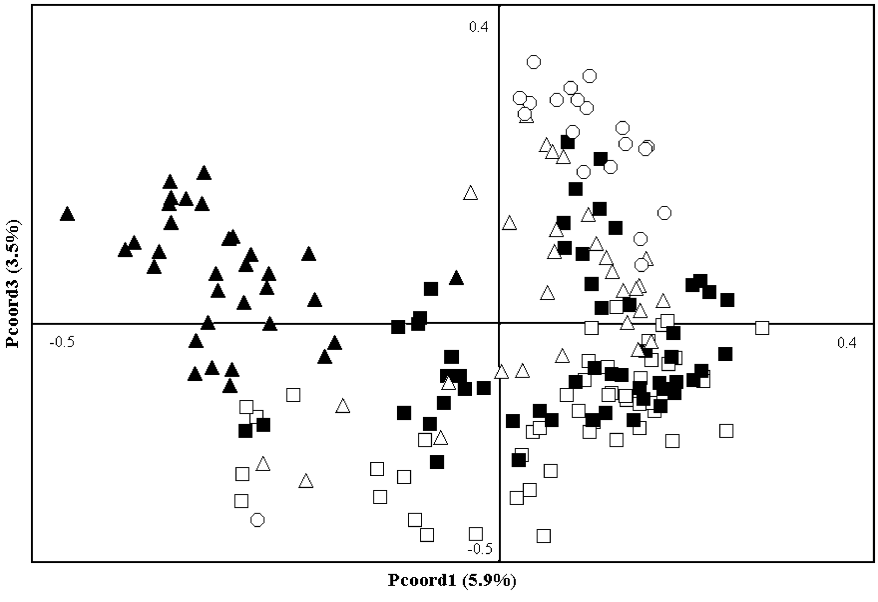

Fig. 3 Ordination of the first and third vectors of principal coordinates analysis on AFLP molecular banding patterns among four California red oaks. Interior populations of *Q. wislizeni* shown by *filled rectangle*, coastal populations of *Q. wislizeni* shown by *open rectangle*, *Q. parvula* shown by *open triangle*, *Q. agrifolia* shown by *open circle*, *Q. kelloggii* shown by *closed triangle*

separation of *Q. kelloggii* from all other red oaks of California (Figs. 2 and 3). *Q. agrifolia* overlapped *Q. parvula* and *Q. wislizeni* in the space of the first two principal coordinates vectors (Fig. 2), but tended to segregate along the third vector (Fig. 3). Although *Q. wislizeni* and *Q. parvula* were interspersed in the plots shown in Figs. 2 and 3, some trends could be observed. First, coastal populations of *Q. wislizeni* had exclusively negative loadings on the third principal-coordinates vector (Fig. 3), whereas interior populations had both positive and negative loadings, although negative loadings were rarely less than –0.1. Interior populations rarely had negative loadings along the second principal coordinates vector, whereas Fig. 2 shows both negative and positive loadings for coastal populations. Second, *Q. parvula* tended to be more closely associated with interior populations of *Q. wislizeni* than with the coastal populations.

Trends revealed by principal coordinates analysis were supported by UPGMA cluster analysis based on a matrix of Jaccard similarities (Fig. 4). The cophenetic correlation (r_{CS}) was 0.91 indicating a very good fit of the cluster analysis to the original distance matrix (Rohlf 1993). With the exception of 25 individuals that did not link to others of the same species or population clusters and are outliers in Fig. 4, species formed distinct clusters that mostly followed population boundaries. All individuals of *Q. kelloggii* formed a distinctive cluster, underscoring the greater differentiation of this species from AFLP fragment profiles. One population of *Q. agrifolia* (A03) grouped closer to some *Q. parvula* and *Q. wislizeni*, than it did to the three other populations of *Q. agrifolia* that

