Population Diversity and Evidence of Introgression Among the Black Oaks of California[1]

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Abstract

The black oaks of California include 4 tree species (California black oak, coast live oak, Shreve oak, interior live oak) that are known to hybridize. Complex patterns of population variation within each species are likely to result from these hybrid combinations and from subsequent introgressions. We have been studying population variation using biochemical and molecular markers and report results from the former here. Diversity is much greater in interior live oak and in Shreve oak than in either of the other two species, and is least in coast live oak. Shreve oak has not received complete acceptance as a valid species, and is considered as synonymous with interior live oak by many ecologists. However, our biochemical data provided a marker that was present in all populations identified as Shreve oak from the central coast, and was extremely rare in populations of interior live oak from the Cascade and Sierra Nevada mountains. This marker occurred at intermediate frequencies in many populations from north western California that were tentatively assigned to interior live oak. We suggest that these two species are recently derived from a common ancestor and that interspecific barriers to fertilization have not yet become complete. Discriminant function analysis on the full biochemical data set suggested a complex pattern of introgression including coast live oak, interior live oak and Shreve oak in these north coastal populations. These studies of population variation help us to understand the genetic architecture of the black oaks of California and may provide valuable information in the search for resistance to sudden oak death (SOD).

Introduction

Four members of the black oak group (Lobatae) are native to California; coast live oak (*Quercus agrifolia*), interior live oak (*Q. wislizenii*), Shreve oak (*Q. parvula* var. *shreveii*) and California black oak (*Q. kelloggii*). Of these, the first three are evergreen oaks and the last is deciduous. All four species occur along the California coast and interior live oak and California black oak are also naturally distributed in the Sierra Nevada and Cascade mountains. In 1995 a disease was reported affecting tanoak (*Lithocarpus densiflorus*), that was subsequently attributed to a strain of *Phytophora* (Garbelotto and others 2001). This sudden oak death (SOD) disease is now reaching epidemic levels on tanoak, coast live oak and black oak (McPherson and others 2000), and has also been reported on Shreve oak and other unrelated

species. The species being affected by this new disease are typical components of the coastal woodland vegetation of California. If the disease continues to spread, the impact on biodiversity could be catastrophic with potential loss of genetic diversity and of species. Several approaches are necessary in the attempt to control future spread of the disease, including the search for genetic resistance among the host species. Preliminary inoculation studies suggest that natural variation in resistance to SOD may exist in coast live oak (D. Rizzo, personal communication). With this in mind, the research reported here explores genetic architecture in the natural range of these species. Understanding levels of genetic variation and its partition within and among populations may be very helpful in developing a program aimed at identifying resistant genotypes, for setting guidelines for seed collection zones and for gene conservation.

In general, oaks, that are long-lived, outcrossed, wind pollinated species, are expected to exhibit a genetic architecture in which most variation is within rather than between populations (Hamrick and Godt 1989). Indeed, this has been the pattern observed for most species of oak that have been studied (Manos and Fairbrothers 1987, Guttman and Weigt 1988, Schnable and Hamrick 1990, Kremer and Petit 1993, Muller-Starck and others 1993). Little is known of the genetic variation among and within populations of the four California black oak species reported on here, but, as in most oak species, interspecific hybridization may be an important source of variation. Our earlier ecological genetic data suggested differentiation of central coast populations of coast live oak from those north of Cloverdale and south of Ojai (Dodd and others 1993a, 1997), which we attributed to possible introgression with Shreve oak in central California (Dodd and others 1993b, 1994, 1997). Recently, our molecular data have shown genetic similarity between interior live oak, Shreve oak and coast live oak, suggesting their close phylogenetic relationship (Kashani and Dodd 2002; Kashani, in press). We should therefore also expect hybridization to occur readily between Shreve oak and interior live oak. Indeed, the distributional limits of these two species in the Coast Ranges are unclear. It was therefore one of the objectives of this work to elucidate the taxonomic status of populations from the northern Coast Ranges that we attribute to the interior live oak complex.

