INTERSPECIFIC AND INTRASPECIFIC POLLINATION PATTERNS OF VALLEY OAK, *QUERCUS LOBATA,* IN A MIXED STAND IN COASTAL CENTRAL CALIFORNIA

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Pollination patterns within *Quercus lobata* and interspecific hybridization between *Q. lobata* and *Quercus douglasii* were studied in a coastal central California mixed woodland. We first identified hybrids by means of microsatellite genotyping and assignment tests. Hybrids were rare, both among adults (4 of 190, 2.1%) and among acorns collected from *Q. lobata* trees (6 of 392, 1.5%). These low rates of hybridization at both early and late life history stages suggest that fertility barriers, rather than natural selection against hybrids, limit hybridization between these two species. However, hybrid adults, although rare, may facilitate gene flow between the two species. Acorns collected from a hybrid tree had both *Q. lobata* pollen donors (11 of 30, 37%) and *Q. douglasii* or hybrid pollen donors (19 of 30, 63%). After removing hybrid acorns from the analysis, we used paternity assignment to track pollination patterns within *Q. lobata*. Of 108 acorns, only 32 (30%) were assigned to candidate pollen donors within 200 m of the maternal tree, indicating that the majority of effective pollen travels more than 200 m. Individual trees had acorn crops with many different sires and an average effective number of pollen donors (N_{ep}) of 219 per tree. Indirect methods using correlated paternity estimated mean pollination distances of ~100 m and mean N_{ep} of 5.2 per tree, values much lower than those derived directly from paternity assignments.

Keywords: *Quercus*, hybridization, microsatellites, pollination, paternity assignment, interspecific gene flow.

Introduction

In plants, pollination patterns are a critical component of the ecological and evolutionary dynamics of plant populations. Rates and patterns of effective pollen movement will determine the size of a plant's reproductive neighborhood, the connectivity of populations, and the potential effects of habitat fragmentation (Levin and Kerster 1974; Loveless and Hamrick 1984; Smouse and Sork 2004). The application of highly variable DNA microsatellite markers has improved the study of pollen dispersal because it allows for the direct assignment of paternity to seeds or seedlings, thus tracking the trajectory of successful pollen (Ashley 2010). In addition to characterizing the distance and direction of pollination patterns, microsatellite analysis also allows precise estimates of rates of hybridization. In the genus *Quercus* (oak), hybridization is common, and reproductive barriers between species appear to be weak (Whittemore and Schaal 1991; Muir et al. 2000; Williams et al. 2001). In this study, we characterized both inter- and intraspecific pollination patterns in a mixed stand of oaks. We identified adults and acorns that were the result of hybridization between valley oak, *Quercus lobata*, and blue oak, *Quercus douglasii*. We also characterized intraspecific pollination within one species, *Q. lobata*, using both paternity assignment and an indirect method that uses correlated paternity among acorn crops to model pollen dispersal curves.

Many species of oaks are interfertile, yet most species maintain distinct morphological, ecological, and genetic identities (Whittemore and Schaal 1991; Craft et al. 2002; Dodd and Afzal-Rafii 2004). Interspecific hybridization explains observations of plants that are intermediate in morphology (Jensen et al. 1993; Howard et al. 1997; Gonzalez-Rodriguez et al. 2004). However, genetic introgression, particularly of cytoplasmic genomes, has been widely reported, and morphologically distinguishable hybrids are typically much rarer than would be predicted from estimates of cytoplasmic gene flow (Whittemore and Schaal 1991; Dumolin-Lapegue et al. 1999; Belahbib et al. 2001). Hypervariable nuclear markers such as microsatellites can provide additional insight into patterns of gene exchange between species. Microsatellites exhibit biparental inheritance, such that both parental species contribute alleles, and high allelic diversity allows F_1 and even backcrossed hybrids to be identified if the parental species are sufficiently differentiated. A number of studies have estimated rates of hybridization in *Quercus* by means of multilocus microsatellite genotypes combined with Bayesian statistical procedures that do not rely on a priori morphological classification (Craft et al. 2002; Gugerli et al. 2008; Burgarella et al. 2009). In some studies, hybrids are reported to occur at low frequencies. For example, hybrids between two Mediterranean evergreen oaks, *Quercus suber* and *Quercus ilex*, comprised less than 2% of adults in areas where their ranges overlap (Burgarella et al. 2009). In a mixed stand of oaks

