POLLEN MOVEMENT IN DECLINING POPULATIONS OF CALIFORNIA VALLEY OAK, *QUERCUS LOBATA*: WHERE HAVE ALL THE FATHERS GONE?

Victoria L. Sork[1, 2]

Frank W. Davis[3]

Peter E. Smouse[4]

Victoria J. Apsit[1]

Rodney J. Dyer[1]

Juan F. Fernandez-M[1]

Bill Kuhn[5]

[1] Department of Biology, University of Missouri, St. Louis, Missouri, 63121-4499

[2] Current address: Department of Organismal Biology, Ecology, and Evolution, and Institute of the Environment, University of California-Los Angeles 90095-1606

[3] Donald Bren School of Environmental Science and Management, University of California, Santa Barbara, California, 93106

[4] Department of Ecology, Evolution & Natural Resources, Rutgers University, New Brunswick New Jersey 08901

[5] Department of Geography, University of California, Santa Barbara, California, 93106

Date of Receipt:

Correspondence to: Dr. Victoria L. Sork
UCLA
Organismal Biology, Ecology & Evolution
Box 951606
Los Angeles, CA 90095-1606
Email: vsork@conet.ucla.edu
FAX: 310-206-0233

Running Title: Pollen movement in California Valley oak

Key Words: California Valley oak, *Quercus lobata*, pollen flow, genetic isolation, TWOGENER

Abstract

The fragmented populations and reduced population densities that result from human disturbance are issues of growing importance in evolutionary and conservation biology. A key issue is whether remnant individuals become reproductively isolated. California Valley oak (*Quercus lobata*) is a widely distributed, endemic species in California, increasingly jeopardized by anthropogenic changes in biota and land use. We studied pollen movement in a savanna population of Valley oak at Sedgwick Reserve, Santa Barbara County, to estimate effective number of pollen donors (N_{ep}) and average distance of effective pollen movement (δ). Using TwoGener, our recently developed hybrid model of paternity and genetic structure treatments that analyzes maternal and progeny multilocus genotypes, we found that current $N_{ep} = 3.68$ individuals. Based on an average adult density of $d = 1.19$ stems ha^{-1}, we assumed a bivariate normal distribution to model current average pollen dispersal distance (δ) and estimated $\delta = 64.8$ m. We then deployed our parameter estimates in spatially explicit models of the Sedgwick population to evaluate the extent to which N_{ep} may have changed, as a consequence of progressive stand thinning between 1944 and 1999. Assuming that pollen dispersal distance has not changed, we estimate N_{ep} was 4.57 individuals in 1944, when stand density was 1.48. Both estimates indicate fewer effective fathers than one might expect for wind-pollinated species and fewer than observed elsewhere. Results presented here provide a basis for further refinements on modeling pollen movement. If the trends continue, then ongoing demographic attrition could further reduce neighborhood size in Valley oak resulting in increased risk of reproductive failure and genetic isolation.

INTRODUCTION

California Valley oak (*Quercus lobata* Neé), one of the State's most familiar and evocative icons, is among the largest and longest lived of the North American oaks, attaining trunk diameters of up to 4 m, heights of 12 to 25 m, and ages of 300 years or more. Unfortunately, this endemic species has been declining steadily for 200 years, due both to landscape alteration and to restricted recruitment within remnant stands (Griffin 1971, Bolsinger 1988, Brown & Davis 1991, Adams *et al.* 1992). Compared to other foothill oak species, it has been and will probably continue to be disproportionately impacted by land conversion, because the species prefers level, fertile sites that are valuable for agricultural and development purposes. Since 1945, over 400,000 ha of foothill oak woodlands in California have been cleared for range improvement or development, and predictions of future loss run as high as another 100,000 ha by the year 2010 (Bolsinger 1988). Roughly, 90% of Valley oak woodland is owned privately, and most stands are in areas predicted to undergo rapid development in the near future (Davis *et al.* 1998). Less than 5% of the species' range is internal to formally designated reserves, with protection concentrated in Monterey and Santa Clara Counties (Greenwood *et al.* 1993, Davis *et al.* 1998). Remnant populations at the southern end of the species' range are fragmented by residential and agricultural development, and are converting slowly to grasslands through stand thinning (Brown & Davis 1991). In northern Santa Barbara County, Valley oak tree cover and density are steadily declining, due to poor recruitment of saplings and trees. Brown and Davis (1991) document 21% attrition among overstory Valley oaks and no new establishment between 1938 and 1989 in any of their 12 surveyed populations.

