

Range Expansion through Pollen Dispersal: Hybrid Studies in California Red Oaks

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Abstract

The red oaks of California comprise four species that hybridize over relatively large geographic areas. Two of these, coast live oak (*Quercus agrifolia* Née) and interior live oak (*Q. wislizeni* AC. D.) hybridize readily in northern California at the limit of the geographic range of coast live oak. In oaks, the chloroplast genome is inherited maternally and therefore backcrossed individuals maintain a history of past hybrid events. We analyzed five microsatellites in over 500 individuals of coast live oak and in almost 300 individuals of interior live oak to determine geographic patterns of haplotype diversity and to detect shared haplotypes among species. In northern California, coast live oak and interior live oak shared a fixed haplotype that appeared to be of interior live oak origin. This suggests that coast live oak pollen dispersing northwards had hybridized with interior live oak and subsequent backcrossing produced coast live oak phenotypes with an interior live oak chloroplast genome. Bayesian estimates of gene flow indicated asymmetric gene flow between groups of populations in Marin County, California and Sonoma/Mendocino County, with gene flow northwards six times greater than in the other direction. A range of hybrid and later generation genotypes may provide genetic resources that will buffer the adverse effects of climate change.

Key words: Quercus, genetic structure, chloroplast DNA, pollen, seed, hybridization

Introduction

The extent of hybrid zones depends on the balance between dispersal that leads to recombinant genotypes and selection that sieves out parental and hybrid genotypes according to their relative fitnesses. Interpretation of the importance of dispersal and selection and the likelihood of hybrids being fitter than parental types has fueled the debate over the importance of hybrid zones in evolution. At one extreme, reinforcing selection is seen as maintaining species isolation so that hybrid zones are narrow and transient (Mayr 1963; Wagner 1969, 1970; Hardin 1975). At the other extreme hybrid zones are viewed as a very potent source of genetic recombination and diversity (Anderson 1949; Stebbins 1959; Arnold 1997).

Interspecific transfer of genes through introgressive hybridization is expected to increase levels of genetic diversity, to provide new gene combinations on which selection can act and may lead to speciation if reproductive isolation is established. However, introgression is difficult to identify. In polymorphic populations, introgression may have gone unnoticed because appropriate methods for its detection were not used, or introgression may have been invoked without any experimental evidence to support it. Out of 165 reports of introgression in plants, only 65 cases were found that justified this claim (Rieseberg and Wendell 1993). However, the

frequency of introgression is probably underestimated as phylogenetic closeness may mean that there are few diagnostic features to separate parental taxa. Also, differences between introgressed individuals and the parent species follow a decay function with time since first introgression occurred (Rieseberg and Wendell 1993).

Recently, polymorphic sites in the chloroplast genome have provided very useful markers of clonally inherited DNA, much like the mitochondrial genome in animals. Many examples have been found in which the gene trees from this cytoplasmic marker are inconsistent with gene trees from nuclear DNA (Rieseberg & Soltis 1991; Rieseberg *et al.* 1996). Although this may be due to lineage sorting of ancestral polymorphisms in chloroplast and nuclear DNA (Comes & Abbott 2001), it appears that in many instances it is due to the introgression of chloroplast DNA from one species into another (Wolfe & Elisens 1995; Jackson *et al.* 1999; Kornkven *et al.* 1999). This so-called chloroplast capture provides a record of earlier hybrid events that may be masked in the nuclear genome by generations of backcrossing.

Quercus (oaks) has had a long history as a difficult taxon that defies the biological species concept (Burger 1975; Van Valen 1976), because of the seemingly unlimited potential for hybrid combinations within a taxonomic section (Hardin 1975; Nixon 2002). Although hybridization is widespread among oak taxa, it is still unclear how important it is as a source of novel genotypes that contribute to evolution of the genus. Most hybrid combinations of the white oaks of eastern North America occur in nature (Hardin 1975), but only rarely are generations beyond the F_1 detected. The few molecular studies have found little evidence for extensive nuclear gene flow in oaks. Nuclear recombinants were found over short distances within a mosaic structured hybrid zone between the white oaks *Q. grisea* and *Q. gambelii* (Howard *et al.* 1997). However, contrasting patterns from nuclear and chloroplast genes were reported in several white oaks from eastern North America (Whittemore and Schaal 1991). Chloroplast genes were exchanged readily among geographically close non-conspecifics, whereas a nuclear ribosomal gene followed taxonomic boundaries. Other reports from nuclear genes suggest little evidence for hybrid gene exchange (Guttman and Weigt 1989; Ducousso *et al.* 1993), however, this may be more lack of detection than lack of gene exchange as differentiation of parental species was very low. Recently, Dodd and A.-Rafii (2003) have shown various degrees of introgression among four sympatric red oaks in California using genomic DNA markers.