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in Romania, 3.4% of adults were first-generation hybrids (Curtu et al. 2007). In a study of *Quercus virginiana* and *Quercus geminata*, species common in the southeastern United States, 5.5% of adults showed mixed ancestry (Cavender-Bares and Pahlich 2009). In contrast, other studies have reported higher rates of hybridization. For example, a study of three species of Mexican oaks (*Quercus hypoleucoides*, *Quercus scytophylla*, and *Quercus sideroxyla*) found that hybrids, including backcrosses and probable triple hybrids, dominated in the zone of contact (Penaloza-Ramirez et al. 2010). Triple hybrids were considered to have resulted from crossing an F_1 hybrid from two species with a pure individual of a third species. A study of four species of European oaks found that the percentage of hybrid individuals ranged from 10.7% to 30.5% in different stands (Lepais et al. 2009). Most of these studies examined only adult trees, however, and thus could not identify whether hybridization is controlled by prezygotic reproductive barriers between species or whether postzygotic barriers selecting against hybrids occur at a later life history stage. Prezygotic barriers could involve one or more mechanisms, including phenology, gamete incompatibility, or developmental failure. Postzygotic barriers could involve intrinsic hybrid breakdown, extrinsic or environmental selection, or hybrid sterility. In oaks, ecological differentiation, rather than prezygotic isolation, has been used to promote an ecological species concept for maintaining species boundaries (Van Valen 1976).

In this study, we compared hybridization rates at two life history stages, acorns and adult trees, to help distinguish between these possibilities. As a second objective of this study, we characterized intraspecific pollen-mediated gene flow in *Q. lobata*. Pollination patterns were characterized using microsatellite genotyping and paternity assignment. The high variability of microsatellite loci often allows a single compatible pollen donor to be identified or, alternatively, the exclusion of all candidate fathers sampled within a circumscribed study area. Paternity assignment studies of pollination have now been applied to a range of temperate and tropical tree species, and we are now beginning to resolve long-standing questions about pollination patterns, distances, and the shape of pollen dispersal curves (reviewed in Ashley 2010). These studies have demonstrated that rates of pollen immigration are often quite high and that average pollen dispersal distances are tens to hundreds of meters in many tree species. These high rates of long-distance pollination have been documented in several species of *Quercus* (Dow and Ashley 1996, 1998; Streiff et al. 1999; Nakanishi et al. 2004; Valbuena-Carabaña et al. 2005; Pakkad et al. 2008; Slavov et al. 2009) and in two species of *Populus* (Pospíšková and Šálková 2006; Slavov et al. 2009).

An important limitation to using parentage assignment to study pollination is that it requires exhaustive sampling of the candidate pollen donors. The approach can therefore be applied only to systems where the pool of pollen donors is a reasonable size to sample and genotype and tracked pollination events will be limited to the area that includes the sampled pollen donors. In our case, paternity assignment was a reasonable approach for *Q. lobata*, but *Q. douglasii* occurs at higher densities, and it was not feasible to sample enough of the candidate pollen donors for a paternity study. To circumvent these limita-

tions, several indirect approaches, starting with TWOGENER (Smouse et al. 2001) and later refinements such as KINDISP (Austerlitz and Smouse 2002; Austerlitz et al. 2004; Robledo-Arnuncio et al. 2006), were developed as less laborious approaches to study pollen dispersal. These indirect methods evaluate the genetic differentiation of pollen pools sampled by different maternal trees spread across the landscape. Only a few studies have applied both methods at the same study site in order to compare the results and to "ground truth" the indirect approaches, but these have raised some concerns. Slavov et al. (2009) compared both approaches in two populations of black cottonwood (*Populus trichocarpa*). In their study, the indirect correlated-paternity approach (KINDISP) severely underestimated pollen dispersal distances, approximating mean pollination distances six times smaller than that based on paternity analysis. In a study of a bat-pollinated tropical tree, *Hymanea courbaril*, de Lacerda et al. (2008) also found that the indirect method underestimated pollen dispersal distances. In addition to differences in pollen dispersal distances, Craft and Ashley (2010) found that the effective numbers of pollen donors based on paternity assignment were higher than correlated-paternity estimates in *Quercus macrocarpa*. Additional studies that apply both approaches are needed to better evaluate the utility and accuracy of the indirect approaches and in our case to determine whether this would provide reliable results for pollination studies in other species, such as *Q. douglasii*, where paternity assignment would be difficult.