For this work we have used biochemical markers (cuticular hydrocarbons) that have proven highly effective in identifying Shreve oak (Dodd and others 1997). Variation in cuticular hydrocarbon profiles is under strong genetic control, as shown by genetic studies of crop plants (Bianchi 1987, Jenks and others 1992) and by heritability estimates in coniferous species (Dodd and A. Rafii 2000) and in Mediterranean oaks.[4]

Methods

Foliage was collected from 29 populations of interior live oak and Shreve oak (referred to as the interior live oak complex hereafter) and from 27 populations of coast live oak. Sampling of the interior live oak complex was designed to include central California coast populations ascribed to Shreve oak, Sierra Nevada populations of interior live oak and a geographic series of populations connecting these two groups that approximately formed an inverted U around the northern Central Valley. Sampling of coast live oak was from throughout its range in the

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[4] Unpublished data on file, University of California at Berkeley, California.

northern and southern Coast Ranges, extending into Baja California. In addition 5 populations of California black oak were sampled from the Coast Ranges and the Sierra Nevada.

Mature foliage was collected at the end of the season from an average of 10 widely spaced trees per population. Only mature foliage from adult trees was sampled to avoid developmental and seasonal changes in cuticular lipid composition. The cuticular waxes were extracted by washing leaves in *n*-hexane for 3 minutes. The hydrocarbon fraction of the cuticular lipid extract was separated from other wax constituents by filtering the extract through a column packed with 0.5 g of 70-230 mesh silica gel. The hydrocarbon extract was analyzed on an HT-5 (0.25 mm internal diameter; 25 m length) capillary column in a Varian 3400 gas chromatograph. Alkanes were identified by comparing retention times with those of commercial standards. Chromatographic peak areas of identified compounds were expressed as a percentage of the total alkane extract.

Results

A homologous series of long chain alkanes ranging from 21 to 33 carbons was detected, with odd chain alkanes with carbon chain lengths from 25 to 33 predominating *(table 1)*. For all species, nonoacosane was the dominant alkane in the cuticular hydrocarbon mixture.

Table 1—*Mean percentage composition of cuticular hydrocarbons. Standard errors in parentheses. Major discriminating hydrocarbons shown in boldface.*

Alkane	Interior live oak	Shreve oak	Coast live oak	California black oak
C_{21} Heneicosane	0.16 (0.01)	1.90 (0.40)	trace	trace
C_{22} Docosane	trace	3.04 (0.64)	trace	0.21 (0.03)
C_{23} Tricosane	0.17 (0.03)	0.14 (0.02)	0.04 (0.003)	0.54 (0.08)
C_{24} Tetracosane	0.34 (0.04)	0.23 (0.03)	0.05 (0.004)	0.67 (0.10)
C_{25} Pentacosane	1.30 (0.09)	0.64 (0.08)	0.52 (0.03)	1.18 (0.13)
C_{26} Hexacosane	1.52 (0.08)	0.73 (0.07)	0.29 (0.02)	0.97 (0.07)
C_{27} Heptacosane	7.71 (0.31)	3.97 (0.41)	3.51 (0.12)	**14.40 (0.69)**
C_{28} Octacosane	2.84 (0.09)	2.68 (0.14)	2.11 (0.06)	2.14 (0.10)
C_{29} Nonacosane	77.02 (0.85)	49.52 (2.29)	**88.10 (0.35)**	78.60 (1.08)
C_{30} Triacontane	1.69 (0.14)	2.37 (0.12)	1.41 (0.04)	0.30 (0.04)
C_{31} Hentriacontane	7.02 (0.81)	**32.08 (1.97)**	3.90 (0.29)	0.85 (0.15)
C_{32} Dotriacontane	0.11 (0.02)	0.73 (0.07)	0.03 (0.004)	0.01 (0.004)
C_{33} Tritriacontane	0.12 (0.02)	1.96 (0.33)	0.05 (0.01)	0.03 (0.01)

Species Differences

California black oak was characterized by relatively high levels of heptacosane and low levels of hentriacontane, and coast live oak by the dominance of nonacosane *(table 1)*. The cuticular hydrocarbon profile of Shreve oak was the most distinctive, including very high levels of hentriacontane and greater than trace amounts of the very short chain alkanes, heneicosane and docosane.