The fragmented population structure and reduced population densities experienced by Valley oak are familiar themes in conservation biology (Gilpin 1987, Ledig 1992). Many tree populations, naturally distributed over large, continuous stretches of landscape, are now divided into patches having little or no genetic exchange among them. Many species may not be adapted to such fragmentation and their persistence in a region may depend on metapopulation processes of dispersal and recolonization of isolated habitat patches (Gilpin 1987, Ledig 1992, Hanski & Simberloff 1997, Bawa & Seidler 1998). A key issue is whether these fragmented patches, or scattered individuals within them, are becoming reproductively isolated. As fragments become increasingly isolated, effective population sizes decrease, and small fragments lose genetic variation, some of it adaptive (Ellstrand & Elam 1993, Frankham 1995). As individual trees become isolated, theycan lose fitness through a lack of fertilization and fruit set, and their progeny can suffer reduced fitness through increased inbreeding depression, caused by selfing or mating with close relatives (e.g., Barrett & Kohn 1991, Holsinger & Vitt 1997). That can only exacerbate the recruitment problems. For many tree species, genetic isolation will be prevented through pollen rather than seed movement, even though both processes are important means of maintaining the integrity of a metapopulation.

It is not known whether Valley oak individuals and patches are becoming genetically isolated. Until recently, it has not been feasible to study pollen movement on a landscape scale (Sork *et al.* 1998). However, a new analytical method, dubbed TWOGENER (Smouse 1998, Smouse *et al.* 2001), allows us to examine pollen flow across a landscape, by combining the two-generation gametic inference of parentage analysis (e.g., Chakraborty *et al.* 1988, Devlin *et al.* 1988) with the survey methods of population structure analysis. TWOGENER uses spatially referenced pollen pool structure to estimate the effective number of pollen donors in a mating neighborhood and the decay parameter of the pollen dispersal curve. That decay parameter can then be deployed to describe landscape-scale patterns of pollen movement for geo-referenced individuals. It can also be used in spatially explicit geographic models (e.g, Walsh & Davis 1994, Goodchild *et al.* 1996), for demographic simulation of different conservation/management scenarios.

In this paper, we describe pollen movement across the landscape in one stand of California Valley oak (*Quercus lobata*), located in the Sedgwick Reserve in central coastal California, where we have documented demographic attrition of adult trees over the last 50 years. We will first ask two questions: (1) what is the effective number of pollen donors per tree, acting as a maternal parent? (2) How large is the effective pollination neighborhood? Then, using our estimate of the decay parameter for pollen dispersion, we will use spatially explicit pollination models to provide a preliminary exploration of the impact of population decline over the last 50 years on genetic connectivity at the study site. The approach we use here extends and applies earlier work on contemporary pollen movement (Austerlitz & Smouse 2001a, Smouse *et al.* 2001) to provide practical input into the debate on Valley oak conservation. The survival of Valley oak is the focus of intense public scrutiny (Griggs 1990, Pavlik *et al.* 1995). Many Counties have adopted or are considering strong Valley oak conservation measures aimed at preserving or increasing stand densities, provoking angry debates among environmentalists, agricultural and development interests. Those measures will be more effective if the target densities and mandated spatial arrays have a credible scientific rationale.

Materials and Methods

Study Species

Valley oak (*Quercus lobata* Neé) is found mostly in the Central Valley of California, and in the surrounding valleys and foothills, ranging from near Shasta Lake southward to the Santa Monica Mountains. The species is generally restricted to deep loamy soils below 600 m of elevation, but some populations occur above 1500 m in Southern California (Griffin & Critchfield 1972). The savanna community type is found on valley floors, Quaternary terraces and some broad ridge tops in the Coast Ranges. Denser gallery forests are found along the margins of rivers, especially in the Central Valley, but not in valleys directly exposed to coastal winds, as the species is sensitive to salt aerosols (Ogden 1980).

Quercus lobata is a deciduous, wind-pollinated, monoecious tree species that flowers in March through April. In general, the genus *Quercus* is thought to have an incompatibility system (Hagman 1975, Ducousso *et al.* 1993). But, for this same population, we estimated the mating system of Valley oak to be 96% outcrossing that was significantly less than 100%, which suggests that if an SI system exists, it is not fully effective (Sork *et al.* In press). Acorns mature in late September through early November of the same year of flowering. Acorns are dispersed by gravity, acorn woodpeckers, scrub jays, and possibly by small rodents. They germinate within 4 - 8 weeks of maturation.

Study Site

The study was conducted at the Sedgwick Reserve, along the valley floor of Figueroa Creek (N 34°42’, W 120° 2’), 10 km northeast of Santa Ynez, California. Sedgwick Reserve is a 2380 ha area managed for research, education and conservation of native biodiversity, and is administered by the University of California Natural Reserve System and UC Santa Barbara. Since 1944, open oak woodland and savanna at Sedgwick

Reserve has experienced a 20% reduction in overstory tree density, including the loss of roughly equal numbers of Valley oak and Coast live oak (*Q. agrifolia*) (Davis *et al.*, Unpublished data).

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 360m to 405m above sea level. Soils are deep silty loams, derived from Quaternary alluvial and colluvial deposits. Cultivation of the valley floor was obvious in 1944 photos, but had ceased before 1967. Annual precipitation for this typical savanna oak woodland site averages roughly 38 cm/yr, nearly all of which falls between December and March.