Pollination in *Q. lobata* has been evaluated in two previous studies conducted at the Sedgwick Reserve in Santa Barbara County, California. A study using the TWOGENER approach estimated an average pollination distance of 65 m and an effective number of pollen donors of only 3.68 individuals, leading the authors to lament, "Where have all the fathers gone?" (Sork et al. 2002). In a more recent study using paternity assignment, pollination distances were estimated to be approximately twice as large as in the earlier study (114 m), and the estimate of the effective number of pollen donors more than doubled, to 10.1 individuals (Pluess et al. 2009).

The goal of our intraspecific pollination study was to use both direct and indirect methods to characterize contemporary pollen flow at a different and contrasting study site at Hastings Natural History Reservation, a site closer to the center of the species range that has higher densities of trees and extensive surrounding woodlands. Our direct approach involved using paternity assignment at a spatial scale of at least 200 m. Our indirect approach involved an assessment of correlated paternity among progeny arrays of different trees at a larger spatial scale.

Overall, there were three objectives of our study: (1) to compare the rate of hybrids in adult trees and acorns, which can provide information regarding the stage of the life cycle in which reproductive barriers occur; (2) to characterize pollination patterns within *Q. lobata* directly, using paternity assignment; and (3) to compare paternity assignment results with those obtained using correlated paternity, an indirect but more convenient and widely applicable approach. Taken together, the results of this study provide a detailed view of intra- and interspecific pollination dynamics of *Q. lobata* at our study site.

Material and Methods

Study Species

The primary study species, *Quercus lobata* (valley oak), is endemic to California, with a distribution from Shasta County south through the Central Valley and lower-elevation foothills and valleys of the Sierra Nevada. It also occurs on Santa Cruz and Santa Catalina Islands (Griffin and Critchfield 1972; Little 1979). Extensive savannas in this region were once covered with valley oaks, but much of its habitat has been cleared for farmland, vineyards, and other development. It is the largest North American oak, with trees standing 10–25 m tall and 0.5–0.7 m diameter at breast height (dbh; Munz 1973). Valley oaks have an average life span of 100–200 yr and may live up to 500 yr. *Quercus lobata* flowers from February through early April (W. D. Koenig, unpublished data). Female flowers contain six ovules but produce a single acorn. Seeds mature in late September to early November of the same year of pollination. Our other study species, *Quercus douglasii* (blue oak), is also endemic to California, circling the Central Valley and being broadly sympatric with *Q. lobata*. *Quercus douglasii* occurs in habitats ranging from open savanna to denser woodland and is generally smaller than *Q. lobata*, with trees standing 6–20 m tall and 36–60 cm dbh (Burns and Honkala 1990). The mean overlap of flowering time was 75% for these two species at Hastings, calculated using flowering data collected between 1991 and 1996 (Koenig et al. 2008) with the method of Egghe and Goovaerts (2007). At Hastings, the densities of *Q. lobata* and *Q. douglasii* are ~17.8 and ~96.9 trees/ha, respectively.

Study Site

Trees were sampled at Hastings Natural History Reservation, a 950-ha reserve located in the Sierra de Salinas, on the most northerly end of the Santa Lucia Ranges of central coastal California. Hastings is located ~40 km inland at an elevation of 460–950 m asl. The climate at Hastings is moderate, with occasional snowfall in winter, the late summer air temperature reaching more than 38°C, and an extended dry season from June to September. Three species of oak are common at lower elevations at Hastings, *Q. lobata*, *Q. douglasii*, and *Quercus agrifolia*. The latter belongs to the distantly related red oak section Lobatae (Manos and Fairbrothers 1987; Nixon 1993; Manos et al. 1999). At Hastings, *Q. lobata* dominates the riparian forests along with *Q. agrifolia*. All *Q. lobata* individuals used in our study were tagged and mapped by means of a handheld global positioning system unit.

Microsatellite Genotyping

We sampled leaves from 133 adult trees identified as *Q. lobata*, 57 adult trees identified as *Q. douglasii*, and five individuals identified as putative hybrids because of ambiguous morphological characteristics. DNA extraction was done using the DNeasy Plant Mini kit (Qiagen). All *Q. lobata* adults and acorns were genotyped at eight microsatellite loci: QpZAG9 and QpZAG1/5 were developed for *Q. petraea* (Steinkellner et al. 1997), MSQ4 and MSQ13 were developed

