

Mating Patterns in a Savanna Population of Valley Oak (*Quercus lobata* Neé)¹

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Abstract

California valley oak is threatened by landscape alteration and failing recruitment in remnant stands. Its reproductive ecology is a key element of the seedling recruitment process. We first examine the mating system, to determine the extent of inbreeding in a population at Sedgwick Reserve, in Santa Barbara County. We then quantify variation in germination success and acorn size, evaluating their spatial patterns across the site. We collected acorns from 21 mapped focal trees in fall 1999, measured their average seed weight and germination success, and identified their multilocus genotypes. Using a mixed mating model, we observed significant, but modest selfing (outcrossing rate: $t_m = 0.96$) and no mating among relatives ($t_m - t_s = 0.0$). The effective pollen donor number was estimated to be between 5 and 7 individuals, depending on the inbreeding coefficient of the adults. These mating results indicate relatively little inbreeding but low numbers of pollen donors. Mothers differed significantly in seed weight (range: ~ 4 - 10 g) and germination percentage (range: 0 - 90 percent), and a bivariate analysis showed a gradient across the study site. Such a pattern suggests that environment conditions influence acorn size and germination success. Future work will address whether isolated individuals are at risk of selfing, for the expression of inbreeding depression on seed traits, or a reduction in the effective pollen donor number.

Introduction

Valley oak (*Quercus lobata* Neé) is one of California's most distinctive oak species. Its massive size and majestic canopy, combined with its longevity, make it a signature element of California's foothills, valleys and floodplains. Unfortunately, land use changes and restricted recruitment within remnant stands have caused its steady decline for 200 years (Adams and others 1992, Bolsinger 1988, Brown and Davis 1991, Griffin 1971). Because the species prefers level, fertile sites that are valuable for agricultural and development purposes, valley oak will continue to be vulnerable to the impact of land conversion. Numerous studies have estimated the losses thus far and predicted future declines (Bolsinger 1988, Brown and Davis 1991, Davis and others 1998, Greenwood and others 1993). Knapp and others (2001) recently reported reduced acorn production in blue oak (*Quercus douglasii*) trees

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with few local neighbors, and concluded that reduced pollen flow to isolated individuals might be the cause. If low adult density reduces acorn production in oaks, then progressive demographic attrition and stand thinning may jeopardize recruitment, through reduced acorn availability. However, before we can determine whether valley oak may be reproductively vulnerable, we need more information about the mating system of this species.

The mating system of a plant can be summarized with estimates of the outcrossing rate, mating with relatives, and probability of two progeny having the same mother and father (Ritland 1983, 1990; Ritland and Jain 1981). Such information is helpful, because it provides an indication of the extent of inbreeding allowed by the mating process. For species with a history of outcrossing, typical of most tree species, inbreeding can lead to inbreeding depression, expressed through reduced seed set or progeny fitness (Ellstrand and Elam 1993). In addition, information about the probability that two progeny share the same father can reveal the extent of diversity in pollen donor pool and can provide an indicator of the effective number of pollen donors for a given maternal plant (Ritland 1989). For outcrossing species, the effective number of donors may be a more sensitive indicator of mating than the outcrossing rate per se. It takes only one other tree to ensure outcrossing, but from an evolutionary perspective, it is critical to know whether that outcrossing represents few or many other individuals. Moreover, because mating system parameters are relatively easy to estimate, this information can be gathered for several sites to facilitate comparison among populations occupying different sorts of landscapes (Sork and others 1999).

The possibility that valley oak adults may be at risk for reproductive isolation has been addressed elsewhere through a study of pollen movement at Sedgwick Reserve, Santa Barbara Co. (Sork and others 2002). That study used a novel analytical method, dubbed TwoGener (Smouse 1998, Smouse and others 2001), which combines the two-generation gametic inference of parentage analysis (e.g., Chakraborty and others 1988, Devlin and others 1988) with the survey methods of population structure analysis. With TwoGener, we can estimate not only the effective number of pollen donors in a mating neighborhood (N_{ep}), but also the average distance of successful pollen movement (δ). With certain assumptions, we can estimate the decay parameter of the pollen dispersal curve and can then deploy that curve to describe landscape-scale patterns of pollen movement for geo-referenced individuals (Austerlitz and Smouse 2001). Our analysis of the data available to date has yielded a surprisingly small estimate of the effective number of pollinators, $N_{ep} = 3.68$ individuals, and an estimated average distance of successful pollination of $\delta = 64.8$ m (Sork and others 2002). The effective pollen donor values are much lower than those observed for wind-pollinated species in closed-forest settings in Missouri Ozark forests. For white oak (*Quercus alba*), we estimate that $N_{ep} \sim 8$ individuals and $\delta = 11$ m (Smouse and others 2001); for shortleaf pine (*Pinus echinata*), we estimate that $N_{ep} \sim 10$ individuals (extracted from Dyer and Sork 2001). Although the pollen dispersal estimate is higher for valley oak than for either of these closed-forest canopy species, we had anticipated an even longer average successful pollination distance, given the savanna setting of our population. Interestingly, the scale of pollination distance observed in valley oak is comparable to the 60-meter scale at which Knapp and others (2001) observed an association between reduced acorn crop size and small numbers of near neighbors in *Q. douglasii*. Both the Blue oak (Knapp and others 2001) and valley oak (Sork and others 2002) results point to the need for a better understanding of mating patterns in California savanna-oak settings.