Since the proportion of hentriacontane present in the cuticular wax profile appeared to be a good marker for separating the Shreve oak from interior live oak, it

was tested as a possible indicator of hybridization between these two species. A frequency plot of percentage composition of hentriacontane for all individuals of interior live oak and Shreve oak suggested a trimodal distribution, in which individuals could be classified as low (0-10 percent of total hydrocarbons), medium (11-36 percent) or high (39-60 percent) chemotypes. The frequencies of these 3 chemotypes in all populations of the interior live oak complex are plotted *(fig. 1)*. With the exception of one population from Brickyard Road (Pop 23), all Sierran populations were composed entirely of individuals with low levels of hentriacontane. Populations from central coastal California were composed mostly of individuals with medium, or high levels of hentriacontane. North coastal populations varied in proportions of low, medium and high chemotypes, but only the medium and low chemotypes were present in northern populations (populations 16 and 17) connecting the Coast Ranges with the Cascade/Sierran distribution.
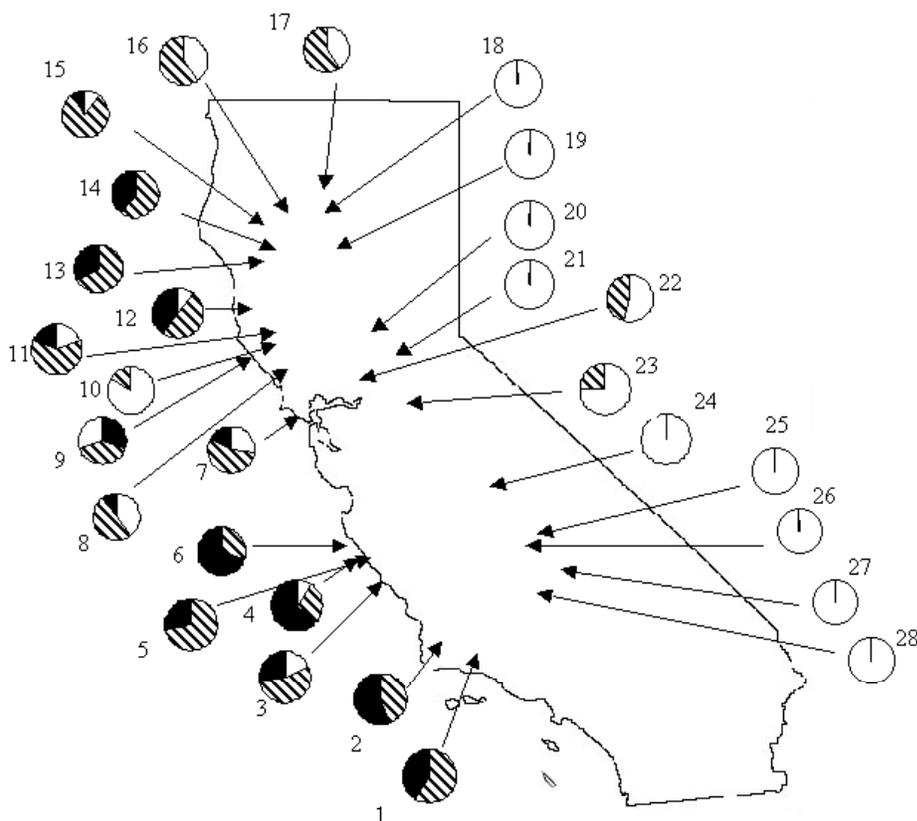

Figure 1—Proportions of hentriacontane chemotypes in populations of interior live oak complex. White sector in pie chart low chemotype, black sector high chemotype, hatched sector medium chemotype.