for *Q. macrocarpa* (Dow et al. 1995; Dow and Ashley 1996), QpZAG15 and QpZAG11 were developed for *Q. robur* (Kampfer et al. 1998), and QM69–2M1 and QM57–3M were developed for *Q. myrsinifolia* (Isagi and Suhandono 1997). A subset of six of these loci (QpZAG9, QpZAG1/5, QpZAG11, QpZAG15, MSQ4, and MSQ13) was used to genotype *Q. douglasii* adults, and these six were used for identification of hybrid adults and acorns. PCR was conducted using 50–100 ng of genomic DNA in 10 μL of PCR mix with the following reagents: 0.5 mM of 10 mM dNTP mix (Denville Scientific), 0.04 μM of the forward primer with the fluorescently labeled M13 (−21) universal primer, 0.6–0.8 μM of reverse primer, 1.0 μg/μL bovine serum albumin, and 0.25 U of Taq polymerase (Biotherm Taq; eEnzyme) with Biotherm buffer (5×). PCR conditions were as described in Craft and Ashley (2007). PCR products (1.5 μL) were analyzed in a capillary DNA sequencing machine (Applied Biosystem 3730), using a LIZ500 ladder (Applied Biosystems). All microsatellite genotypes were scored by analyzing the raw data using Applied Biosystem GeneMapper software, version 3.7.

Adult Hybrid Identification

Descriptive statistics, such as allele frequencies, observed heterozygosities, expected heterozygosities, and genetic distances, were calculated using GenAlex, version 6.3 (Peakall and Smouse 2006). Species assignments and hybridization were evaluated using the programs STRUCTURE, version 2.2 (Pritchard et al. 2000; Falush et al. 2007), and NewHybrids, version 1.1 beta (Anderson and Thompson 2002). Information on species identity was not included in the initial analysis to determine which individuals best represented "pure" species and could be used to assign acorns in later analyses. Both programs implement a Bayesian clustering approach and Markov chain Monte Carlo simulations to provide estimates of an individual's identity using posterior probabilities. The assumptions for each program and the interpretation of probabilities are somewhat different. NewHybrids gives the probability that each individual belongs to one of the two parental species or to the hybrid category or categories (several hybrid categories can be defined). The posterior probabilities of STRUCTURE give the estimated proportion of an individual's genome that originated from each cluster (the user defines the number of clusters, K). The two models are similar when specifying two clusters for the STRUCTURE model.

For STRUCTURE analysis, we used the admixture model assuming correlated allele frequencies and set $K = 2$, corresponding to the two species. We collected data from 250,000 iterations after an initial burn-in of 50,000 iterations. We used a threshold of $q \leq 0.85$, the posterior probability of an individual genotype belonging to a single genetic cluster, to categorize individuals as hybrids.

For NewHybrids, data were collected from 50,000 iterations after an initial burn-in of 10,000 iterations. The probability of each individual being pure *Q. lobata*, pure *Q. douglasii*, or an F_1 hybrid was calculated. All four combinations of priors (Jeffreys' vs. uniform) were tested for the allele frequency (θ) and mixing proportion (π) parameters; choice of priors only significantly affected the q and assignment for one individual (details are presented in "Results"). Because

we were using only six microsatellite loci, we had relatively low power to identify backcrossed individuals; thus, we did not attempt hybrid categorization beyond the F_1 generation. An individual was classified as a hybrid if the probability that it belonged to the F_1 category was greater than the probabilities for membership in either Q. lobata or Q. douglasii.

Acorn Hybridization

In 2007, 424 acorns were collected from 21 trees, 20 identified as Q. lobata (394 acorns) and one putative hybrid, Tree QL167 (30 acorns). Acorns were germinated, and 0.1 g of acorn-sprouted root tissues was ground to a powder and used for DNA extraction. DNA extraction and microsatellite PCR procedures for genotyping acorns were as described above for leaves. NewHybrids was used to test the species identity of acorns, using the 184 adults identified as pure Q. lobata and Q. douglasii as standards incorporated into the analysis using the z option. A burn-in period of 30,000 iterations preceded data collection from 100,000 iterations of the model. Unlike the earlier analysis, the model was run to identify both first- and second-generation hybrids because we included acorns from a putative F_1 hybrid (QL167). We did not use STRUCTURE for the acorn analysis because the large number of half-sib acorns might have artificially skewed the posterior probabilities, as described in Pritchard et al. (2007).

Pollen Pool Analysis

The maternal trees from which acorns had been collected were between 10 and 1855 m apart (mean = 725 m, median = 718 m) and thus were sufficiently spaced across the landscape that a correlated-paternity approach could be used (Smouse et al. 2001). Using the full set of eight microsatellite loci and 424 acorns, we calculated the correlated paternity among maternal sibships using the software package POLDISP, version 1.0c (Robledo-Arnuncio et al. 2007). We used the KINDIST procedure to estimate the relationship between correlated paternity among sibships and pairwise distance. We then used KINDIST to estimate the dispersal function parameters, using the two one-parameter and three two-parameter dispersal distributions available in KINDIST.

