Gene Flow Among Populations of Three California Evergreen Oaks[1]

Richard S. Dodd[2] Zara A. Rafii[3] Nasser Kashani[4]

Abstract: *Intraspecific variability within the oak genus has been the source of considerable taxonomic confusion. In part, this variability seems to arise from the relatively facile hybridization of oak species. Our biochemical data suggest that coastal populations of* Quercus wislizenii *should be ascribed to* Q. parvula *and that, in restricted regions, hybridization between this species and* Q. agrifolia *accounts for difficulty in their separation. Low levels of interspecific gene flow occurring over a wider geographic range may account for morphological variability in the species.*

Oak species are characterized by unusually high levels of morphological variability which often pose serious taxonomic problems. This variation may be attributed to high intrinsic levels of genetic variation, to high levels of phenotypic plasticity, and to high potential gene flow among species. Undoubtedly, all three sources of variation are important. Reports of unusually high levels of genetic variation at the species level (Dodd and others 1993a; Guttman and Weight 1989; Rafii 1988; Schnabel and Hamrick 1990; Schwarzmann and Gerhold 1991) are as expected for species of late seral stages, which are predominantly outcrossed and long-lived (Hamrick and Godt 1990). Relatively little is known about the genetic controls of phenotypic plasticity, particularly in the genus *Quercus*. However, the high degree of within-tree morphological variability suggests that phenotypic plasticity is an important source of variation in oaks.

It is the third source of variation, gene flow among species, that is the focus of this paper. Although many oak species are sufficiently distinct that identification presents no great problems, there are instances in which morphological convergence results in taxonomic confusion. This is particularly true in regions of sympatry, where the separation of species is sometimes problematic. In these instances, hybridization has commonly been cited as the source of morphological confusion. Reports of hybrids in oaks have usually taken one of two forms: (1) infrequent individuals that are intermediate between parental forms or (2) populations in which individuals show a range of morphological variation including characteristics of either parents and all levels of intermediacy. Because genetic incompatibility is believed to be absent or infrequent in *Quercus* (Stebbins 1950), the potential for interspecific crossing should be relatively high. It is, therefore, the absence, or low frequency, of hybrids that is perhaps more remarkable under conditions of sympatry.

Reports of low levels of hybridity in nature may be more apparent than real because of the difficulty of identifying crossed individuals. Anderson (1948) pointed out that F1 hybrids are generally morphologically intermediate between either parent, but future generations of hybrids and backcrosses include high proportions of individuals that closely resemble either one or the other parent. Because of high levels of morphological variation within parental taxa, field biologists might fail to recognize individuals as hybrid. The need for more specific genetic markers is essential for the detection of interspecific gene flow. Recently, low levels of gene flow have been demonstrated among morphologically "typical" members of eastern North American white oaks, using chloroplast DNA (Whittemore and Schaal 1991). This evidence, together with molecular studies of parapatric species from other genera, suggests that introgressive gene flow may be more prevalent than previously thought.

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[2]Professor, Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA 94720, and Professeur, Groupe de Biologie Quantitative, Université Paul Sabatier, 118 Route de Narbonne, 31062 Toulouse Cedex, France

[3]Directeur de Recherche, Institut Méditerranéen d'Ecologie et de Paléoécologie, UA 1152, Université d'Aix-Marseille III, 13397 Marseille, Cedex 13, France

[4]Graduate student, Wood Science and Technology, University of California Forest Products Laboratory, 1301 South 46th Street, Richmond, CA 94801

Evaluation of the role of hybridization in evolution depends on an adequate means for identifying gene flow and on an understanding of the ecological preferences of parental species and the occurrence of ecological gradients in sympatric zones. To shed some light on these processes, we have been studying intraspecific variation in natural allopatric and sympatric populations of evergreen oaks in California (Dodd and others 1993a, b) and in the Mediterranean Basin (Rafii and Dodd 1992; Rafii and others 1993). Earlier work using acorn steroids and acorn fatty acids as biochemical markers provided strong evidence to support field observations of hybridization between *Q. wislizenii* and *Q. agrifolia* and at the same time indicated significant differentiation between Sierra Nevadan and coastal populations of the former species. To further investigate diversity in these species we have analyzed epicuticular wax composition of a wider geographic range of populations of the two species, together with individuals of *Q. parvula*, including some from the type locality of variety *shreveii*, which was formerly attributed to *Q. wislizenii*.