GIS mapping

Individual trees in the study area were mapped using a 1993 digital panchromatic orthophoto with 1 m^2 resolution, produced by the U.S. Geological Survey (U.S. Department of the Interior 1992). The map of tree locations and species identity was updated with 1:24,000 true color air photos collected for the County of Santa Barbara in July 1997 and by field surveys during 1999 and 2000. Most of the 312 mapped trees were *Quercus lobata* (n = 153), the evergreen *Q. agrifolia* (n = 104), or *Q. douglasii* (n = 40) that, along with *Pinus sabiniana* (n = 8) rimmed the study area, on slightly higher and drier sites (Fig. 1). Remaining trees included the riparian species *Platanus racemosa* (n = 6) and a single individual of *Schinus molle*. Based on our experience with modern photos, we discriminated Valley oaks from Coast live oaks with high confidence, using canopy tone, texture and size. We distinguished Valley oaks from Blue oaks with less confidence, based on canopy size, shape and site characteristics. We mapped 39 trees present in 1944 and absent by 1999 scattered throughout the study area (Fig. 1). The estimated density of Valley oak in the study area is thus 153 trees in 130 ha or 1.19 trees/ha. Because no new trees were recruited during this period, this represents a reduction in population size and density (from 1.48 trees ha^{-1} in 1944 to roughly1.19 trees ha^{-1}) of 20.3% over the period. Tree point locations and identities were stored as digital information, using ArcInfo Geographic Information System (GIS) software.

[Fig. 1 – about here]

Field sampling design

In fall 1999, we selected 21 reproductive Valley oak adults at the Figueroa Creek study site (Fig. 2). Our sampling intent was to construct a hexagon-shaped grid with seven triangular clusters (three trees each) at the 6 vertices of the hexagon and in the middle. The point of this design was to sample at different spatial scales, because we were uncertain whether average pollination distance would be in the 50m or the 500m ranges. The inter-female distances within each cluster ranged from about 50-150 m, and distances between clusters ranged between 250 and 750 meters (maximum distance across the hexagon is 1040 m; see Fig 2).

[Fig. 2 -- about here]

We collected up to 100 acorns from each female and planted the seeds in the greenhouse at UM-St. Louis. Our goal was to assay 15 progeny per maternal plant, based on sample size analyses reported in Smouse et al. (2001). However, after germination, several trees did not yield sufficient numbers of offspring to reach the target sample size. We assayed 4-16 progeny from each mother, for a total of N = 211 seedlings. As leaves emerged, we removed leaf tissue for progeny genotypes, for purposes of allozyme and DNA microsatellite analyses. In Spring 2000, we collected newly emerging leaves of the maternal trees, placed samples in zip-lock plastic bags, and kept them on ice until permanent storage in an ultra-cold freezer (-80°C.).

Laboratory methods

We used a combination of ten allozymes and one microsatellite as the genetic markers for this study. We chose this strategy because the allozyme loci provided expeditious and inexpensive genetic information, while the microsatellite locus gave us valuable genetic resolution (Smouse *et al.* 2001).

For the allozyme markers, we extracted plant enzymes by grinding in 1 ml of a modified phosphate buffer (Alvarez-Buylla & Garay 1994) with mortar and pestle, absorbing the exudates onto chromatography paper wicks. We stored the wicks at –70ºC until analysis. We followed similar procedures for the maternal leaf tissue. We conducted the electrophoresis on 10.5% potato starch gels (Sigma, St. Louis). We assayed seven enzyme systems (Soltis *et al.* 1983, Kephart 1990, Sork *et al.* 1993) on four gel/electrode buffer systems: fluorescent esterase (*Fe*, EC 3.1.1.1, 1 and 3), leucine aminopeptidase (*Lap*, EC 3.4.11.1), and phosphoglucose isomerase (*Pgi*, EC 5.3.1.9, 1 and 2) on a modified system 8 (Soltis *et al.* 1983); malate dehydrogenase (*Mdh*, EC 1.1.1.40) and phosphoglucomutase (*Pgm*, EC 2.7.5.1) on morpholine citrate pH 7.2 (Clayton & Tretiak 1972); menadione reductase (*Mnr,* EC 1.6.99.-) on system 34 (Poulik 1957); and triosephosphate isomerase (*Tpi*, EC 5.3.1.1, 1 and 2) on system 6 (Soltis *et al.* 1983). All protocols for staining enzymes are from Soltis *et al*. (1983).