We also calculated the probability of paternal identity (PPI) and the effective number of pollen donors, N_{ep}, using the indirect approach of within-sibship correlated paternity to compare the same estimates derived from a direct approach based on parentage analysis (parentage analysis is described below). We calculated an estimate of PPI directly from the paternity assignments using the method described by Smouse and Robledo-Arnuncio (2005). The PPI can be estimated for each maternal plant by calculating r_{gg}, using Smouse and Robledo-Arnuncio's equation (10a) (or eq. [2b]). The method estimates PPI by taking the sum of the rates of paternal matching within each sibship, based on sampling without replacement. The authors recommend using r_{gg} instead of q_{gg} (the sum of the squared proportions of acorns sired by a particular father) because the former is unbiased when PPI is low, as is often the case with wind-pollinated trees. Here we report the weighted average of r_{gg} and estimate of PPI for the "average female," using Smouse and Robledo-

Arnuncio's equation (6b). We calculated the estimated "average" PPI in two ways, one using only the assigned pollen donors and another using both the assigned and unassigned (outside the study plot) pollen donors. In the latter case, we assumed that each acorn pollinated by a tree outside the plot had a different father, as recommended by Smouse and Robledo-Arnuncio (2005). We estimated N_{ep} by taking the inverse of the PPI for the "average mother." However, Smouse and Robledo-Arnuncio (2005) note that this estimate of N_{ep} is known to be biased, especially when the number of assigned offspring is low.

Acorn Parentage Assignment

For paternity assignment of acorns, we selected five maternal trees for which all adult Q. lobata trees (112) that occurred within a 200-m radius of each maternal tree could be sampled (fig. 1). These candidate fathers were also part of the hybridization study described above. In the paternity assignment, the five maternal trees were also included as candidate fathers. A total of 115 acorns were included in the paternity analysis, including five acorns that had been classified as hybrids. We determined parentage using the maximum likelihood approach implemented by the program CERVUS (Marshall et al. 1998; Kalinowski et al. 2007). CERVUS calculates a likelihood ratio for each candidate parent-offspring pair over all loci given the genotypic data of the population and calculates δ, the difference in the likelihood ratio (LOD) scores between the most likely parent (i.e., the candidate with the highest LOD score) and the second most likely parent. Confidence levels of δ are estimated through simulations of parental and offspring genotypes, using allele frequencies observed in the population. When calculating confidence for parental assignments, the user has the option of selecting LOD scores or δ as the criterion for successful assignment. The results for both methods were similar (data not shown). We present the results for the more commonly used δ. We used the 80% confidence level, although values for the 95% confidence level are also given in "Results." A limitation of the CERVUS method is that paternity assignments can be very sensitive to simulation input values, including error rates and the proportion of candidate fathers sampled, but these values are generally unknown (Ashley 2010; Jones et al. 2010). We ran CERVUS at three error rates (0%, 1%, and 5%) and with three different proportions of candidate fathers sampled (25%, 50%, and 75%). The latter is equivalent to the proportion of seeds with an assigned pollen donor (within-stand pollinations). We simulated 10,000 offspring.

Results

All eight loci were highly variable in *Quercus lobata*, with 11–20 alleles per locus and with observed and expected heterozygosities ranging from 0.606 to 0.902 and from 0.595 to 0.870, respectively (table 1). The cumulative paternal exclusion probability given one known parent was 0.998. The six loci used to genotype *Quercus douglasii* were also highly variable (table 1). The two species were well differentiated, with Nei's genetic distance being 0.80. For the six loci scored in both species, 33 of the 88 alleles observed in Q. lobata were

Fig. 1 Map of the *Quercus lobata* and hybrid adult trees at Hastings Natural History Reservation that were used in this study. All marked trees were used in the adult hybridization study. Large squares are seed trees used for the acorn hybridization study and pollen pool analysis; open squares are *Q. lobata* individuals, and closed squares are hybrids. Diamonds are the five seed trees used for the acorn hybridization study, pollen pool analysis, and acorn paternity assignment; open diamonds are *Q. lobata* individuals, and the closed diamond is a hybrid. A circular area with radius of 200 m is shown for each of the five trees. Three of the latter trees are in close proximity and cannot be distinguished on the map. Black squares are adult *Q. lobata* within 200 m of the seed trees used for paternity assignment. White circles are hybrid individuals used in the adult hybridization study and included in the paternity assignments if they were within the 200-m radii.

not observed in *Q. douglasii*, and most of the shared alleles occurred at different frequencies between the two species. The estimated allele frequency differential between the two species, δ (Shriver et al. 1997), ranged from 0.390 for MSQ13 to 0.915 for MSQ4 (table 1).