The first objective of this paper is to evaluate the mating system of the Sedgwick Reserve population of valley oak, through Ritland's MLTR program (1990), using those same first year data from our pollen movement study (Sork and others 2002). Ritland's approach provides a population estimate of the extent of inbreeding (through selfing and mating between relatives) and it also provides a separate estimate of the effective number of pollen donors, which latter can be compared with a corresponding estimate, independently available from TwoGener. The data reported here are from one site and one year, but they represent an initial step toward a larger understanding of variation in mating patterns of valley oak populations across different landscapes, and the extent to which those processes are contributing to the ongoing recruitment challenge for threatened populations. Eventually, successful management of valley oak will require an approach that allows us to compare not only populations, but also individual trees, using specific information about the spatial locations of near neighbors and local tree density. We will use the results of this study to seed a discussion of future directions for our enquiries.

The second objective is to report findings on two fitness-related components of the reproductive biology of valley oak: seed size and germination success. Germination success is key to seedling recruitment, and seed size is often associated with early seedling survival and height growth. Inbreeding depression can have negative impact on either trait (Ellstrand and Elam 1993), even though maternal genotype and maternal environment can have a large influence on successful seedling establishment. As an initial inquiry into these two fitness-related components of valley oak recruitment, we quantify the extent of their variation among study trees, and we assess whether the variation is distributed randomly across the landscape. A nonrandom spatial distribution can indicate either the genetic consequences of restricted gene flow or the spatial pattern of environmental effects. For example, with restricted gene flow, adjacent (related) trees may have similar reproductive traits. Alternatively, spatial pattern in these traits may indicate the impact of environmental gradients. Our motivation for this analysis is to determine whether there are spatial patterns in the features determining recruitment success, which can then be pursued in future studies. Because density also varies within our site and density may affect inbreeding, we include this factor in our analysis of seed size and germination.

Materials and Methods

Study Site

We conducted our work at the Sedgwick Reserve, along the valley floor of Figueroa Canyon (N 34°42', W 120° 2'), 10 km northeast of Santa Ynez, California. Sedgwick Reserve is a 2,380 ha area, managed for research, education and conservation of native biodiversity by the University of California Natural Reserve System and UC Santa Barbara. The study trees in Figueroa Canyon are located on the valley floor and surrounding hill slopes in a broad, shallow basin, roughly 130 ha in extent and ranging in elevation from 360 m to 405 m above sea level. In fall 1999, we selected 21 reproductive valley oak adults at the study site, arrayed in an approximate hexagon-shaped grid, with six triangular clusters (three trees each) at the six vertices of the hexagon, and one in the middle (*fig. 1*). The inter-female distances within each cluster ranged from about 50-150 m; the maximum distance across the hexagon was 1,040 m.