The red oaks of western North America include 3 evergreen and one deciduous species. Fine-scale habitat differentiation among the species results in some differences in geographic ranges, but areas of sympatry are extensive. In most cases, the four species are morphologically distinct, but in some areas a range of intermediate phenotypes may be encountered. Hybrids have been reported for all species combinations based on morphological or biochemical traits (Brophy and Parnell 1974; Tucker 1980; Vasey 1980; Nason *et al.* 1992; Dodd *et al.* 1993; Dodd *et al.* 2002). Recently, we demonstrated widespread hybridization and introgression among the red oaks in northern California and showed that extrinsic selection pressure promoted the persistence of a variety of introgressant forms at a landscape level (Dodd & A.-Rafii 2003).

Here, we explore the possible role of hybridization as a means of dispersal and colonization of coast live oak at its northern range limit, by making use of micro-

satellite variation in the chloroplast and nuclear genomes.

Materials and Methods

Leaf samples were collected from 505 individuals from 41 populations of coast live oak (*Quercus agrifolia* Née) and 272 trees from 23 populations of interior live oak (*Q. wislizeni* A. DC.) for chloroplast DNA analysis and from 499 individuals from 28 populations for nuclear DNA analysis. Populations were selected to cover the geographic range of the two species. The leaves were stored in plastic zipper-loc bags at -20°C.

Total genomic DNA was extracted from the leaf samples using a simplified CTAB (cetyltrimethyl ammonium bromide) method (Cullings 1992).

Chloroplast microsatellites

Five pairs of primers developed for the amplification of chloroplast microsatellite loci (*udt1*, *udt3*, *udt4*, *ucd4*, *udt5*) in *Q. petraea* and *Q. robur* (Deguilloux et al. 2003) were chosen to amplify chloroplast DNA in coast live oak. The PCR reaction solution (10µl) contained four dNTPs (0.2mM each), 2.5 mM of MgCl₂, 0.2µM of each primer, 10x reaction buffer, 25ng DNA and 1 unit of Amplitaq polymerase (Applied Biosystems, Foster City, CA). Amplifications were performed on a Techne Genius thermal cycler with the following profile; 5 min denaturing at 95°C, followed by 25 cycles of 1 min denaturing at 94°C, 1 min annealing at the primer T_m (see Table 1 in Deguilloux et al. 2003) and 1 min extension at 72°C, with a final extension of 72°C for 8 min. The PCR product (0.75 µL) was mixed with a solution of 8 µL of formamide and 0.5 µL of 350 ROX size standard (Applied Biosystems, Foster City, CA) and electrophoresed on an ABI 3100 automated sequencer (Applied Biosystems, Foster City, CA). Results were analyzed with GENESCAN 3.7 and GENOTYPER 3.7 software (Applied Biosystems, Foster City, CA).

Nuclear microsatellites

Six pairs of primers developed for the amplification of nuclear microsatellite loci (*quru-GA-0A01*, *quru-GA-0C11*, *quru-GA-0C19*, *quru-GA-1C08*, *quru-GA-1F02*, *quru-GA-2F05*) in *Quercus rubra* (Aldrich et al. 2002) were used to amplify nuclear DNA in coast live oak. Amplifications were performed in a standard polymerase chain reaction (PCR) mixture containing a buffer of 2.5mM Tris-HCl (pH 8.0), 12.5 uM EDTA, 125 uM DTT. We added 2.5 mM MgCl₂, 2.5mM each of the amplification primers, 2.5uM of each dUTP, 250ug/mL BSA and 0.0375 units/uL of Taq DNA Polymerase (Invitrogen). To facilitate PCR multiplexing, we used a touchdown program to optimize for differences in annealing temperature. The PCR reaction began with one activation cycle at 95°C for 10 min and then used the following cycle parameters: a denaturation phase of one minute at 94°C, one minute at 60°C and 35 seconds at 70°C for two cycles. The second phase followed for 18 cycles: 45 seconds at 93°C, 45 seconds at 59°C (reducing the annealing temperature by 0.5°C each cycle) and 45 seconds at 70°C. Following this phase were 20 cycles of 30 seconds denaturing at 92°C, 30 seconds at 50°C and 1 min extension at 70°C. This was followed by a final extension phase of 5 min at 72°C.