For microsatellite genotypes, we extracted total genomic DNA from fresh leaves from the greenhouse for the seedlings and from frozen leaf tissue from the maternal trees. We ground a sample of 0.1 g from each leaf with liquid nitrogen, using a mortar and pestle. After grinding, 1 ml of extraction buffer (Lefort & Douglas 1999) was added, and the sample was vortexed for 10 s and inverted enough times to homogenize the mixture. The samples were then incubated for 15 min at 65 ºC and tubes inverted 2-3 times every 5 min. Next, 0.750 µl of chloroform/isoamil-alcohol (24:1) was added to each sample and thoroughly agitated to make an emulsion. We separated phases by centrifuging for 5 min at ca. 8,000 g. The upper phase was then transferred to a new 1.5 ml tube. An additional centrifuging for 1 min was performed if debris or protein precipitate was still present. Chilled isopropanol (500 µl) was added and mixed to precipitate DNA. Next, samples were centrifuged for 1 min, and the supernatant was eliminated carefully. Finally, we rinsed the DNA pellet twice with 1 ml of chilled 70% ethanol and resuspended it in 200 µl of TE (10 m*M* Tris-HCl pH 8.0, 1 m*M* EDTA).

Total DNA was diluted 1:50 for the PCR reaction. Preliminary analysis revealed two useful primers, QpZAG110 and QpZAG46, originally developed for *Quercus petrea* (Steinkellner *et al.* 1997). We used only QpZaG110 for this study. PCR reactions were carried out at concentrations of 1.5 m*M* of $MgCl_2$, 0.2 µ*M* of each primer, 0.2 m*M* of each dNTP's, one unit of *Taq* polymerase buffer B (PROMEGA Corp.) and approximately 20 ng of DNA template. Reaction cycles consisted of an initial denaturing of 2 min at 94 ºC, 35 cycles of 1 min at 94 ºC, 30 s at 50 ºC, and 30 s at 72 ºC, with a final extension time of 5 min at 72 ºC. PCR products were separated using standard acrylamide sequencing gels (Biorad Sequi Gen system®) and visualized using silver staining (Bassam *et al.* 1991). Gels were scanned and allele sizes were scored, based on a 10 bp (30-330 bp) DNA ladder (Gibco BRL ®), using software developed by R. Dyer (ALLELESIZER, www.umsl.edu/~biology/Dyer/software).

TWOGENER analysis

To characterize the pollen structure of the population, we conducted a TWOGENER analysis (Smouse *et al.* 2001), a molecular analysis of variance (Excoffier *et al.* 1992) on the male gametic genotypes, obtained by subtracting the female gametic contribution from each diploid seedling genotype. A partition of male gametic variation into among- and within-female components yields an intra-class correlation measure Φ_{ft} of 'pollen pool structure', analogous to an F_{st} partition, but with females (rather than populations) as the strata and individual male gametes (rather than individual diploid individuals) as replicates within strata. Using the estimate of Φ_{ft}, we extracted derivative estimates of the average distance of pollination (δ), the effective number of pollinators (N_{ep}) and the effective pollination neighborhood (A_{ep}) (Smouse *et al.* 2001).

GIS analysis and modeling

The GIS was used to calculate inter-tree distances and to generate maps of probabilities of effective pollen dispersal from each tree. For this exercise, we treated the planar center of each tree as a point source of pollen, although in reality, each tree constitutes a volume source as well as a volume trap for airborne pollen. Thus, patterns of inter-tree pollen flow were estimated on the basis of inter-tree (center point) distances, without requiring any complex GIS modeling. In addition to modeling pollen flow under 1994 and modern conditions, we explored the sensitivity of our findings to a range of pollen dispersal parameters (σ), which probably vary a bit, as a function of stand density and year-to-year variation in weather during the flowering period.

Results

Genetic resolution

The allele frequencies for all 10 allozyme loci and the 1 microsatellite locus, extracted from the derived male gametes, are presented in Table 1. Statistical precision is a function of the polymorphic variation of the genetic battery, conveniently described in terms of the average exclusion probability, defined as $E_L = 1 - \Pi_l (1 - E_l)$, where E_l is the exclusion probability for the *l*-th locus and E_L is the corresponding multi-locus value (Selvin 1980, Chakraborty *et al.* 1988). The more polymorphic the genetic battery, the greater is E_L and the greater the statistical precision available for estimation of Φ_{ft} and δ. In our sample of 211 offspring, the single microsatellite locus yielded 17 alleles, while the 9 polymorphic allozyme loci included 2-5 alleles each (Table 1). The individual allozyme loci had much lower parentage resolution than the single microsat, but collectively they yielded $E_A \sim 0.6903$. For the 9 allozyme loci and one microsatellite combined, we obtained a multilocus exclusion probability of $E_L \sim 1 - (1 - E_A)(1 - E_M) = 1 - (1 - 0.7513)(1 - 0.6903) = 0.9231$ (Table 1), ample genetic resolution.

[Table 1 – about here]

Pollen pool structure

Even with our limited replication within females ($\tilde{n}_0$ 11.07), the AMOVA results (presented in Table 2) present a striking departure from the null (broadcast pollination) hypothesis ($\hat{\Phi}_{ft} = 0.136$; $p \leq 0.001$). To a very considerable degree, different females are being pollinated by different sets of males. Smouse *et al* (2001) recommended within-female replication on the order of $n \sim [\Phi_{ft}]^{-1}$. On the strength of earlier work with *Quercus alba*, we were anticipating $\Phi_{ft} \sim 0.06$, and attempted to sample 15 -16 progeny per female. In retrospective view of our results, *n* of 7 - 8 would have been ample to estimate the average amount of differentiation among females for Valley oak.