Methods

Foliage was collected from approximately 10 individuals from each of 26 populations of *Q. agrifolia* and seven populations of *Q. wislizenii*. Sampled individuals of the latter species included five Sierra Nevadan populations (allopatric from *Q. agrifolia*), a Central Valley population from Roseville (near Sacramento), and a coastal population from Ornbaum (near Gualala). Because *Q. wislizenii* and *Q. parvula* have commonly been treated as synonymous, individuals from the type locality of *Q. parvula* var. *shreveii* at Palo Colorado Canyon and from nearby locations at Santa Cruz and Pfeiffer Big Sur and individuals of *Q. parvula* var *parvula* from the Purisima Hills were included in the analyses. In addition, three putative hybrid populations from Hopland, Yorkville, and Santa Cruz were included. Population locations are numbered in *fig. 1*, and numbers correspond to the population names in *tables 1, 2, 3*.

Within each population, mature trees at least 50 m apart were selected, and foliage from different sides of the outer crown was sampled. Sampling was carried out after late summer, and further analyses were carried out only on mature foliage. Epicuticular waxes were extracted by submerging a random sample of 10 whole leaves (to avoid extraction of internal lipids) per tree in 10 ml of hexane for 3 minutes. Hydrocarbons were separated from other wax constituents by filtering the extract through a mini-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) column in a Varian 3400 gas chromatograph equipped with a flame ionization detector. Alkanes were identified by comparing

Table 1—Percentage occurrence of low, medium, and high levels of hentriacontane in foliar wax extracts of Quercus parvula *and of hybrid populations of* Q. agrifolia *and* Q. wislizenii

Collection locality	Low	Medium	High
P1 Palo Colorado	0	50	50
P2 Pfeiffer Big Sur	0	50	50
P3 Purisima Hills	0	0	100
P4 Santa Cruz	0	0	100
Hybrid Populations			
H1 Hopland	43	43	14
H2 Yorkville	100	0	0
H3 Santa Cruz	88	6	6