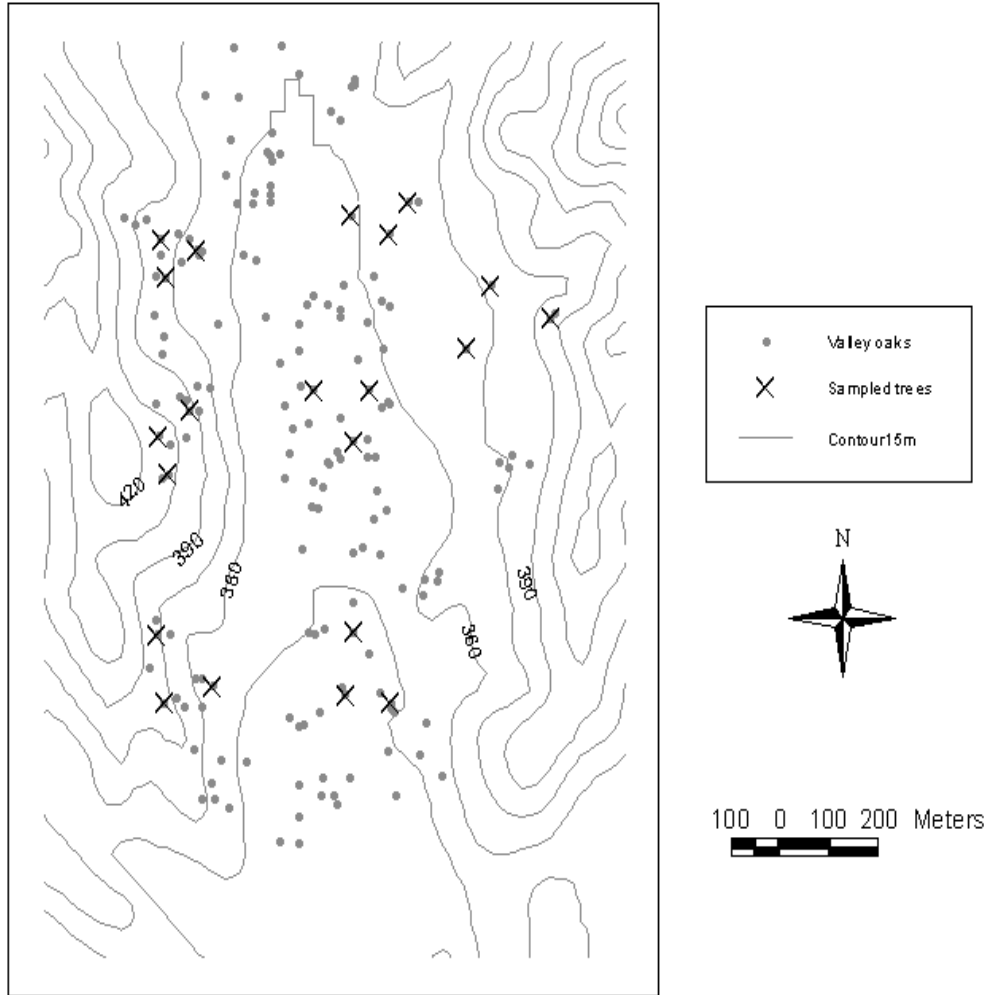


Figure 1—Map of all valley oak adults at study site in Sedgwick Reserve, Santa Barbara Co., California.

Valley oak density for the study site and for neighborhoods surrounding individual trees were estimated using mapped tree locations. ArcInfo™ (ESRI, Inc). A loose minimum bounding rectangle around 153 mapped valley oaks encompassed roughly 128 hectares, yielding a stand density of 1.19 trees/ha. Second order analysis of K-function statistics (Getis and Franklin 1987) indicates that trees are significantly clustered in the study area at all spatial scales. Thus, if a tree’s neighborhood is defined as a circle of radius 150 m, roughly 3 standard deviations in our Gaussian pollen dispersal model (Sork and others 2002), centered on the tree canopy, the average neighborhood density for the 21 study trees is $\bar{D} = 1.62$ trees/ha. The local neighborhood density, however, varies considerably for the 21 focal trees, ranging from $D = 0.14 - 3.54$ trees/ha (*table 1*). If we view local density as a signal of environmental effects that might affect seed traits, these raw stem counts are appropriate measures.

Table 1—Local and weighted densities of conspecific adults surrounding each study tree, number of acorns sampled, mean proportion germinated, and mean acorn weight of 21 valley oak study trees collected in fall 1999 at Figueroa Creek, Sedgwick Reserve, Santa Barbara County, California.

Study tree	Local density (<i>D</i>)	Weighted density (Δ)	Number sampled	Proportion germinated	Acorn weight (g)
1	0.57	1.39	70	0.600	8.66
2	0.99	1.26	83	0.518	5.24
3	0.71	0.41	6	0.674	8.50
4	0.42	0.10	51	0.118	8.29
5	0.28	1.00	7	0.572	5.93
6	0.14	0.05	108	0.334	8.57
7	1.84	2.59	59	0.187	6.98
8	2.12	6.12	21	0.248	7.47
9	2.55	3.21	12	0.083	5.76
10	1.27	2.50	19	0.421	4.97
11	1.56	3.24	75	0.147	6.73
12	1.27	3.10	24	0.125	4.06
13	1.84	5.52	14	0.357	8.23
14	1.27	2.47	76	0.132	5.99
15	1.56	3.59	27	0.185	9.97
16	1.84	5.66	38	0.316	7.22
17	2.55	5.73	12	0.000	9.09
18	2.26	4.68	63	0.571	5.96
19	2.83	3.31	27	0.556	4.88
20	2.41	3.88	142	0.908	6.94
21	3.54	6.50	61	0.082	6.74

The blue oak findings (Knapp and others 2001) imply that density is more appropriately viewed as a statement about pollen availability. To account for the steep exponential drop in probability of pollination with tree separation distance (Sork and others 2002), we also calculated a local neighborhood density, in which a neighboring tree’s contribution to local density (Δ) was scaled by its distance (δ_i) to the focal tree, using the formula:

$$(\delta_i) = \exp\{-0.0002*d_i^2\},$$

and

$$\Delta = \sum_i \delta_i \text{ for all trees within 150 m of the focal mother,}$$

where the exponential coefficient (0.0002) is $(2\sigma^2)^{-1}$ (see Austerlitz and Smouse 2001, 2002), with σ^2 estimated as 2,500 m² (Sork and others 2002). These weighted local densities also vary among the 21 focal mother trees (*table 1*). The average value is 3.15, but the values ranged over two orders of magnitude, from 0.05 - 6.50.