[Table 2 – about here]

Average Pollination Distance

Austerlitz and Smouse (2001a) have worked out the formal theory for both the bivariate normal and bivariate negative exponential pollen flow models, but the results are comparable. We will use the more tractable bivariate normal theory, for which the expected value of Φ_{ft} takes the form

$$\Phi_{ft} = \frac{1}{8\pi\sigma^2 d}$$

where σ^2 is the variance in pollen flow distance and d is the density of potential pollen donors, across the landscape in question.

We estimate the modern stand density to be roughly 1.19 adult Valley oaks per hectare. Since we have d in terms of the number of adults per hectare, we will express σ^2 in comparable hectare $(100\ \text{m})^2$ units. Inserting our observed estimate of $\hat{\Phi}_{ft} = 0.136$, we obtain an estimate of $\tilde{\sigma}^2 = 0.2459$ hectares (Austerlitz & Smouse 2001a), assuming isotropic pollen flow, which translates into an estimate of the average distance flown by a successful male gamete of δ units, where

$$\hat{\delta} = \hat{\sigma}\sqrt{\frac{\pi}{2}} \sim .648$$

or about 65 m for Valley oak at Figueroa Creek.

Effective pollination neighborhood

Another way to look at this is to imagine a circle, centered at a focal female, and containing a certain number of genetically randomized adults (serving as males, and denoted N_{ep}). Now assume that each of these males contributes pollen to the focal female with equal probability (no distance effect), and that no other males (outside the circle) contribute. Reciprocally, these males contribute only to this female. Separate sets of N_{ep} idealized ('effective') males, one genetically random set per female, would yield the same value of male gametic divergence among females as the realized value of Φ_{ft} that we obtained from the TWOGENER analysis. In the real world, some males (generally the closest) will provide far more gametes than their 'fair share', and others (those more distant) will provide far less, and any given male will contribute to different females, but the 'effective number of males' (N_{ep}) is a standardized measure of the stochastic equivalence of a small number of equally probable contributors and a larger number of *unequally contributing* males. Austerlitz and Smouse (2001a) show that

$$N_{\mathrm{ep}} = 4\pi\sigma^2 d \,,$$

or < 4 males with our numbers. Another way to say this is that each subset (of ~ 4 males), each male contributing equally to a given female (and no others), would yield the inter-mother variation among male gamete pools that we actually observed for Valley oak. The adults are so sparse on the landscape (1.19 per hectare) that pollination would be expected to show a spatial component, but given the ability of wind-dispersed pollen to move large distances, the severe localization of successful pollination is a surprise. To gauge the implications of that result, we can also describe the effective neighborhood area, A_{ep}, over which the idealized males are distributed (Austerlitz & Smouse 2001a). For Valley oak, we have

$$A_{\mathrm{ep}} = 4\,\pi\sigma^2 \sim 3.09 \text{ hectares,}$$

a circle of radius r_{ep} ~100 m, drawn around each focal female.

The average distance of successful pollination, $\delta = 64.8$ m, is an average, of course, and there is a long tail to the distribution, in every direction. Small amounts of pollen are probably coming in from substantial distances, but with a density of $d = 1.19$ adults per hectare, the bulk of the pollen is drawn from very few males, everything else being equal.

Spatially explicit pollen donor neighborhood

Another way to visualize the patterns of pollen movement is to view the relative contribution of pollen donors for pollen recipients in different parts of the stand (Fig. 3). We selected four focal trees and modeled which of the neighboring trees would act as pollen donors. The circles on the map are centered on mapped trees and their area is proportional to the estimated relative contribution of each tree to fruit production by the reference tree, as indicated by our model (Fig. 3).

[Fig. 3 --about here]

Because our modeling is influenced by our estimate of σ, we include here an examination of the implications of varying σ for our estimates of pollen donor neighborhoods. Here, we arbitrarily selected one of the interior trees, Tree 57, to be a focal individual. From our TWOGENER result, we computed the likelihood of a male at distance z from Tree 57 contributing to Tree 57's fruit production. Knowing the distance of every tree from Tree 57, we calculated the point probability (probability mass function) for the bivariate normal, $[N(0,\sigma^2)]^2$, for each tree. In other words, we centered the 2-D Gaussian distribution on Tree 57 and estimated the likelihood of a pollen source as a function of distance from that reference female, treating potential donors as point sources. Except for the nearest neighbors to Tree 57, these likelihoods are all miniscule (see Fig. 3). The relative contribution (likelihood of parentage) of any tree to Tree 57's acorn production is simply its pollination probability, divided by the sum of pollination probabilities for all mapped trees. If the stand consisted of Tree 57 and only one other tree, that tree's relative pollen contribution would be 1.0, no matter how far it was from Tree 57. In 1999, trees within 100 m of Tree 57 have by far the greatest relative contribution, although the value declines predictably as σ increases (Fig. 4). If $\sigma = 25$ m, about half of the observed standard deviation, the nearest neighbor would account for 48% of cumulative probability of male parentage; but if $\sigma = 100$ m, twice the observed value, our simulations predict that that same tree would account for only 11% of the total male parentage.