To further investigate possible introgression in north coastal populations of the interior live oak complex, linear discriminant function analysis was carried out. A training data set and a test data set were created to investigate the strength of species separations based on the total cuticular hydrocarbon data set. The populations for the training data set included all non-hybrid populations of coast live oak and of

California black oak, as these species are highly distinctive morphologically, and Sierran populations of interior live oak and central coast populations of Shreve oak. Remaining populations of the interior live oak complex, mainly from the northern Coastal ranges were included in the test data set. The training data set was used to derive discriminant functions that best separated the species and the test data set was used to ascribe individuals of uncertain affinity to species, based on the derived discriminant functions. The training data set produced a good separation of species with an overall error rate of 8.2 percent *(table 2)*. Classification error rates were 2.3 percent and 6.1 percent respectively for coast live oak and California black oak, whereas interior live oak and Shreve oak both had error rates of about 12 percent.

Table 2—*Percentage classification to species from discriminant function analysis.*

Field identification	Pct of individuals classified into species by discriminant function classification on training data set			
	Coast live oak	Cal black oak	Shreve oak	Interior live oak
Coast live oak	97.7	0.4	1.5	0.4
Cal. Black oak	4.1	93.9	0	2.0
Shreve oak	6.0	0	88.0	6.0
Interior live oak	7.6	3.5	1.4	87.5
	Pct of individuals classified into species by test data set			
Interior live oak	21.6	0	44.1	34.3

All individuals of the test data set were assigned to the interior live oak complex in the field, partly because morphological separation of interior live oak and Shreve oak was not certain, and partly because distinction of these two taxa in the northern Coast Ranges was being investigated. Only 34 percent of individuals from the test data set were assigned to interior live oak *(table 2)*. Twenty two percent were assigned to coast live oak and 44 percent were assigned to Shreve oak. No individuals from the test data set were assigned to California black oak. The proportion of individuals in populations assigned to the three species are shown in a distribution map *(fig. 2)*. Individuals from the northernmost populations at Douglas City and Whiskeytown were all assigned to interior live oak. Remaining populations included varying proportions of individuals assigned to coast live oak*,* interior live oak and Shreve oak.
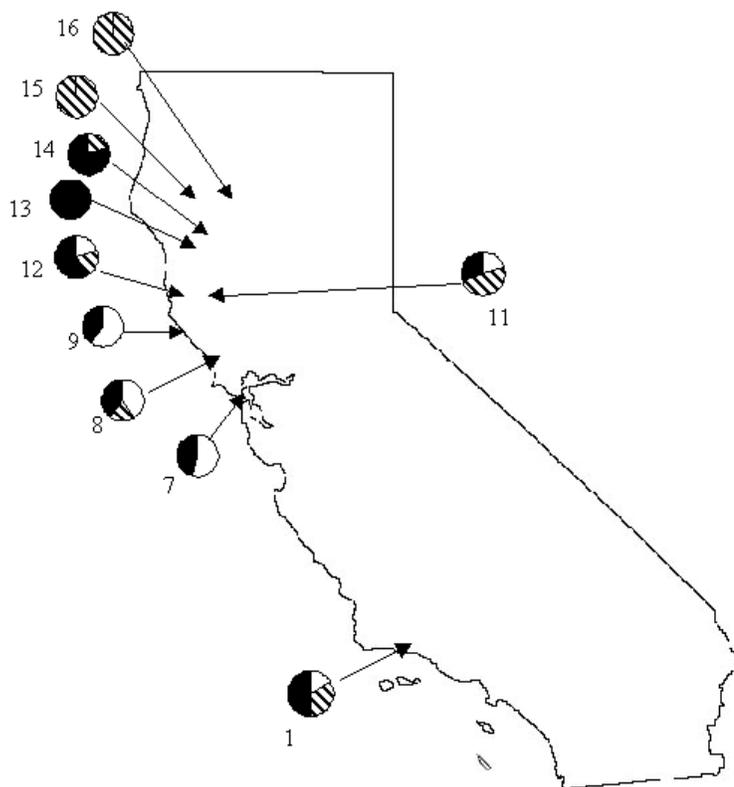
Figure 2—Proportions of individuals in populations of the interior live oak complex from the Coast ranges attributed to species from discriminant function analysis. White sector in pie charts coast live oak, black sector Shreve oak, hatched sector interior live oak.