Collection and Greenhouse Work

We visited the site repeatedly, from October through mid-November 1999, to collect mature acorns, up to 100 acorns for some trees. Individual trees in the study area were mapped using a 1993 digital panchromatic orthophoto with 1 m² resolution, produced by the U.S. Geological Survey (U.S. Department of the Interior 1992). Acorns were shipped to the Sork lab in St. Louis, where they were weighed individually and then planted into 12 in. deep pots. We planted acorns 1 cm below

the soil (Pro-Mix BX), with six to nine acorns from the same family together in one or more pots, depending on family size. We watered acorns as needed and kept them under a 12 hr light regime. We monitored the pots weekly until radicals began to emerge from the soil, and then we monitored every 2-4 days. We marked newly emerging seedlings individually and recorded their dates of emergence. We defined successful germination as emergence of an above ground radical within 90 days of planting. For each maternal plant, we calculated the percentage of germination as the number of acorns germinated, out of the total number of seed planted for that tree.

Laboratory Analysis

For the mating system study, our goal was to assay 20 seedling genotypes for each of the 21 focal trees, but some trees yielded low germination rates. Realized sample sizes for the mating system study ranged from 4-20 progeny per focal tree, with only 19 of the 21 focal trees represented. We used a combination of ten allozyme and one microsatellite loci as the genetic markers for this study, a strategy deployed because the allozyme loci provided expeditious and inexpensive genetic screening, and the microsatellite locus provided valuable genetic resolution (Smouse and others 2001). Details of our methods of identifying the allozyme and microsatellite loci are given elsewhere (for enzymes also see Gram and Sork 2001, Sork and others 1997, Sork and others 2002). Allozyme loci are: *fluorescent esterase* (*Fe*, EC 3.1.1.1.), *leucine aminopeptidase* (*Lap*, EC 3.4.11.1), *phosphoglucose isomerase* (*Pgi*, EC 5.3.1.9), *malate dehydrogenase* (*Mdh*, EC 1.1.1.40), *phosphoglucumutase* (*Pgm*, EC 2.7.5.1), *menadione reductase* (*Mnr*, EC 1.6.99.-), and *triosephosphate isomerase* (*Tpi*, EC 5.3.1.1). The microsatellite primer was QpZAG110, originally developed for *Quercus petrea* (Steinkellner and others 1997).

Data Analysis

Ritland's MLTR program (Ritland 1990) generates three mating system parameter estimates that we focus on in this paper: (a) the outcrossing rate (estimated from multilocus genotypes, t_m , and from the average single-locus estimate, t_m); (b) the probability of mating among relatives ($t_m - t_s$); and (c) the probability of mating among full-sib relatives (the likelihood that two randomly selected offspring from a single mother share the same father, r_p), which can be translated into an estimate of effective number of pollen donors ($N_{ep} = 1/r_p$). MLTR uses maximum likelihood analysis to provide minimal variance estimates of these parameters under a mixed mating model that assumes that a portion of the matings represent selfing and that the remaining portion are due to random non-self mating. We conducted MLTR with an EM algorithm, using 1,000 bootstrap replicates for the estimation of confidence intervals.

We were forced to pool microsatellite alleles into eight categories, due to the limitations of MLTR. We pooled the rare alleles by grouping them into three 'allelic classes', taking care that the new 'allelic classes' did not alter the probability of a mother producing a selfed offspring (e.g., we ensured that rare alleles were not pooled into alleles that were present in the maternal genotype). MLTR was run with two different parameter specifications: (1) We estimated the adult inbreeding coefficient (F) simultaneously with the other mating system parameters. (2) We fixed the adult F -value at zero. We use both strategies for adult inbreeding (F), because we

do not know the inbreeding structure of the adults *a priori*, and these two estimates provide upper and lower bound estimates for the other mating system parameters.

To determine whether seed weight, transformed as $\log_e(\text{seed weight})$, differed between mothers, we conducted a one-way Kruskal-Wallis test (Proc NONPAR1WAY, SAS version 8). For the germination data, we analyzed the number of germinated and non-germinated seeds for each mother by way of a 21 x 2 contingency table (Conover 1999).

We examined the spatial patterns of average $\log_e(\text{seed weight})$ and percent germination (arc sine square root transformed) for the 21 maternal plants, using Canonical Correspondence analysis (Proc CANCELL, SAS version 8). The dependent (response) variables are mean seed weight and percent germination per maternal tree. The independent (predictor) variables were the X and Y spatial coordinates of each tree. Because the model includes precisely two response and two predictor variables, only two canonical axes result, only the first of which turns out to be useful. We also conducted the canonical correspondence analysis using local density and weighted local density values for each maternal tree to see whether these variables improved the model or were more important than the spatial variables.

Results

Mating System Analysis

The number of alleles per locus varied from 2-17. Recall that the 17 microsatellite allele frequencies were pooled into 8 allelic classes because the MLTR program can only handle 8 alleles (for raw allele frequencies at all 11 loci, see Sork and others 2002). The exclusion probability associated with this collection of loci is 92 percent, so genetic resolution of the assay battery is high (Sork and others 2002).