[Fig. 4--about here]

Changes in stand density

To examine the impact of changes in stand density from 1944 to 1999, we simulated the relative contribution of pollen donors to three focal trees (Trees 57, 33, and 102) under the both historical and contemporary stand density conditions (Fig. 5a-c). Our simulations for these individual trees predict that changes in stand density from 1944 to 1999 would have had much larger effects on trees that lost immediate neighbors than those that did not. According to that model, such an outcome is possible because the predicted pollination neighborhood for a given female is so small (A_{ep} ~ 3.1 ha). For example, Tree 57 lost 5 neighbors within a 100m radius, which means that the current near neighbors are likely to play a much larger role now than they would have in 1944 (Fig. 5a). Assuming a constant $\sigma = 49.6$ m, the nearest neighbor today would account for nearly 30% of the total male parentage, compared to 17% in 1944. Tree 33 (Fig. 5b) illustrates the case where the pollen donor neighborhood has changed drastically, while

Tree 102 (Fig 5c) illustrates the case where no immediate neighbors were lost, so that the pollen donor contribution would have changed very little.

[Fig. 5 -- about here]

Discussion

Our results suggest that effective pollen flow among Valley oaks at the study site is highly localized. Admittedly, this conclusion is based on only one flowering season and the results will certainly vary from year to year, depending on local weather conditions (cf. Koenig *et al.* 1994). Our analysis indicates that Valley oak trees at the study site did not have a large number of 'effective fathers' in 1999, with an average effective number of pollen donors equal to 3.68 and a range of 3 - 5 individuals. The equivalent N_{ep} for 1944, assuming the same σ but $d = 1.48/\text{ha}^{-1}$, yields a value of $N_{ep} = 4.57$, suggesting that the number of fathers may have declined in 45 years, due to demographic attrition. Both the 1999 and 1944 estimates are substantially lower than the values reported for two wind pollinated species in Missouri Ozark forests: white oak (*Quercus alba*, $N_{ep} \sim 8$ individuals, Smouse *et al*. 2001) and short leaf pine (*Pinus echinata*, N_{ep} ~10 individuals, extracted from Dyer & Sork 2001). Such low values in Valley oak are somewhat unexpected, because we anticipated that open spacing would favor extensive pollen movement, due to changes in turbulence in a savanna setting (Okuba & Levin 1989).

Using the TwoGener analysis, we estimated that the average distance of successful pollination is almost 6 times greater for *Q. lobata* ($\delta \sim 65$ m) at Sedgwick Reserve than for *Q. alba* in the Ozarks ($\delta \sim 11$ m). However, in spite of greater pollen dispersal, the density of adults available for pollination is reduced by a factor of 78 at Sedgwick (1.19 stems ha^{-1}), relative to the Ozarks (92.8 stems ha^{-1}). Pollen (at least successful pollen) is clearly moving farther under savanna than under closed canopy conditions, but the differential movement is not sufficient to compensate for the difference in adult density. To the extent that we can compare two different oak species in different settings, it appears that even with increased pollen flow, populations in open landscapes exhibit a reduction in the effective number of pollen donors.

The degree of pollen pools separation among sampled females is gauged by our estimate of Φ_{ft}, and the *effective* number of pollen donors per female, N_{ep}, follows directly from Φ_{ft}. As with all estimates of *effective* population size (*idealized* pollen donors, in this case), N_{ep} covers a multitude of sins. The modeling of neighborhood area is based on the assumptions that pollen movement is bivariate normal, that all adults are equally likely (*a priori*) to fertilize a pollen recipient, and that there is no inbreeding or spatial population structure among adults. N_{ep} is decreased by adult inbreeding or spatial population structure (Austerlitz & Smouse 2001b). In this small sample, we were unable to detect meaningful inbreeding or population structure, but such 'adult structure' may play a role in other studies. Any inflation of Φ_{ft} (deflation of N_{ep}) means that our estimate of the average distance of pollen movement, δ, is too small.

It would also be useful to assess whether genetic incompatibility or phenological variation among individuals inflate Φ_{ft}, thus reducing local N_{ep}. For a given value of Φ_{ft}, either genetic incompatibility or phenological variation violates the assumptions of our pollen distance model. The important point is that genetic incompatibility systems and phenological variation reduce the available donor pools for any particular female and exacerbate the tendency for different females to sample different sets of males, resulting in higher values of Φ_{ft} and lower values of N_{ep}.