For our analysis of hybridization among adults, the results from STRUCTURE and NewHybrids were similar but not identical. Both programs identified two trees—one from each species—that had been assigned to the wrong species on the basis of morphology; the labels for these individuals were switched for all subsequent analyses. Both programs determined that all five putative hybrid adults (including QL167) and a single individual identified in the field as *Q. douglasii*

were likely of mixed ancestry. NewHybrids found three *Q. lobata* adults that likely had mixed ancestry. STRUCTURE identified these same three individuals and two additional *Q. lobata* individuals as likely hybrids. One *Q. douglasii* individual was identified as a hybrid only when using Jeffreys' prior for allele frequencies (θ); it was classified as *Q. douglasii* when using STRUCTURE or NewHybrids with the uniform prior for θ. We therefore estimated that hybrids comprised 5%–6% of the adult population, similar to our previous estimate at this site using fewer individuals and fewer loci (Craft et al. 2002). The 12 trees identified by either program as being of mixed ancestry were excluded from future analyses characterizing the gene pools for acorn assignment. Thus, when testing the spe-

Table 1

Descriptive Statistics for Eight Microsatellite Loci for *Quercus lobata* and Six Loci for *Quercus douglasii*

Locus		Q. lobata					Q. douglasii			
	n	Alleles	Unique alleles	H_O	H_E	n	Alleles	H_O	H_E	δ
MSQ13	131	13	9	.881	.756	55	21	.927	.861	.390
MSQ4	130	15	12	.902	.870	52	19	.788	.912	.915
QpZAG9	130	20	11	.752	.801	57	22	.877	.928	.592
QpZAG1/5	130	12	9	.647	.702	55	15	.800	.884	.529
QpZAG11	124	12	7	.724	.717	52	9	.596	.667	.811
QpZAG15	129	16	7	.606	.797	56	14	.571	.570	.529
QpZAG57-3M	126	12	na	.814	.774	na	na	na	na	na
QpZAG69-2M1	122	11	na	.664	.595	na	na	na	na	na

Note. The parameter δ is a calculation of the diagnostic power for each locus, as described by Shriver et al. (1997). H_E = expected heterozygosity, H_O = observed heterozygosity, na = not applicable.

cies identities of acorns, 183 adults were used as genetic standards: 55 *Q. douglasii* and 128 *Q. lobata*.

Of the 394 acorns collected from 20 pure *Q. lobata* adults, NewHybrids identified seven as having mixed ancestry when Jeffreys' prior was used for θ (only five of these seven were identified as hybrids when the uniform prior was used). Thus, the hybridization rate among *Q. lobata* acorns (1.3%–1.8%) was even lower than that among adults. The hybrid acorns were from five different maternal plants. We also tested 30 acorns collected from the hybrid adult QL167. Of these, 19 (63%) were produced by pollinations from *Q. douglasii* trees.

Results from KINDISP (Robledo-Arnuncio et al. 2007) indicated that among-sibship correlated paternity decreased as distance ($R^2 = 0.155$) between maternal trees increased and stabilized at ~800 m. The two bivariate, one-parameter dispersal functions, normal and exponential, yielded average pollination distances of 82 and 108 m, respectively. We were unable to obtain useful results from the two-parameter dispersal distributions. The geometric and bivariate Student's t functions yielded infinite values for the central moments estimates, which the authors suggest is likely an artifact of extrapolating the observed data beyond a limited spatial scale of analysis. The exponential power function did not complete parameter estimation within a reasonable time period (2 d).

We calculated the PPI from the indirect method of correlated paternity within sibships, PPI(I). For individual mater-nal trees, within-sibship correlated paternities ranged from 0.096 to 0.336 (mean = 0.190). The effective number of pollen donors, N_{ep}, is calculated as the inverse of PPI, so the indirect approach yielded N_{ep} estimates ranging from 10.4 to 2.97 (mean = 5.2).