We estimate the multilocus outcrossing rate (t_m) for valley oak to be 96 percent, indicating some degree of selfing (table 2). Simultaneous estimation of t_m , t_s , and F in the mixed mating model yields a significant estimate for inbreeding in the adult ($F = 0.243$). The estimates of the single locus outcrossing rate (t_s), which include both self-mating and mating with relatives, were both ~ 0.96 , the same as the multilocus estimate (t_m), regardless of whether F for the adult population was set to $F = 0$ or was allowed to float ($F = 0.243$). Thus, the resulting estimate of the portion of consanguineous mating was 0.00. The range of estimates of effective number of pollen donors, $N_{ep} = 1/r_p$, was 4.7-7.1 individuals, depending on whether we use $F = 0$ or $F = 0.243$. Either way, N_{ep} is the same order of magnitude we had estimated earlier, using TwoGener methods (Sork and others 2002).

Table 2—Mating system parameters for valley oak population at Sedgwick Reserve, Santa Barbara Co. based on two versions of the MLTR model: F fixed at 0 and F was jointly estimated (Floating F) by seeding the program with $F = 0.10$.

Mating system parameters	Fixed F	Floating F
Parental F estimate (SD)	0.000 (set)	0.243 (0.081)
Multilocus t estimate (SD)	0.957 (0.020)	0.957 (0.020)
Single locus t estimate (SD)	0.956 (0.011)	0.957 (0.012)
Consanguineous matings, t_m-t_s (SD)	0.001 (0.012)	-0.001 (0.011)
Correlation of t estimate, r_s (SD)	0.048 (0.014)	0.046 (0.013)
Correlation of p estimate, r_p (SD)	0.214 (0.045)	0.141 (0.033)
Effective number of donors, N_{ep}	4.7	7.1

Germination and Seed Weight

Germination ranged from 0 – 91 percent (*table 1*) and was significantly different among mother trees (Contingency Table, $df = 20$, $\chi^2 = 334.9$, $P \ll 0.001$). Average acorn weight, varied from 4-10 grams (*table 1*) and differed significantly among mother trees (Kruskal-Wallis test, $\chi^2 = 365.77$, $df = 20$, $P = 0.0001$).

This variation in germination and acorn weight is not distributed randomly throughout the study area. The canonical correspondence analysis indicated a linear spatial trend in bivariate combination of mean percent germination and seed weight (*table 3*). The adjusted canonical correlation for the first canonical axis is 0.58, relatively high but not quite significant for the degrees of freedom in our model (*table 3*, $p = 0.066$, $df = 4, 34$). As summarized by the first canonical axis, the study site has an underlying spatial gradient with smaller acorns and lower germination success in the southwest corner and larger acorns and higher germination in the northeast corner (*fig. 2*). This trend does not correspond to elevation patterns shown in *figure 1*. Soils vary somewhat across the site, ranging from silty clay soils developed on Pleistocene colluvium to silty clay loams derived from recent alluvial deposits. However, this variation occurs along an east-west rather than north-south axis, and there is no apparent relationship between fruit characteristics and soil. With the exception of the trees at the south end of the plot, trees within clusters showed similar reproductive phenotypes (*fig. 2*). We also conducted a canonical correspondence model that included the density variables, but the results were not significant and are not presented. At this time, we have no additional genetic or environmental variables to associate with this nonrandom distribution of acorn traits.

Table 3—Summary of results of canonical analysis of germination percentage (arc sine square root transformed) and seed weight (\log_e transformed) versus the spatial X- and Y-coordinates of 21 study trees.

Parameter	Canonical axis 1	Canonical axis 2
Canonical correlation	0.626	0.078
Adjusted correlation	0.586	----
Eigenvalue	0.644	0.006
Likelihood ratio	0.605	0.994
F-test	2.430	0.110
Numerator df	4	1
Denominator df	34	18
Probability > F	0.066	0.745

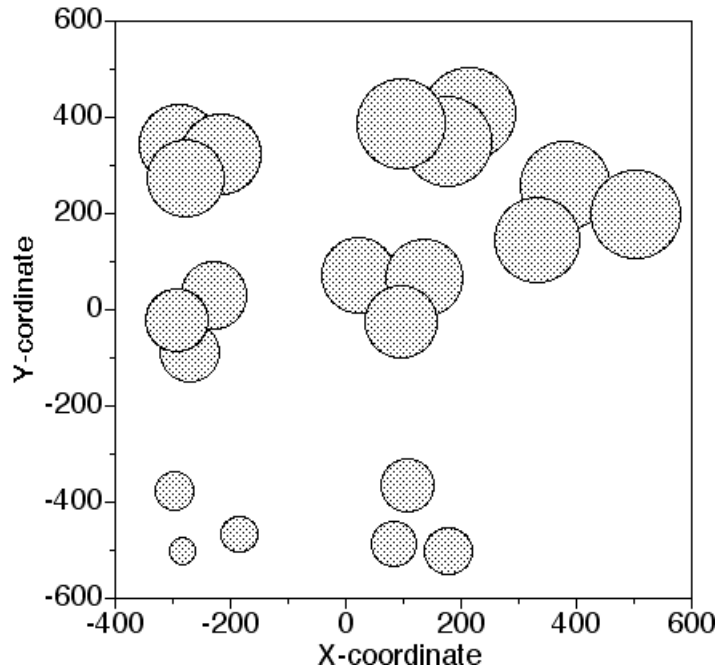


Figure 2—Map of the spatial pattern of the first canonical axis (CA 1) of multivariate combination of germination and mean acorn weight per study tree. Bubble size is positively associated with percent germination and acorn size. (See *table 4* for correlation of reproductive variables with CA 1.)