We now will address the assumption of our model concerning circular neighborhoods, which may or may not be satisfactory for wind-pollinated species. We should be able to extend the model to include anisotropic pollen flow, but to apply this extension to the Valley oak situation will require much larger sample sizes of mothers and progeny than we have for this initial study. Moreover, it would be ideal to "ground truth" our results by using direct paternity analysis to verify the location of fathers with respect to maternal tree location. That analysis will also require large sample sizes of progeny, enabled by a mast year for Valley oak, as well as paternal genotypes for the area and better genetic resolution, currently under development.

An additional question remains concerning the estimates in σ (and hence δ) from Valley oak at Sedgwick, versus those from wind-pollinated species in Ozark forests. Is this difference merely a statement about the changes in inter-adult spacing or are airfoil and turbulence issues involved? To the limited extent that the available data will allow comparative inference, they do not suggest a tradeoff between inter-adult density (d) and average pollination distance (δ). To resolve the issue, we will need comparative studies of pollen movements for the same species (*Q. lobata*, in this case) under different density conditions; those studies are currently underway. At the moment, all we can say is that reduced adult density appears to be coupled with a reduction in the number of effective pollen donors, in spite of the fact that the average successful male gamete is moving farther, from male source to female target.

Sampling consideration

Our estimate of Φ_{ft} is highly significantly divergent from the null hypothesis value of ‘0’, but is still not tightly estimated. Using *F*-distribution methods from Searle *et al.* (1992), we can place a rough 95% confidence interval on the point estimate, obtaining $0.04 < \Phi_{ft} < 0.33$. There is no overlap with ‘0’, of course, but the confidence interval is more forgiving than is ideal. That large confidence interval translates into correspondingly large uncertainty for all the derivative parameters. Careful analysis of sample allocation issues now suggests (Irwin *et al*. in prep; Austerlitz and Smouse in prep.) that we will need larger numbers of mothers to provide tighter confidence intervals. Our intent is to follow up this initial study of *Quercus lobata* with additional field sampling, some of it at the Sedgwick Reserve. We have 153 adult Valley oaks to choose among, and our intention is to sample a larger number for the follow up, bringing our total closer to (say) 100 mothers. The results to date suggest that 7-8 seedlings per mother should provide ample and 10-15 should provide abundant replication. Given an average distance ($\delta \sim 65$ m) of successful pollination, we probably need to sample more pairs of adults at closer quarters than we have. The adults are not randomly distributed across the landscape, and where density will permit, we plan to sample multiple trees per cluster.

Local density

An important next question is the extent to which local density influences the patterns of pollen movement. Our simulations based on TwoGener parameters indicate that changes in local stand density may affect pollen donor neighborhood drastically. If a focal tree loses several near neighbors and pollen movement is indeed restricted, the focal tree could become reproductively isolated. In contrast, those trees that do not lose neighbors will show relatively little change in neighborhood. These simulations are based on the assumption that sigma does not change when stand density changes. That assumption may be valid for this study, given the minor changes in density between 1944 and 1999, but it does need to be tested empirically by estimating σ (and δ) for mothers with differing local conspecific densities. That work is also under way.

Modeling limitation

The results of our simulations illustrate that spatial modeling of pollen movement may be highly informative, but our findings also suggest that the estimate of neighborhood area is very sensitive to our estimate of σ. Our modeling also assumes that aerodynamic processes are constant over space and time. We do not account for pollen interception by intervening Valley oaks or other trees (especially live oaks that might effectively shield other trees if they are close enough). An empirical examination of σ under different conditions of conspecific tree density and general canopy closure is essential to address this modeling limitation.

An additional limitation of our initial spatial modeling is our treatment of canopies as points rather than areas or volumes. It might be more appropriate to model canopies as areas or volumes, but we still do not know the extent to which this approach would influence our simulations of pollen donor neighborhood. Because canopy volume determines the amount of pollen production and the physical structure of the landscape, it would be valuable to explore more elaborate modeling in the future.

Conservation implications

Undoubtedly, the most critical challenge for the maintenance of Valley oak woodland and savanna in the region is the attrition of existing trees, with the concomitant failure of recruitment. The findings reported here suggest that increasing isolation of extant individuals may hinder future reproduction. The likelihood of reduced reproduction is supported by the work of Knapp *et al.* (2001), who found that acorn crop size was positively associated with number of neighboring trees within 60 meters in a thinned stand of *Quercus douglasii*. They concluded that reduced pollen availability is likely to limit reproduction. Clearly, more work is needed to understand the impact of landscape features and stand density on pollen movement, but our conclusion that pollen movement is restricted is not likely to change. As neighborhood size becomes further reduced, trees will become reproductively isolated, experiencing reduced seed set, with an increasing probability that their seedlings will exhibit reduced fitness, if such isolation increases selfing. Efforts must continue to improve seedling recruitment and survival, but we must simultaneously develop conservation strategies that preserve large stands of Valley oaks, with adequate densities, so that pollination itself is maximized.