Genetic Diversity

Plots of frequency distributions of percentage composition were skewed, with a strong tendency towards tri-modality for 5 of the cuticular alkanes. This type of frequency distribution can be explained by the presence of three chemotypes for each of the 5 alkanes; low, medium and high chemotypes. The frequencies of the 3 chemotypes by species and by population within species were calculated, and the resulting chemotype frequencies were used to estimate chemotypic diversity using Shannon's diversity index:

$$D = -\Sigma \log_3 p_i(p_i)$$

where p_i is the proportion of individuals with each of the i chemotypes.

Chemotypic diversity was least in coast live oak and greatest in interior live oak and Shreve oak *(table 3)*. The unassigned populations were no more diverse, on average, than either of the two latter species. Average diversity estimated over all individuals, ignoring population structure, is a measure of total diversity for the species. For coast live oak, this estimate did not differ from the average of populations. However, in the remaining species total average diversity was much greater than average of populations, suggesting much greater within population variation in these species.

Table 3—*Shannon's diversity index for populations of oak species. Numbers in parentheses refer to populations numbered on figures 1 and 2.*

Coast live oak				Cal black oak		Interior live oak		Shreve oak		Unassigned populations	
Population	D		D	Population	D	Population	D	Population	D	Population	D
Cloverdale	0.04	Lompoc	0.04	Redding	0.12	Kern R. (25)	0.10	Purisima (2)	0.09	Cachuma (1)	0.10
Franz Valley	0.02	Ojai	0.02	Legget	0.08	Wofford Hts. (28)	0.08	Plaskett Cr. (3)	0.15	Mt. Tamalpais (7)	0.11
Crane Park	0.04	Valencia	0.02	Round Vlly.	0.06	Kernville (27)	0.02	Palo Colorado 1 (4)	0.17	Geysers (8)	0.14
Point Reyes	0.06	Cleveland 1	0.02	Petaluma	0.11	Cal. Hot Spr. (26)	0.11	Palo Colorado 2 (5)	0.19	Gualala (9)	0.16
Pacheco Pk.	0.08	Cleveland 2	0.02	San Pablo	0.05	Mariposa (24)	0.13	Big Sur (6)	0.13	Orrs Spr. Rd. (12)	0.13
Pacheco Pass	0.08	Fallbrook	0.02			Brickyard (23)	0.12			Lower Lake (10)	0.06
Fremont Pk.	0.08	Peutz Valley	0.03			Roseville (22)	0.13			Clearlake (11)	0.16
San Juan Rd.	0.09	San Ysabella	0.04			American R., Cool (21)	0.17			Farley (13)	0.05
Parkfield	0.10	Vallecitos (BC)	0.02			Hwy 70 (20)	0.17			Round Valley (14)	0.06
San Miguel	0.02	San Antonio (BC)	0.02			Red Bluff (19)	0.19			Douglas City (15)	0.18
Paso Robles	0.04	La Mission (BC)	0.02			Redding (18)	0.07			Whiskeytown (16)	0.13
Blk. Mountain	0.03	Santo Thomas (BC)	0.07			Lake Shasta (17)	0.11				
Cambria	0.05	San Pedro Martir (BC)	0.05								
Population mean			**0.05**		**0.08**		**0.12**		**0.14**		**0.12**
All individuals			**0.05**		**0.21**		**0.22**		**0.22**		**0.20**