Our 1999 data do not reveal a strong correlation between germination percentage and mean seed weight ($r = -0.09$; *table 4*), but we do find that both germination and seed weight are positively associated with the North/South axis (Y) and the bivariate W1 axis, which is correlated more strongly with Y-axis ($r = 0.96$) than the X-axis ($r = 0.51$). The first canonical axis, W1, is loaded almost evenly by mean seed weight and germination percentage ($r = 0.44, 0.41$, respectively, *table 4*). The correlations of local and weighted local density with germination were negative but not significant ($r = -0.25, -0.29, p = 0.28, 0.20$, respectively). The correlations with seed weight were also not significant ($r = -0.17, 0.02, p = 0.46, 0.92$, respectively).

Table 4—Correlations among reproductive and spatial variables associated with 21 study trees. Germination percent and acorn weight were transformed as described in text. W1 and W2 are the canonical axes for the reproductive variables. V1 and V2 are the first two canonical variables associated with the X- and Y-coordinate. Correlations are above the diagonal and significance values are below.

	Germ	Weight	X	Y	W₁	W₂	V₁	V₂
Germ	1.000	-0.086	0.271	0.402	0.438	0.055	0.700	0.714
Weight	0.711	1.000	0.157	0.407	0.408	-0.059	0.651	-0.759
X	0.234	0.496	1.000	0.236	0.511	0.860	0.320	0.067
Y	0.070	0.067	0.304	1.000	0.956	-0.294	0.598	-0.023
W ₁	0.047	0.067	0.018	0.000	1.000	0.000	0.626	0.000
W ₂	0.811	0.800	0.000	0.196	1.000	1.000	0.000	0.078
V ₁	0.000	0.001	0.158	0.004	0.002	1.000	1.000	0.000
V ₂	0.000	0.000	0.774	0.922	1.000	0.738	1.000	1.000

Discussion

Mating System

The population of valley oak at Sedgwick Reserve has a very high outcrossing rate (96 percent) but no apparent mating among relatives ($t_m - t_s = 0$). The high outcrossing rate is expected for wind-pollinated trees species and has been observed for other oak species (e.g., $t_m = 100$ percent for *Quercus rubra* (Schwarzmann and Gerhold 1991) and *Q. alba*⁶ and *Q. velutina* (Fernandez and others 2002). Given our previous finding that average pollen movement is ~ 65 m (Smouse and others 2001), the lack of mating among relatives is a bit unexpected, but such a result may indicate that neighbors at our study site are not closely related. We have preliminary evidence showing a lack of significant autocorrelation among adults,⁶ which is consistent with that interpretation, but it is based on the 21 study trees only. In sum, the mating system we examined during the 1999 field season indicates a population with no mating among relatives but some degree of selfing.

This study supports our earlier finding that valley oak has relatively few effective pollen donors (Sork and others 2002); we estimated $N_{ep} = 4.7-7.1$ ‘effective fathers’ for $F = 0$ and $F = 0.243$, respectively. We have far too few adults to estimate the parental inbreeding coefficient with any precision (the confidence interval extends from $0.0 \leq F \leq 0.60$), and it is difficult to say anything definitive about F under those conditions. The lower value here is compatible with the range of N_{ep} estimates (3 - 5) obtained from our TwoGener analysis, but the larger point is that all of these estimates indicate very few effective pollen donors. Pollen can move long distances in this savanna setting, and there are probably long distance pollination events contributing to the genetic variation we encounter in the seedlings of these focal mothers, but they represent minor contributors to the total seedling pool; most pollination is exceedingly local. Our *Quercus lobata* values are at the lower end of the range of values reported for other studies that use MLTR to estimate N_{ep} . For example, Western larch (*Larix laricina*) has ~ 35 effective pollen donors (El-Kassaby and Jaquish 1996), *Pinus washoensis* has ~ 14 effective donors (Mitton and others 1997) and *Grevillea iaspicula*, an endangered shrub in the Proteaceae, has 2 - 3 effective pollen donors (Hoebe and Young 2001). In Missouri Ozark forest with high tree density, our TwoGener estimate of N_{ep} indicated that *Quercus alba* had ~ 8 effective pollen donors (Smouse and others 2001). Based on an MLTR analysis, *Quercus velutina* in a closed Ozark forest had ~ 6 effective donors. Thus, the low number of effective donors that we have observed in valley oak indicates that, in spite of the open landscape conditions that should permit pollen to move more freely across the landscape (Okuba and Levin 1989), most pollination is very localized.