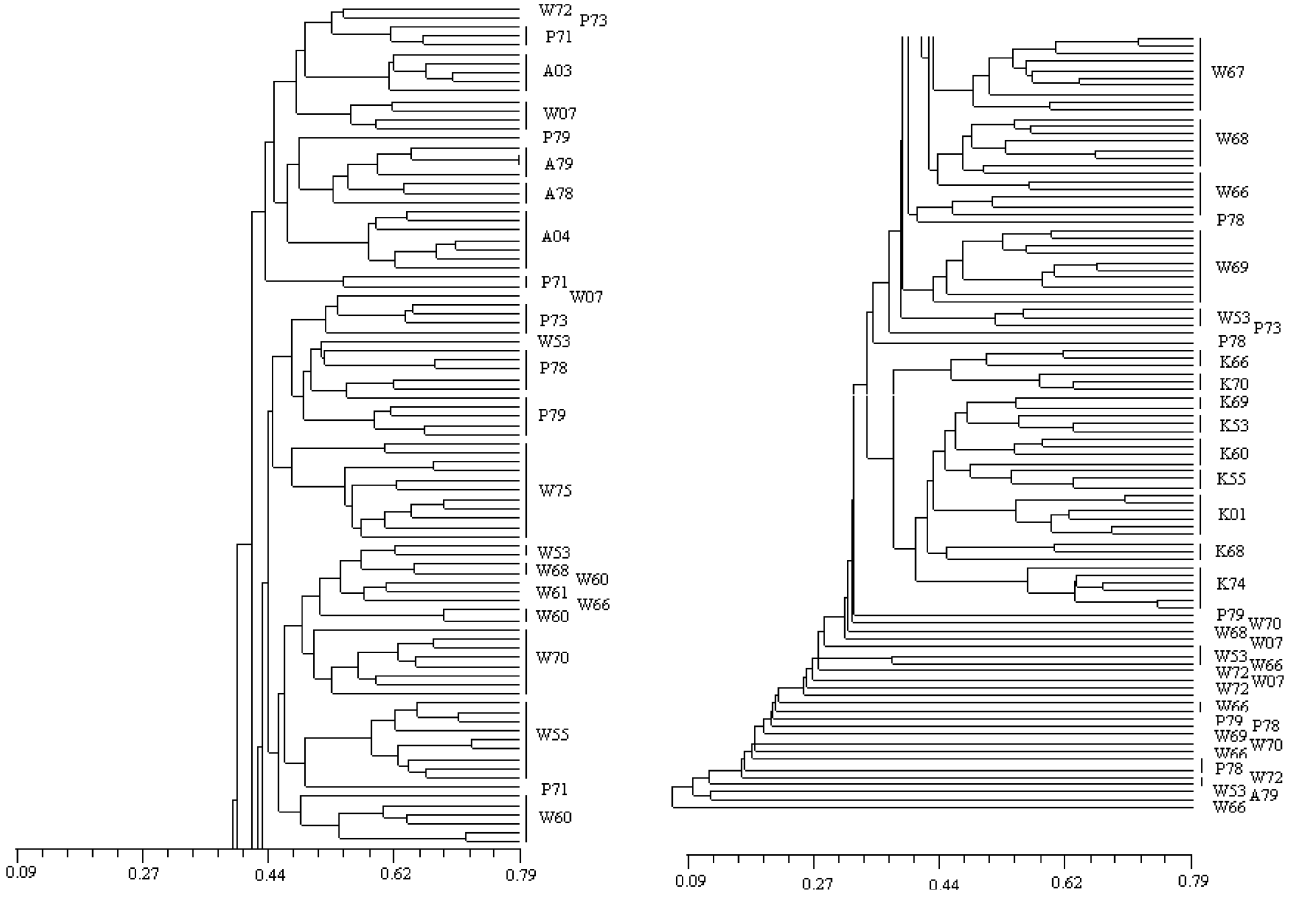

Fig. 4 UPGMA cluster analysis of individuals of four species of California red oak based on Jaccard's coefficient of AFLP molecular-banding patterns. See Fig. 1 for population codes

Table 3 Matrix of mean population within species genetic distances D_{ST} (standard deviations in parentheses) and pairwise unbiased genetic distances (Nei 1978) among four species of California red oaks. Total sample size (*N*)

Species	*N*	D_{ST}	*Q. wislizeni*	Coastal	Interior	*Q. parvula*	*Q. agrifolia*
Q. wislizeni	104	0.05 (0.02)					
Coastal	38	0.05 (0.02)					
Interior	66	0.05 (0.02)		0.05 (0.02)			
Q. parvula	30	0.05 (0.02)	0.06 (0.02)	0.06 (0.02)	0.05 (0.03)		
Q. agrifolia	20	0.09 (0.02)	0.10 (0.03)	0.10 (0.03)	0.09 (0.03)	0.09 (0.03)	
Q. kelloggii	33	0.12 (0.03)	0.13 (0.03)	0.13 (0.03)	0.14 (0.03)	0.14 (0.03)	0.17 (0.03)

formed a single cluster that included one individual of *Q. parvula*. With the exception of unresolved individuals, *Q. parvula* tended to group with *Q. agrifolia* and with interior populations of *Q. wislizeni*. Most coastal *Q. wislizeni* formed a group with individuals from the same population in most cases sharing common nodes. This group split interior populations of *Q. wislizeni* into central Sierran populations (W72, W75, W07) and more northern Sierra Nevada and Cascade foothill populations.