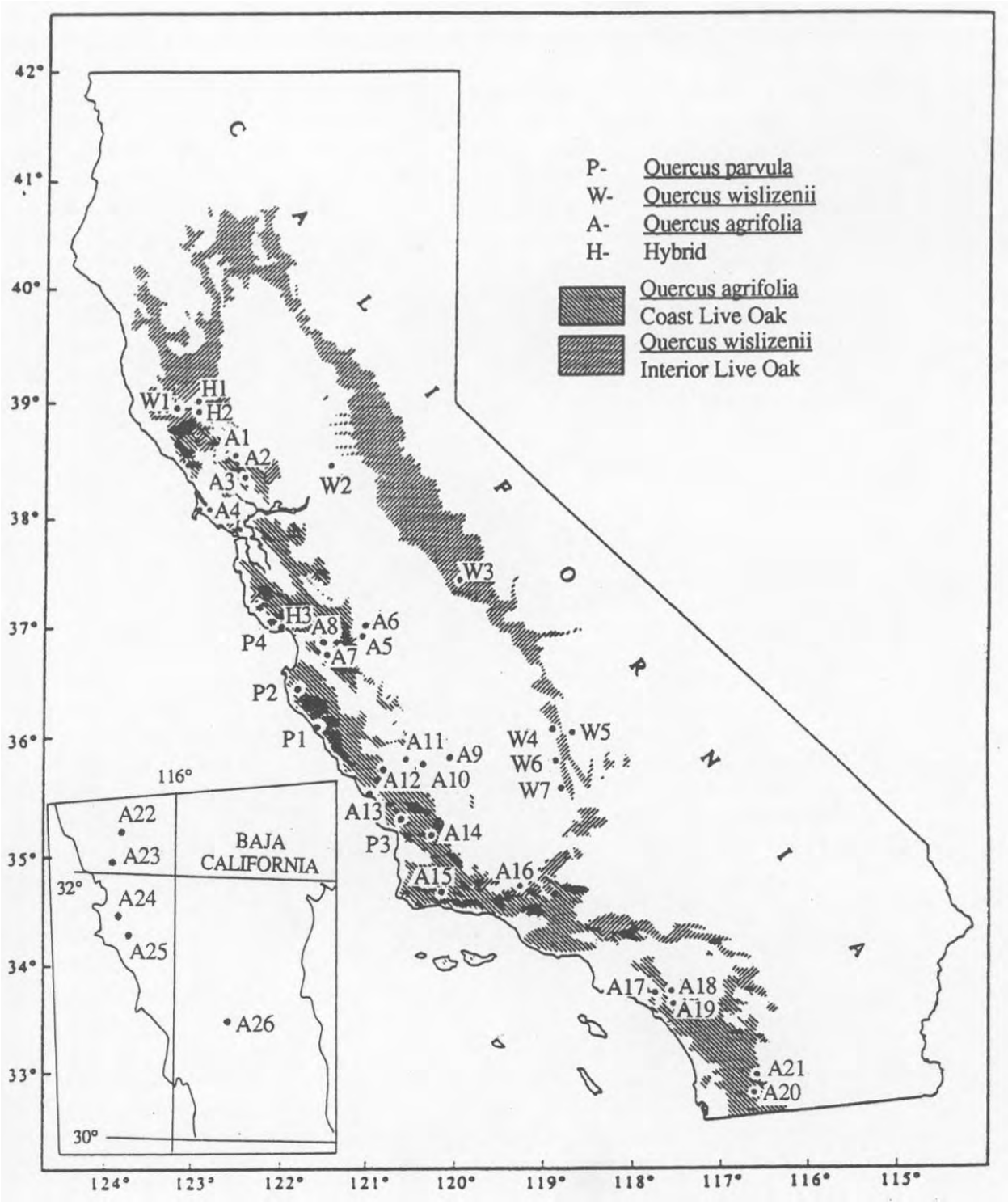

Figure 1—Geographic distribution of *Quercus agrifolia* and *Q. wislizenii* in California and populations sampled in California and Baja California.

retention times with commercial standards and by comparing gas chromatography - mass spectrometric (GC-MS) analyses with a library of mass spectra (D. Henneberg, Max-Planck Inst., Mulheim, Germany). Chromatographic peak areas of identified compounds were expressed as a percentage of the total alkane extract.

Table 2—Percentage occurrence of low, medium, and high levels of hentriacontane in foliar wax extracts of Quercus agrifolia

Collection locality	Low	Medium	High
A1 Cloverdale	90	10	0
A2 Franz Valley	100	0	0
A3 Crane Park	100	0	0
A4 Point Reyes	90	10	0
A5 Pacheco Creek	90	10	0
A6 Pacheco Pass	80	20	0
A7 Fremont Peak	62	38	0
A8 San Juan Road	70	30	0
A9 Parkfield	80	20	0
A10 San Miguel	100	0	0
A11 Paso Robles	89	11	0
A12 Black Mountain	100	0	0
A13 Cambria	90	10	0
A14 Lompoc	100	0	0
A15 Ojai	90	10	0
A16 Valencia	100	0	0
A17 Cleveland 1	100	0	0
A18 Cleveland 2	100	0	0
A19 Fallbrook	100	0	0
A20 Peutz Valley	100	0	0
A21 San Ysabella	100	0	0
A22 Vallecitos Baja California (BC)	100	0	0
A23 San Antonio (BC)	100	0	0
A24 La Mission (BC)	100	0	0
A25 Santo Thomas (BC)	86	14	0
A26 San Pedro Martir (BC)	83	17	0

Table 3—Percentage occurrence of low, medium, and high levels of hentriacontane in foliar wax extracts of Quercus wislizenii

Collection locality	Low	Medium	High
W1 Ornbaum	30	40	30
W2 Roseville	55	45	0
W3 Mariposa	100	0	0
W4 California Hot Springs	90	10	0
W5 Kernville	90	10	0
W6 Wofford Heights	100	0	0
W7 Kern River	100	0	0

Results

A total of 11 alkanes, with carbon chain lengths ranging from C_{20} to C_{33}, were detected in the filtered wax extract. Frequency distributions of the percentage composition of these alkanes (from the more than 400 individuals) were heavily skewed, as shown most strikingly for hentriacontane (C_{31}), in *fig. 2*. This distributional pattern may be explained by the presence of three chemotypes comprising low levels of hentriacontane (up to 10 percent of total hydrocarbons), medium levels (11-36 percent), and high levels (39-60 percent). This trimodal pattern is suggestive of two alleles at a single locus specifying high and low levels of the compound, respectively.