We used fluorescently labeled primers to visualize amplified PCR products on an Applied Biosystems 3100 automated sequencer. A two uL aliquot of PCR product was suspended in 8uL of formamide and 0.5uL of ROX 350 size standard (Applied Biosystems) and denatured for 4 minutes at 93 °C. Genotypes were scored

by length in base pairs using GENESCAN 3.7 and GENOTYPER 3.7 software (Applied Biosystems, Foster City, CA) and recorded in a Microsoft Excel spreadsheet.

Data Analysis

Because the chloroplast genome is inherited clonally, we combined the five microsatellite loci into a single haplotype for each individual. Nuclear microsatellites were treated as independent loci with two alleles.

We used a maximum-likelihood (ML) method based on the coalescent (as implemented in MIGRATE version 2.0.6 (Beerli and Felsenstein 1999, 2001) to assess direction of migration rates between populations from two regions in northern California, of interest under a hypothesis of hybridization by pollen dispersal: 1. southern Sonoma County to northern Mendocino County and 2. Marin County. Starting values of parameter estimates were obtained using F_{ST} on the first run, and the final values input into subsequent runs, which were carried out with differing input trees and random number seeds (and checked for consistency). Ten short chains (50 000 steps, sampling interval 500, first 100 samples discarded as burn-in) and two long chains (500 000 steps, sampling interval 100, first 100 samples discarded) were run, with adaptive heating of chains used in the definitive final run. Ten replicates were performed.

Results

A total of 31 chloroplast haplotypes were detected in coast live oak. The distribution of haplotypes in California suggested about 4 major groups (Fig1). 1. The San Francisco Bay area and northwards, haplotypes 1, 6 and 30 are common. Of these, only haplotype 6 is found outside of this region at York Mtn. Rd. in San Luis

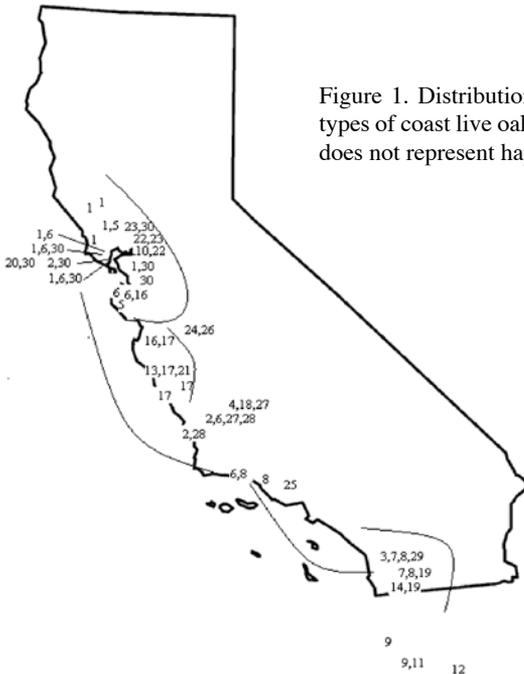


Figure 1. Distribution of chloroplast haplotypes of coast live oak. Sequence of numbers does not represent haplotype evolution.