A UPGMA cluster analysis of populations based on Nei's genetic distances (Nei 1978) showed similarities and some inconsistencies with the phenetic partitioning of individuals described above. However, bootstrap values tended to be low and r_{CS} was 0.71 indicating a poor fit of the cluster analysis to Nei's distance matrix. *Q. kelloggii* populations formed three groups; K66 and K70 grouped closer to the other species than to remaining populations of *Q. kelloggii*, a cluster of six populations (K74, K01, K68, K53, K60, K55) and a single population, K69 (Fig. 5). The remaining populations fell into four clusters and two populations (one *Q. agrifolia* and one *Q. wislizeni* from the Sierra Nevada) remained well separated. Of the four clusters, one comprised two populations of *Q. agrifolia* and a second included two populations of *Q. parvula*. The two remaining clusters separated into coastal and interior *Q. wislizeni*, with two populations of *Q. parvula* and one population of *Q. agrifolia* joining the interior *Q. wislizeni* group. This population of *Q.*

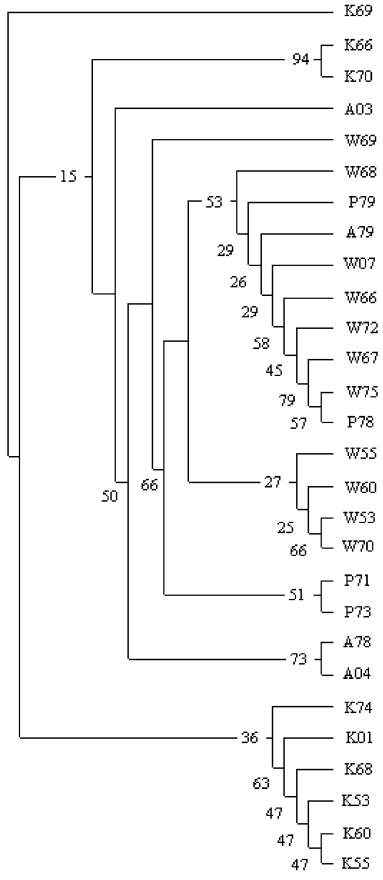

Fig. 5 UPGMA cluster analysis of populations of four species of California red oak based on Nei's (1978) unbiased genetic distances. See Fig. 1 for population codes. Support for branches is given by bootstrap percentages at each node for re-calculated genetic distances from 1,000 bootstrap replicates

agrifolia (A79) was joined by a sympatric population of *Q. parvula* (P79).

Average genetic distances between species were very low for all species combinations (Table 3) and were least between coastal and interior populations of *Q. wislizeni* and between interior populations of *Q. wislizeni* and *Q. parvula*. Genetic distances were highest in species comparisons that included *Q. kelloggii*, and a maximum between *Q. kelloggii* and *Q. agrifolia*. In support of the genetic analysis, only 8.7% of the total variance in AFLP banding patterns was attributable to species differentiation (Table 4) from analysis of molecular variance. Variance at the species level for pooled populations of *Q. wislizeni* and *Q. parvula* was only 1.1% and this variance component was non-significant. Variance among populations was 17.4% for pooled species and 16.0% for pooled populations of *Q. wislizeni* and *Q. parvula* (Table 4). The partition of variance among and within populations is shown for the four species in Table 4, but data for *Q. kelloggii* and *Q. agrifolia* should be treated with caution because of inadequate sampling within these two species. Nevertheless, the data suggest higher population differentiation in these latter two species and least population differentiation in *Q. parvula*.