Paternity assignments were conducted for 115 acorns from five maternal trees using CERVUS (table 2). Varying the error rate (0%, 1%, and 5%) gave identical or nearly identical results; we report assignments using a 1% error rate. The pool of candidate fathers included all *Q. lobata* adults within 200 m of each of the maternal trees (fig. 1). Varying the proportion of candidate fathers sampled did affect the number of assignments. For example, at the 80% confidence level, 25, 33, and 64 fathers were assigned using 25%, 50%, and 75% of candidate fathers sampled, respectively. We focus on the results based on 50% of candidate fathers sampled; we chose this value because it is similar to pollen immigration rates (~50%) previously reported for the genus *Quercus* (Dow and Ashley 1996, 1998; Streiff et al. 1999; Nakanishi et al. 2009; Craft and Ashley 2010). Of the 33 acorns assigned to fathers at the 80% confidence level, 13 were also assigned at the 95% confidence level. Thus, 82 acorns were pollinated from trees beyond the 200-m sampling radius, corresponding to a pollen immigration rate of >70%. For assignments within the sampling area, pollination distances ranged from 12 to 336 m, with a mean pollination distance of 112 m.

Table 2

Results of Maximum Likelihood Paternity Analysis Based on Assignments at 80% Confidence Level in CERVUS

Maternal tree	Acorns	Acorns assigned to fathers (%)[a]	Fathers assigned	Average pollination distance (m)
QAS150	24	6 (25.0)	5	168.6
QAS160	19	5 (26.3)	5	129.5
QAS161	30	10 (33.3)	9	90.5
QAS163	30	11 (36.7)	7	101.1
QAS167	30	1 (9.1)	1	21.6
All	133	33 (28.7)	23[b]	112.0[c]

[a] Percentages are calculated on the basis of the number of acorns that resulted from pollination by *Quercus lobata* pollen.

[b] Represents the number of unique fathers; four fathers were shared among maternal trees.

[c] Average over all assigned acorns.

When using the parentage assignments resulting from assuming 25% or 75% of candidate fathers were sampled, the mean pollination distances were 101 and 117 m, respectively.

Direct estimates of PPI(D_{all}) and PPI(D_{ws}) were calculated from our measured rate of paternal sharing within sibships based on paternity assignments, using the method of Smouse and Robledo-Arnuncio (2005). PPI(D_{all}) includes both within-stand pollinations and outside pollinations, with the assumption that each acorn pollinated from outside the stand had a different father. PPI(D_{ws}) considers only the assigned within-stand pollen donors and can be considered a lower bound on the true PPI. The PPI(D_{all}) estimate (0.00456) was much lower than the PPI(I) estimates (mean = 0.190). Thus, the direct N_{ep} estimates from paternity assignments (219.5) were much higher than those calculated indirectly from correlated paternity. The PPI(D_{ws}) calculation (0.04) was also lower than the indirect estimates. When using the paternity assignments that resulted from entering the assumption sampling of 25% or 75% of possible fathers in CERVUS, PPI(I) was still at least 9.6 times greater than PPI(D_{all}) and was 2.6 times greater than PPI(D_{ws}).

Discussion

Quercus species often hybridize, yet species remain clearly distinguishable in morphology and ecophysiology. This phenomenon has interested evolutionary biologists since Darwin (1859), and it challenges the biological species concept that emphasizes the role of reproductive isolation in maintaining species boundaries (Burger 1975). Indeed, oaks have served as a primary example of species concepts that rely on ecological criteria (Van Valen 1976). This view suggests that environmental selection maintains species identities because hybrid individuals with intermediate characteristics will not be as well adapted as "pure" individuals. Thus, hybrids will be selected against at one or more life history stages. While there have been numerous studies of hybridization in oaks, few have compared rates of hybridization across different life history stages and thus were not able to evaluate the role played by fertility barriers versus differential survivorship as the primary mechanism maintaining oak species. In a study of a mixed stand of *Quercus robur* and *Quercus petraea*, Streiff et al. (1999) found 23 hybrid seeds among 310 sampled (7.4%), whereas 5 of 296 adults (1.7%) showed intermediate phenotypes and thus were presumed hybrids. While this reduction in hybrids from seeds to adults is consistent with selection against hybrids, the conclusion is weakened because hybrids were identified using different criteria in seeds (paternity assignment) and adults (morphology). A study of hybridization between *Q. petraea* and *Quercus pubescens* (Salvini et al. 2008) reported asymmetric pollination, with pollinations from *Q. petraea* to *Q. pubescens* occurring at a much greater rate (25.8%) than in the other direction (less than 5%). High levels of admixture, however, were also found among the adults in the stand; thus, the study did not provide evidence for reduced survivorship of hybrids in later life history stages. In our study, *Quercus lobata* and *Quercus douglasii* are genetically well differentiated at microsatellite loci, allowing good resolution for distinguishing hybrids among both seeds and adults. As previously reported (Craft