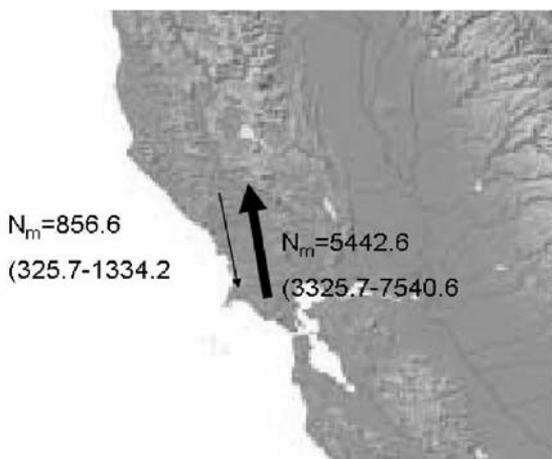


Fig 3. Estimated migration rates (scaled for mutation rate) for coast live oak between Marin County California and Sonoma and Mendocino Counties. Fig 3. Estimated migration rates (scaled for mutation rate) for coast live oak between Marin County California and Sonoma and Mendocino Counties.

north of Ojai was also detected close to Lake Berryessa, whereas haplotype 20 was unique to this locality. Interior populations from Parkfield and Coalinga were distinct from populations further west.

In some regions of sympatry between coast live oak and interior live oak and Shreve oak, sharing of haplotypes occurred. For example, in the Big Sur area 13 and 17 were the only haplotypes detected in Shreve oak and these were also detected in coast live oak. In the interior hills of the Coast ranges at Parkfield, haplotype 27 was detected in interior live oak and in coast live oak. In northern California from Sonoma County to northern Mendocino County haplotype 1 was almost fixed for both interior live oak and coast live oak.

Estimates of migration rate are summarized in Fig 3. Clear evidence of asymmetric gene flow was found between northern and southern groups of populations. In both cases, a source-sink model was supported with gene flow northwards greatly exceeding that in the opposite direction, evidenced by the lack of overlap between the central 90% of the likelihood profiles for the two parameter estimates (Fig 3).

Discussion

In both coast live oak and interior live oak, chloroplast haplotypes were mostly restricted to geographic regions, suggesting that the heavy seeds of oak are dispersed over relatively short distances. In coast live oak at least four biogeographic groups were recognized based on the chloroplast genome. In interior live oak, three Sierran and three coastal groups were detected. In some areas of sympatry, haplotypes were shared between the two species. Interspecific sharing of chloroplast haplotypes in areas of sympatry has been reported for other oak species (Whitte-

more and Schaal 1991, Dumolin-Lapégue et al. 1999).

At the northern limit of the range of coast live oak, we detected a single chloroplast haplotype. The same haplotype was fixed in populations of interior live oak from the area of sympatry and further north to northern Mendocino County beyond the present-day distribution of coast live oak. Possible explanations for this sharing of haplotypes include lineage sorting of ancestral polymorphisms (Comes and Abbott 2001), or chloroplast capture through hybridization (Tsitrone *et al.* 2003). The sharing of different haplotypes in different parts of the geographic range of these species strongly favours chloroplast capture as the most likely explanation for shared haplotypes between coast live oak and interior live oak. Furthermore, since the haplotype was detected in interior live oak well north of the geographic range of coast live oak, it is most likely to be of interior live oak origin. It would appear that pollen swamping from coast live oak results in hybrid progeny that have captured the maternally inherited *cpDNA* of interior live oak. Subsequently, backcrossing of these hybrid progeny to coast live oak has resulted in coast live oak phenotypes with interior live oak chloroplast genomes. Similar effects of pollen swamping have been found in *Eucalyptus* (Potts and Reid 1988, Potts et al. 2003) and in European oaks (Petit et al. 1997, Belahbib et al. 2001).

Our estimates of asymmetric migration rates between two population groups in northern California, suggests that more pollen successful in fertilization is being dispersed northwards than southwards. This could be a result of prevailing wind directions, or to differences in flowering time, with non-native pollen more likely to be present when female flowers are receptive in the north. It will be interesting to follow the phenology of male and female flowers over a latitudinal range in these two species.

It is encouraging that hybridization with interior live oak appears to be facile in northern California where summer temperatures are predicted to increase more than in the southwest (Hayhoe et al. 2004). Whereas coast live oak is adapted to a Mediterranean climate under the influence of summer fog, interior live oak is better adapted to more continental conditions of drought and extreme temperatures. Hybrid products between these two species may offer genotypes that will be well-adapted to the new ecological conditions as suggested by the landscape patterns of introgression reported by Dodd and A.-Rafii (2003).

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