For all individuals combined, the low C_{31} chemoytpe was the most common, followed by medium and then high C_{31} chemotypes. An interesting pattern emerged for the frequency of the different chemotypes among populations. High C_{31} was the most common chemotype in all individuals from the type locality of *Q. parvula* var *shreveii* at Palo Colorado and from nearby populations at Pfeiffer Big Sur, Santa Cruz, and in the Purisima Hills population of *Q. parvula* var *parvula* (*table 1*). For these individuals the low C_{31} chemotype was entirely absent.

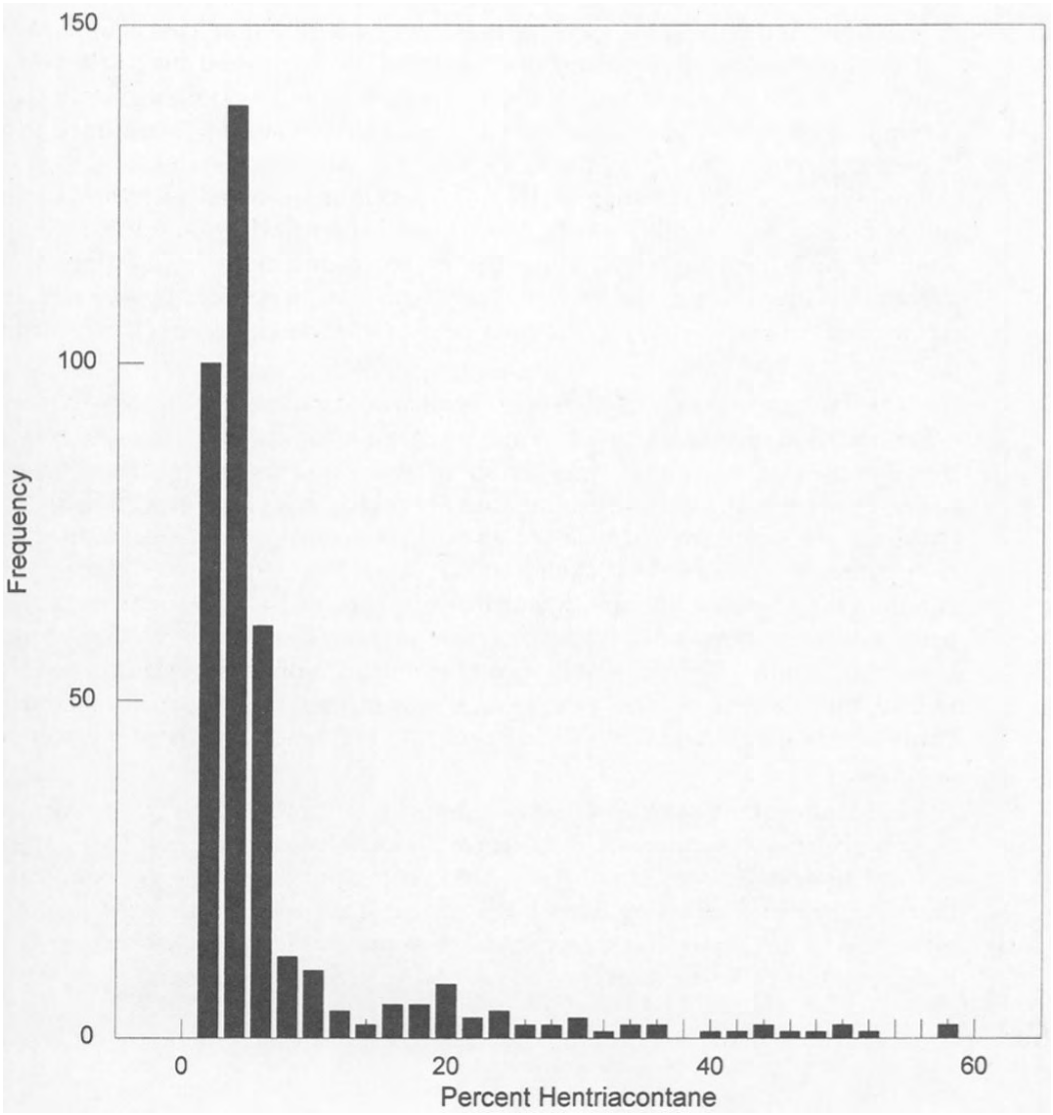

Figure 2—The frequency distribution of the percentage abundance of hentriacontane in wax extracts of leaves of *Quercus agrifolia*, *Q. wislizenii*, and *Q. parvula*.

By contrast, high C_{31} chemotypes were absent in *Q. agrifolia* (*table 2*), and the medium C_{31} chemotype occurred occasionally. Occurrence of the latter chemotype was restricted to populations from Cloverdale, in the north, to Ojai, in the south, and in two outlying populations in Baja California. In most of these populations, the incidence of medium C_{31} chemotypes was at, or below, 10 percent, but increased to 30 percent in a region east of Monterey-Santa Cruz (Pacheco, Fremont Peak, San Juan Road). The occurrence of the medium C_{31} chemotype in central California, in the probable range of *Q. parvula* var *shrevei*, is highly suggestive of gene flow between these two species.

In Sierra Nevadan populations of *Q. wislizenii*, the high C_{31} chemotype was absent (*table 3*), and the medium chemotype occurred in only two individuals (a frequency of less than 4 percent). This marked biochemical differentiation from *Q. parvula* serves to underline the differentiation of these two taxa. Interestingly, the coastal population from Ornbaum included almost equal numbers of the three chemotypes (*table 3*), raising the question as to whether this population might be better ascribed to *Q. parvula*, or to hybrids between the two species. Similar reasoning may apply to the Central Valley population at Roseville, in which 45 percent of individuals were of the medium C_{31} chemotype.