Discussion

Differences in AFLP banding patterns among the four California red oak species were relatively minor. Because very few AFLP fragments were unique to a species, observed differentiation was mostly a result of variations in fragment frequencies. Of the four species, *Q. kelloggii* was the most distinctive, appearing well-segregated in principal coordinate ordinations, hierarchical cluster

Table 4 Partition of variation in AFLP fragment patterns among four species of California red oaks. Partition of variation Φ from AMOVA, fixation indices θ from small population size estimate of Wright's (1978) F_{ST} with 95% confidence intervals obtained by bootstrapping 1,000 replicates over loci

Species	% Variance	Φ	Prob.	θ
All species pooled				
Among species	8.7	0.087	<0.001	0.06 ± 0.01
Among populations within species	17.4	0.190	<0.001	0.22 ± 0.02
Within populations	73.9	0.260	<0.001	
Q. wislizeni and *Q. parvula* pooled				
Between species	1.1	0.011	0.07	0.007 ± 0.01
Among populations within species	16.0	0.162	<0.001	0.20 ± 0.02
Within populations	82.9	0.171	<0.001	
Quercus wislizeni				
Among populations	17.5	0.175	<0.001	0.20 ± 0.02
Within populations	82.5			
Quercus parvula				
Among populations	10.6	0.106	<0.001	0.18 ± 0.03
Within populations	89.4			
Quercus kelloggii				
Among populations	34.2	0.342	<0.001	0.47 ± 0.04
Within populations	65.8			
Quercus agrifolia				
Among populations	24.7	0.247	<0.001	0.35 ± 0.05
Within populations	75.3			

analyses and having the greatest interspecific genetic distances. Average genetic distance between *Q. agrifolia* and *Q. wislizeni* was a little greater than between *Q. agrifolia* and *Q. parvula*. The AFLP analyses showed very little differentiation between *Q. parvula* and *Q. wislizeni*. Genetic distance between these two species was very low and substantial overlap appeared in the ordinations and cluster analyses.

This lack of clear genetic differentiation among oak species is not uncommon, even among species that are morphologically well-defined. Manos and Fairbrothers (1987) reported low genetic distances for isozyme variation among six species of red oak from eastern North America, similar to the genetic distances we found in the present study. Similarly, Guttman and Weigt (1988) found very little isozyme variation among a wide range of species in section Lobatae. The poor genetic differentiation that we observed among species, contrasts with relatively clear morphological and biochemical separation of the taxa. With the exception of populations where hybridization is currently occurring, morphological separation of the four California red oak species generally presents few problems. The large-lobed, deciduous leaves of *Q. kelloggii* contrast sharply with the small sclerophyllous evergreen leaves of the three other species. Time to acorn maturity and distinctive leaf venation patterns clearly separate *Q. agrifolia* from *Q. wislizeni* and *Q. parvula*. The last two, however, present some difficulties in field identification, although pure populations are morphologically and ecologically distinct (Nixon 1980, 2002) and exhibit discrete differences in cuticular wax biochemistry (Dodd et al. 1997, 2002). Presumably, this reflects the difference between AFLP markers that are likely to be mostly neutral to selection, and morphological and biochemical traits that are under selection pressure.

Relationships among the red oaks of California are not clear. According to Nixon (2002), the leaf-venation pattern of *Q. agrifolia* suggests an origin in the lobed-leaved species, whereas this is not true of *Q. wislizeni* or of *Q. parvula*. Furthermore, Nixon suggests that *Q. agrifolia* and *Q. kelloggii* may be related through an extinct lobed-leaved ancestor. Our molecular data do not support this hypothesis; the genetic distance between *Q. kelloggii* and *Q. agrifolia* was the greatest of all interspecific pairwise distances and our AFLP data consistently showed a closer affinity of *Q. agrifolia* with *Q. wislizeni* and *Q. parvula*. This suggests that the three evergreen red oaks are likely derived from a common ancestor with a separate lineage to that of *Q. kelloggii*, or that the latter species diverged earliest from a common ancestor of the California red oaks. According to Wolfe (1980), *Q. kelloggii* appears in the lower Miocene, occupying a summer-wet lowland broad-leaved forest, while *Q. agrifolia* and *Q. wislizeni* appear later in the fossil record and increase in abundance during the late Miocene as the climate in California became drier. Relationships among the three evergreen species remain obscure. According to Nixon (1980), *Q. parvula* and *Q. wislizeni* had independent origins, the former from insular populations of southern California and Baja California, and the latter from an interior lineage. In contrast, Axelrod (1983) proposed the spread of *Quercus shrevioides*, a hypothesized common progenitor of *Q. parvula* and *Q. wislizeni*, from western Nevada into the Sierra Nevada during the early to mid Miocene, which was then replaced by *Quercus wislizenoides* as the climate became drier. The similarity of AFLP banding patterns of *Q. parvula* and *Q. wislizeni* that we have observed favours a recent common ancestry for these two species. The tendency for coastal and interior populations of *Q. wislizeni* to segregate suggests that these two groups of populations have been isolated for some time and may reflect different refugia during Pleistocene glaciations. However, it is curious that our data showed a tendency for *Q. parvula* to have its closest affinities with interior populations of *Q. wislizeni* from the central Sierra Nevada foothills. This region of the Sierra Nevada mountains shows floristic affinities with the Coast Ranges of California, presumably as a result of the inland seas that produced a marine influence in the central Sierra Nevada foothills. This oceanic influence continues today through the San Joaquin delta (Hickman and Roberts 1993).