et al. 2002), putative hybrids comprised only 5%–6% of adults. More surprising from our study, however, was the extremely low number of hybrid acorns. Only 7 of 394 acorns (1.8%) involved fertilization of a *Q. lobata* ovule by *Q. douglasii* pollen. There was no evidence that hybrids were selected against between acorn and adult stages; rather, they were rarely produced. Thus, our results suggest that prezygotic isolating barriers between these two species, rather than postzygotic barriers such as environmental selection, limit gene flow between the species. The higher frequency of hybrid adults than hybrid acorns raises the possibility that hybrids may have a selective advantage through hybrid vigor or a mixture of ecological adaptations.

Interestingly, the acorn crop from the one hybrid maternal tree showed substantial frequencies of pollination by *Q. lobata* and *Q. douglasii* (37% and 63%, respectively). Rare hybrid adults in the stand may thus be conduits for gene flow between species. Our study characterized pollination events from *Q. douglasii* to *Q. lobata* only. If hybrid pollination is asymmetrical, as between *Q. petraea* and *Q. pubescens* (Salvini et al. 2008), hybrid acorns may be more common than we observed.

Conspecific pollinations patterns within *Q. lobata* showed that most pollinations (~70%) occurred over distances greater than 200 m. Such high levels of pollen immigration and long-distance pollination appear to be typical for oaks, with most studies showing greater than 50% pollen immigration (Dow and Ashley 1996, 1998; Streiff et al. 1999; Nakanishi et al. 2004; Chybicki and Burczyk 2010). Interestingly, pollen immigration for *Q. lobata* at another site, Sedgwick Ranch, was reported to be only 18.5%, and average pollination distances were somewhat lower (114 m; Pluess et al. 2009). The two sites are quite different, with the Sedgwick Ranch having a low density of *Q. lobata* and higher nearest-neighbor distances. Thus, differences in the degree of isolation of the sampled trees may be the primary cause of the differences observed in pollen flow between the sites. Alternatively, the apparent differences in pollination distances and pollen immigration may be an artifact of the paternity assignment protocols used. For the Sedgwick study, CERVUS analysis was run with the input parameter that 85% of the candidate paternal trees were sampled, and resulting assignments produced a concordant level of pollen immigration. On the basis of values reported in the literature, we concentrated on an input parameter of 50% of candidate fathers sampled for our analysis, but CERVUS analysis indicated that we had actually overestimated this parameter. Even our most liberal assumption for the proportion of fathers sampled (75%) suggested pollen immigration rates of 40%–45%.

By any measure, acorn crops of individual maternal trees were genetically diverse, and shared paternity of acorns (full sibs) was rare. These low rates of correlated paternity determined through paternity assignment resulted in very low estimates of PPI and high estimates of N_{ep} (mean = 219). This method for estimating N_{ep} is known to be biased, especially since the estimate is greater than the sample size of acorns (Smouse and Robledo-Arnuncio 2005); nonetheless, it is clear that the number of effective pollen donors is very large for this population. It appears that there is no shortage of *Q. lobata* fathers at Hastings Natural History Reservation, in apparent contrast to the situation at Sedgwick Ranch.

Our results using nonparentage approaches based on genetic differentiation between pollen pools (KINDIST; Robledo-Arnuncio et al. 2006) suggest that these approaches were not useful for our system. The normal and exponential dispersal distributions produced estimates of mean dispersal distances that were much lower than those shown by paternity analysis (82 and 108 m, respectively), while fitting bivariate distributions either could not be completed in a reasonable time (exponential power) or resulted in infinite values (geometric and bivariate Student's 2Dt). The authors indicate infinite values are likely an artifact of extrapolating the observed data beyond a limited spatial scale of analysis (Robledo-Arnuncio et al. 2007). The indirect pollen pool approach also produced estimates of PPI that were unrealistically high and thus estimates of N_{ep} that were unrealistically low (mean = 5.2). We speculate that the low within-sibship paternal sharing revealed by paternity assignment is the main reason that the discrepancy between direct and indirect PPI estimates is so large. The indirect methods appear to inflate estimates of paternal sharing when full sibgroups are rare. This is accentuated by the large pollen immigration rate, but the discrepancy is still large even when not considering pollination events from outside the study area, as $PPI(D_{ws})$ was one-fourth of $PPI(I)$. We urge caution when applying indirect approaches, especially when full sibs are infrequent or pollen immigration rates are high.

Acknowledgments

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