Within Species Population Structure

Canonical discriminant analysis was carried out on the matrix of cuticular hydrocarbon composition for coast live oak and for the interior live oak complex. Population mean scores of the first two canonical vectors are plotted *(figs. 3* and *4)*.
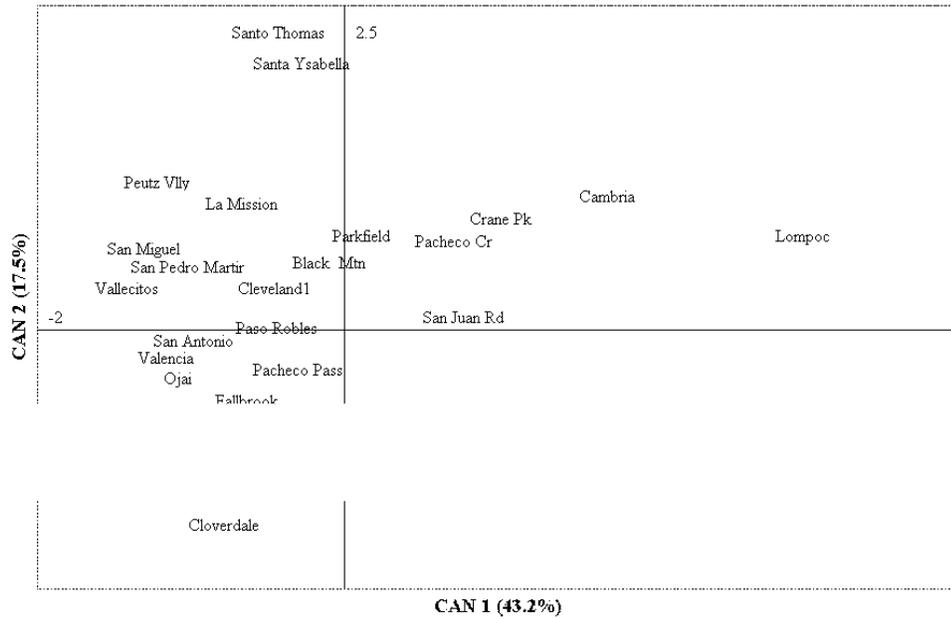

Figure 3—Plot of the first two canonical discriminant vectors of cuticular hydrocarbon composition among populations of coast live oak.
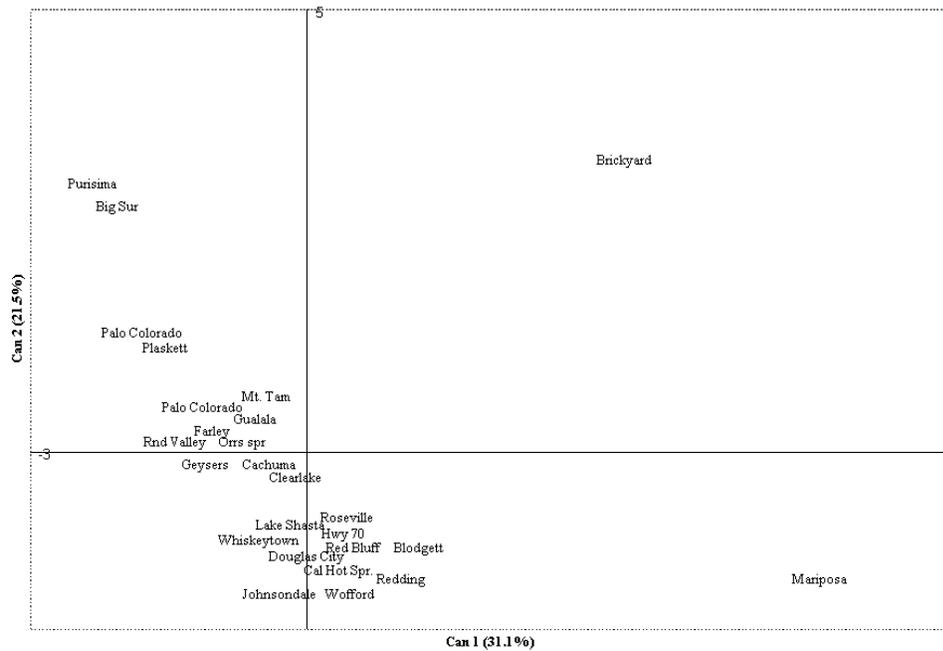

Figure 4—Plot of the first two canonical discriminant vectors of cuticular hydrocarbon composition among populations of the interior live oak complex.