Genetic diversity and structure

Although numbers of populations and sample sizes were relatively small for *Q. agrifolia* and *Q. parvula*, it has been shown that in highly outcrossed species such as oaks, few populations may be needed to sample most of the variation at the population level (Manos and Fairbrothers 1987; Schnabel and Hamrick 1990). Hamrick and Godt (1989) found that species-level gene diversity is most closely correlated with geographic range, and that outcrossing taxa, such as oaks, tend to maintain higher than average levels of gene diversity. Our data revealed similar levels of gene diversity, as measured by expected heterozygosity, for all four species, suggesting that the more-restricted geographic ranges of *Q. parvula* and *Q. agrifolia* had little effect on genetic diversity (however, small sample sizes may have led to an underestimate of genetic diversity).

Population differentiation tended to be relatively high in the species studied here. The data for *Q. agrifolia* and *Q. kelloggii* should be treated with caution, as the full geographic range of these species was not sampled in this study. Population differentiation in *Q. wislizeni* was 0.18 and in *Q. parvula* was 0.11, above the average of 0.07–0.09 expected for long-lived, wind-pollinated woody species (Hamrick and Godt 1989). These values of population differentiation are at, or above, the mean of 0.11 reported for oak species by Hamrick et al. (1992). The higher than average population differentiation that we have found in the California red oaks would suggest that range fragmentation has occurred in the past, probably associated with Pleistocene glaciations and the retreat of oak populations into refugial sites. It will be very interesting to see whether the extraordinarily high

levels of population differentiation that we have found in the limited sampling of *Q. agrifolia* and *Q. kelloggii* are corroborated with a more-intense sampling that we are currently conducting.

Role of hybridization

We have been studying the importance of hybridization on the population structure of the California red oaks (Dodd et al. 1996, 2002) and will present results on AFLP data from hybrid populations in a later report. However, it should be pointed out here that introgression may be contributing to the poor separation of species reported in this study. From biochemical data, Dodd et al. (2002) have shown that red oak populations from northwestern California show evidence of important levels of introgression. *Q. wislizeni* is particularly problematic in this region, and these authors have reported *Q. agrifolia* and *Q. parvula* chemotypes in these populations. Nixon (2002) has also pointed out the confusion that hybridization has created for these populations north of the San Francisco Bay area. Several anomalies that were observed in our cluster analyses of the molecular data might be explained by reticulation due to introgression. One of the populations of *Q. agrifolia* clustered with a sympatric population of *Q. parvula*, rather than with the other populations of coast live oak. Twenty five individuals did not resolve to their population clusters in Fig. 4; this could be explained if these were hybrid or introgressed individuals. Of these 25 individuals, 19 were *Q. wislizeni*, five *Q. parvula* and one was *Q. agrifolia*, suggesting that hybridization may not be equal in all directions and that combinations in which *Q. wislizeni* is the maternal parent may be more common. Some indirect evidence in support of this is the much greater number of AFLP fragments and the greater number of rare bands that were detected in *Q. wislizeni*, suggesting the capture of fragments from other species. Our estimates of genetic distances also provide evidence for important levels of hybridization among these species. In the absence of hybridization, interspecific genetic distances should be much larger than genetic distances among populations within species. Our data showed more or less comparable levels of genetic diversity within and among species, which would be consistent with high levels of interspecific introgression. Within species, genetic diversities were highest for *Q. agrifolia* and *Q. kelloggii*, consistent with the high estimates of population differentiation discussed above. Our current work that includes more extensive population sampling should help to clarify whether these differences among species in population genetic structure are real.

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