We will comment briefly on the differences in estimates generated by MLTR and TwoGener. The mixed mating model uses a maximum likelihood approach to jointly estimate a selfing rate and the effective outcrossing rate assuming random mating throughout the population. If the scale of population exceeds the scale of pollen movement, the assumption of random mating throughout the area is violated. On the other hand, TwoGener makes no assumption about random mating but it does assume no inbreeding in the parental population. If F is large, then TwoGener underestimates the effective number of pollen donors, but we can adjust that estimate for $F > 0$ (Austerlitz and Smouse 2002). If $F = 0.243$, the adjusted TwoGener

⁶ Unpublished data on file at the web address: <http://mofep.conservation.state.mo.us> (this information is only available with permission from VLS).

estimate of $N_{ep} = 4.57$, still somewhat smaller than the corresponding estimate from MLTR, which is $N_{ep} = 7.1$. For conservation, restoration and management purposes, both estimates are telling us that contemporary pollen flow is spatially restricted, severely so.

Variation in Recruitment-relevant Seed Traits

We found a great deal of heterogeneity across study trees in germination and seed weight. As poorly estimated as F is, from this limited sample size, we are hesitant to suggest that inbreeding depression is contributing to that heterogeneity. With greater sample size per tree in future studies, we will be able to test the association between the selfing and biparental inbreeding rates with seed traits. We will also test whether localized density affects those traits once we have a larger sample size of trees and a greater variance in density. At this point, we cannot conclude whether the trend is confined to this set of trees or is a reflection of some larger gradient within Sedgwick Reserve. To determine whether the gradient in seed traits reflect environmental effects alone or whether the adult population has an underlying genetic structure, we are expanding the study area and increasing the sample size of focal mother trees, which should permit a more definitive analysis of the genetic structure of the adult population.

Implications for Management of Valley Oak

Our results indicate that this population of valley oak is highly outcrossing but may have limited pollen movement. Given the ongoing loss of valley oak stands in the region, additional research is needed to assess the relationship between local tree distribution pattern and reproductive isolation (and associated reduction in acorn crop size). Such information could help ongoing oak conservation and restoration efforts in the region to evaluate impacts of additional stand fragmentation and could also guide patterns of restoration plantings. Our ongoing research at Sedgwick Reserve, expanded to study pollen flow and fruit production for a much larger sample of trees over the next several years, is designed to contribute such information.

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References

- Adams, T. E.; Sands, P. B.; Weitkamp, W. H.; McDougald, N. K. 1992. **Oak seedling establishment on California rangelands.** *Journal of Range Management* 45(1): 93-98.
- Austerlitz, F.; Smouse, P. E. 2001. **Two-generation analysis of pollen flow across a landscape. II. Relation between ϕ_{it} , pollen dispersal, and inter-female distance.** *Genetics* 157(2): 851-857.
- Austerlitz, F.; Smouse, P. E. 2002. **Two-generation analysis of pollen flow across a landscape. III. Impact of within-population structure.** *Genetical Research* 78(1): 271-278.
- Bolsinger, C. L. 1988. **The hardwoods of California timberlands, woodlands, and savannas.** Portland, OR: U.S. Department of Agriculture Resource Bulletin.
- Brown, R. W.; Davis, F. W. 1991. **Historic mortality of valley oak in the Santa Ynez Valley, Santa Barbara County, CA.** In: Standiford, R., technical coordinator. Proceedings of the symposium on oak woodlands and hardwood rangeland management. October 31-November 2, 1990. Forest Service, U.S. Department of Agriculture; 202-207.
- Chakraborty, P.; Meagher, T.; Smouse, P. E. 1988. **Parentage analysis with genetic markers in natural populations. I. Paternity exclusion and expected proportions of offspring with unambiguous paternity.** *Genetics* 118(3): 527-536.
- Conover, W. J. 1999. **Practical nonparametric statistics.** 3rd Edition. New York: John Wiley & Sons;
- Davis, F. W.; Stoms, D. M.; Hollander, A. D.; Thomas, K. A.; Stine, P. A.; Odion, D.; Borchert, M. I.; Thorne, J. H.; Gray, M. V.; Walker, R. E.; Warner, K.; Graae, J. 1998. **The California Gap Analysis Project—Final Report.** University of California, Santa Barbara. Web address: http://www.biogeog.ucsb.edu/projects/gap/gap_rep.html
- Devlin, B.; Roeder, K.; Ellstrand, N. C. 1988. **Fractional paternity assignment: theoretical development and comparison to other methods.** *Theoretical and Applied Genetics* 76(3): 369-380.
- Dyer, R. J.; Sork, V. L. 2001. **Pollen pool heterogeneity in shortleaf pine, *Pinus echinata* Mill.** *Molecular Ecology* 10(4): 859-866.
- El-Kassaby, Y. A.; Jaquish, B. 1996. **Population density and mating pattern in western larch.** *Journal of Heredity* 87(6): 438-443.
- Ellstrand, N. C.; Elam, D. R. 1993. **Population genetics of small population size: Implications for plant conservation.** *Annual Review of Ecology and Systematics* 23:217-242.
- Fernández-M., J. F.; Idol, J.; Sork, V. L. 2002. **High outcrossing rates but small pollination neighborhood in a forest population of *Quercus velutina* (Fagaceae) in a Missouri oak-hickory forest.** Unpublished report supplied by author
- Getis, A.; Franklin, J. 1987. **Second-order neighborhood analysis of mapped point patterns.** *Ecology* 68(3): 473-477.
- Gram, W. K.; Sork, V. L. 2001. **Association between environmental and genetic heterogeneity in forest tree populations.** *Ecology* 82(7):2012-2021
- Greenwood, G. B.; Marose, R. K.; Stenback, J. M. 1993. **Extent and ownership of California's hardwood rangelands.** Sacramento: California Department of Forestry and Fire Protection Strategic and Resources Planning Program.
- Griffin, J. R. 1971. **Oak regeneration in the upper Carmel Valley, California.** *Ecology* 52(5): 862-868.