For coast live oak, the first four canonical vectors were significant at the P<0.0001 level and together accounted for 79 percent of the variance. The plot of the first two vectors showed a geographic trend, in which southernmost populations were on the negative side of the origin along the first canonical vector *(fig. 3)*. With the exception of Cloverdale and Paso Robles, central and northern populations were close to the origin or on the positive side along the first vector. Within these two main geographic groups, no clear geographic, or ecological trend was evident.

The first 8 vectors were significant at P<0.0001 for the interior live oak complex, of which the first 4 explained 75 percent of the variance. The plot of the first two vectors showed a trend from populations of Shreve oak in the upper left quadrant through unassigned populations close to the origin and populations of interior live oak in the lower half of the plot, either close to the origin or in the lower right quadrant *(fig. 4)*. Three populations assigned to interior live oak were well separated from all others. These three populations from Brickyard Road, Lower Lake and Mariposa, were mixed with California black oak and their cuticular hydrocarbon profiles showed some affinities with this species.

Discussion

Morphological differentiation of Shreve oak and interior live oak has proven difficult, with the result that the distributions of these two species in the California Coast Ranges is inconclusive. According to Tucker (1993), the former species is found in the Southern Coast Ranges and in the San Francisco Bay Area, but Shreve oak is still not fully accepted by the scientific community. Whereas, molecular markers have not been successful in separating these two taxa (Kashani and Dodd 2002), cuticular lipid composition appears to provide a remarkably powerful method for their distinction. Unusually high levels of hentriacontane appear to be unique for Shreve oak, among the four black oak species native to California. In pure populations of interior live oak, coast live oak and California black oak, only the low hentriacontane chemotype is present.

Hybridization among oak species is common, and is reported to occur among these four black oak species (Tucker 1980). In our earlier work (Dodd and others 1997), we reported on a low frequency of medium hentriacontane chemotypes in central coastal populations of coast live oak, and concluded that this was due to introgression of Shreve oak genes. Our more extensive analyses of coastal and interior populations of the interior live oak complex support this earlier interpretation of chemotypic variation, and suggest that cuticular biochemistry is a sensitive indicator of introgressive gene flow. Among our unassigned populations of this group, discriminant function analysis on the full cuticular hydrocarbon data, placed some individuals to coast live oak. Populations with individuals assigned to coast live oak fell entirely within the distributional range of this species, providing indirect support for the sensitivity of this method. All individuals were identified morphologically as belonging to the interior live oak complex, and no morphologically identified hybrids were included in the analysis. Results of the discriminant analysis therefore suggest that introgressive gene flow is important in these populations and introgression of coast live oak genes appeared to be greatest in the coastal populations at Mt. Tamalpais and at Gualala. Remaining individuals in each of these unassigned populations were attributed to Shreve oak or to interior live

oak and never to California black oak, even though hybrids among all four species are known to occur.

In the unassigned populations, individuals attributed to Shreve oak exceeded those attributed to interior live oak, except in the extreme north at Douglas City and Whiskeytown. In these two latter populations all individuals were attributed to interior live oak. The canonical discriminant function plot and the map of hentriacontane chemotypes strongly suggest a transition in the Cascade Mountains from a coastal to a Sierran form of interior live oak. Should coastal forms all be treated as Shreve oak? We suggest that central coast populations at low elevations, typically occupying redwood forest should be treated as Shreve oak. This region may extend as far north as the San Francisco Bay. However, it seems likely that Shreve oak and interior live oak are poorly differentiated, probably from a recent ancestor and that in the northern Coast Ranges, a complex mix of the two taxa, along with hybrids and introgressants, exists, probably varying in proportions according to ecological preferences. The Shreve oak form gradually falls out eastward to the Sierra Nevada, probably as summer drought limits its success. Among the four species studied chemotypic diversity was greatest in interior live oak and in Shreve oak, which might be expected if these two species were relatively poorly differentiated and were forming hybrid complexes.

Sudden oak death has been reported on coast live oak, California black oak and on Shreve oak, but not yet on interior live oak. It is not yet clear whether lack of reports of infection on the latter species are due to its occurrence on drier sites, due to misidentification or due to genetic resistance. The present work points out the biosystematic complexity of these black oak populations, which needs to be understood in attempting to find resistant genotypes.

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