Among the three populations identified as hybrid in the field, medium and high C_{31} chemotypes were recorded in both the Hopland and Santa Cruz populations, but only low C_{31} chemotypes were identified at Yorkville (*table 1*). Our data from steroid chemistry and morphology suggested highest levels of hybridization at Hopland followed by a decline from Yorkville to Santa Cruz (Dodd and others 1993a). In this earlier work, hybridization was assumed to be between *Q. agrifolia* and *Q. wislizenii*. However, presence of medium and high C_{31} chemotypes at Hopland suggest that *Q. parvula* is involved in hybridization rather than *Q. wislizenii*. Consistent with our earlier findings, hybridization at Santa Cruz is probably low, since the medium and high chemotypes were individuals identified in the field as *Q. wislizenii* type (probably *Q. parvula*). The hydrocarbon data provide no evidence for hybridization between *Q. parvula* and *Q. agrifolia* at Yorkville.

The patterns described above for hentriacontane were repeated by some other hydrocarbons and provide some interesting insights into the diversity of this complex of evergreen oaks. First, *Q. parvula* exhibits a marked genetic differentiation from interior populations of *Q. wislizenii*. Indeed, it is more distinct from *Q. wislizenii* than the latter species is from *Q. agrifolia*, supporting recognition of this taxon (Tucker 1993). Second, the frequency of chemotypes common in *Q. parvula*, but rare in the other two species, increases with increasing proximity to the range of *Q. parvula*, suggesting gene flow over a relatively broad geographic range. Third, chemotypes characteristic of *Q. parvula* appeared in two of the putative hybrid populations, suggesting the presence of hybrids between this species and *Q. agrifolia*, but only at Hopland were these chemotypes common.

In local regions such as Hopland, hybridization between *Q. parvula* and *Q. agrifolia* may be common, constituting a local hybrid swarm. This pattern of local hybrid success may be attributable to specific ecological conditions that favor hybrid progeny. Our biochemical data indicate that interspecific gene flow is not restricted solely to these regions, but that low levels of gene flow may be occurring over a relatively large geographic range.

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