- Hoebe, S. E.; Young, A. G. 2001. **Low neighborhood size and high interpopulation differentiation in the endangered shrub *Grevillea iaspicula* McGill (Proteaceae).** *Heredity* 86(4): 489-496.
- Knapp, E. E.; Goedde, M. A.; Rice, K. J. 2001. **Pollen-limited reproduction in blue oak: implications for wind pollination in fragmented populations.** *Oecologia* 128(1): 48-55.
- Mitton, J. B.; Latta, R. G.; Rehfeldt, G. E. 1997. **The pattern of inbreeding in Washoe pine and survival of inbred progeny under optimal environmental conditions.** *Silvae Genetica* 46(4): 215-219.
- Okuba, A.; Levin, S. A. 1989. **A theoretical framework for data analysis of wind dispersal of seed and pollen.** *Ecology* 70(2): 329-338.
- Ritland, K. 1983. **Estimation of mating systems.** In: Tanksley, S. D; Orton, T. J., eds. *Isozymes in plant genetics and breeding, part A* Amsterdam: Elsevier Science Publishers B.V.; 289-302
- Ritland, K. 1989. **Correlated matings in the partial selfer, *Mimulus guttatus*.** *Evolution* 43(4): 848-859.
- Ritland, K. 1990. **A series of FORTRAN computer programs for estimating plant mating systems.** *Journal of Heredity* 81(3): 235-237.
- Ritland, K.; Jain, S. 1981. **A model for the estimation of outcrossing rate and gene frequencies using n independent loci.** *Heredity* 47(1): 35-52.
- Schwarzmann, J. F.; Gerhold, H. D. 1991. **Genetic structure and mating system of northern red oak (*Quercus rubra* L.) in Pennsylvania.** *Forest Science* 37(5): 1376-1389.
- Smouse, P. E. 1998. **Thoughts on a genetic structure-like approach to pollen flow.** In: Sork, V. L.; Campbell, D.; Dyer, R. J.; Fernandez, J.; Nason, J.; Petit, R.; Smouse, P. E.; Steinberg, E., eds. *Theoretical and empirical approaches to the study of gene flow in fragmented, managed, and continuous populations* Santa Barbara: National Center for Ecological Analysis and Synthesis Research Paper Number 3. Web address: [http://www.nceas.ucsb.edu/papers/geneflow/data/Gfpart2.html#Smouse percent20essay](http://www.nceas.ucsb.edu/papers/geneflow/data/Gfpart2.html#Smouse%20essay)
- Smouse, P. E.; Dyer, R. J.; Westfall, R. D.; Sork, V. L. 2001. **Two-generation analysis of pollen flow across a landscape. I. Male gamete heterogeneity among females.** *Evolution* 55(2): 260-271.
- Sork, V. L.; Nason, J.; Campbell, D. R.; Fernández, J. F. 1999. **Landscape approaches to the study of gene flow in plants.** *Trends in Ecology and Evolution* 14 (6): 219-224.
- Sork, V. L.; Davis, F.; Smouse, P. E.; Apsit, V. J.; Dyer, R. J.; Fernández, J.; Kuhn, B. 2002. **Pollen movement in declining populations of California valley oak, *Quercus lobata*: Where have all the fathers gone?** *Molecular Ecology* In press.
- Sork, V. L.; Koop, A. L.; de la Fuente, M. A.; Foster, P. A.; Raveill, J. 1997. **Patterns of genetic variation in woody plant species in the Missouri Ozark Forest Ecosystem Project (MOFEP).** In: Brookshire, B. L.; Shifley, S. R., eds. *Proceedings of the Missouri Ozark Forest Ecosystem Project symposium: an experimental approach to landscape research, 1997 June 3-5, St. Louis, MO.* St. Paul, MN: U.S. Department of Agriculture Forest Service North Central Forest Experiment Station; 233-249
- Steinkellner, H.; Fluch, S.; Turetschek, E.; Lexer, C.; Streiff, R.; Kremer, A.; Burg, K.; Gloessl, J. 1997. **Identification and characterization of (GA/CT)-n-microsatellite loci from *Quercus petraea*.** *Plant Molecular Biology* 33(6): 1093-1096.
- U.S. Department of the Interior, Geological Survey. 1992. **Standards for digital orthophotos.** Reston, VA.