When it is necessary to achieve Valley oak recruitment through planting programs, it may well be preferable to take advantage of the opportunity to increase genetic diversity. Pollen flow is now so limited that these programs should sample seeds from a large number of trees within the region, rather than from a few local individuals. While it is legitimate to view ‘local material’ as being locally adapted, the use of highly variable seed pools permits local selection pressures to weed out the poorly adapted (sometimes inbred) genotypes from local fertilization, while promoting genetic diversity (Templeton *et al.* 1990).

Valley oak is in jeopardy, but ample individuals remain and sufficient public interest exists that is still possible to develop a workable conservation strategy that allows for the persistence of sustainable populations. Long-term sustainability will depend on a variety of demographic and evolutionary processes. An effective strategy will require the integration of genetic and ecological information, and it is becoming increasingly clear that we must pay attention to the spatial context of the populations to be preserved.

Acknowledgments

The authors would like to thank F Austerlitz, AJ Irwin, KA Mylecraine, and J Nason for helpful comments on the manuscript. VLS, VJA, RJD, and JF were supported by the UM Research Board and UM-St. Louis Research Award programs, VLS by NSF-DEB-0089445, FD by the Santa Barbara County Oak Restoration Project**,** and PES by the NJ Agricultural Experiment Station, USDA/NJAES-17106, McIntire Stennis-17309, and NSF-BSR-0089238. Elements of this work were conducted as part of the Gene Flow Dynamics Working Group supported by the National Center for Ecological Analysis and Synthesis, a Center funded by NSF (Grant #DEB-0072909), the University of California, and the Santa Barbara campus.

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INFORMATION BOX

This study is part of a collaborative research effort developing spatial approaches to contemporary pollen movement. VLS has interests in evolutionary ecology, population genetics, and conservation, FWD in geography, ecology, and spatial modeling, and PES in population genetics and biostatistical modeling. VJA and JF study conservation genetics in tropical and temperate trees, RJD's expertise is population genetic statistical modeling, and BK studies the geography of California oaks.

Figure Legends

Fig. 1 Map of study area at Figueroa Creek, Sedgwick Reserve, Santa Barbara Co. showing individuals of all tree species--*Quercus lobata*, *Q. douglasii*, *Q. agrifolia*, *Pinus sabiniana*, *Platinus racemosa*, and *Schinus molle*. Blue dots indicate *Q. lobata* trees used in study; red dots indicate *Q. lobata* trees that were present in 1944 but no longer alive.

Fig. 2 Aerial photo of study area showing the sampling design of triangular sets of maternal trees nested within a "hexagon" array.

Fig. 3 Individual Valley oak adults at the study site, showing estimated probability of each tree contributing to fruit production for four focal pollen recipients based on bivariate normal distribution with $\sigma = 49.6$ m, and contributing area of each tree of 256 m^2, based on a canopy radius of roughly 9 m.

Fig. 4 Relative likelihood of neighboring trees contributing to acorn production by an interior *Q. lobata* tree (Tree 57) as a function of distance. Each data point represents a tree within a 200m radius of the focal tree. Relative contribution from each neighboring tree is the probability of a pollen source at that inter-tree distance, divided by the sum of probabilities for all trees in the stand. The analysis treats each tree as a point. The three lines show relative contribution, based on mapped tree locations in 1999 and on $\sigma = 25$, 50, or 100 m. For example, for $\sigma = 50$ m, the relative pollen contribution from the nearest tree, which is 39 m from Tree 57, is 0.29 or 29%. For $\sigma = 100$, the relative contribution would be only 0.12.

Fig. 5. Predicted relative contribution of neighboring trees to fruit production in 1944 vs. 1999, as a function of separation distance, for three focal trees: Tree 57 (a), Tree 33 (b), and Tree 107 (c). Curves are based on TWOGENER analysis with $\sigma = 49.6$. Each data point represents a mapped Valley oak up to a distance of 150 m.

Table 1 Allele frequencies and exclusion probabilities (E_l) of one microsatellite locus and nine allozyme loci for Valley Oak (*Quercus lobata* Neé) at Sedgwick Reserve, Santa Barbara County, California. Overall exclusion probability (E_L)= 0.923.

allele	***QpZAG* 110**	***Tpi-1***	***Tpi-2***	***Mnr***	***Pgm***	***Mdh***	***Lap***	***Fe-1***	***Pgi-1***	***Pgi-2***
1	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	0.048	0.068	0.068	0.024	0.024	0.000	0.000	0.000	0.005	0.005
3	0.121	0.801	0.923	0.749	0.218	0.117	0.029	0.000	0.181	0.181
4	0.155	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.015
5	0.193	0.131	0.010	0.227	0.758	0.883	0.952	0.981	0.804	0.765
6	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
7	0.021	0.000	0.000	0.000	0.000	0.000	0.019	0.019	0.000	0.034
8	0.161									
9	0.110									
10	0.063									
11	0.043									
12	0.011									
13	0.005									
14	0.005									
15	0.011									
16	0.021									
17	0.005									
E_l	0.752	0.172	0.070	0.175	0.171	0.092	0.047	0.019	0.145	0.191
# alleles	17	3	2	3	3	2	3	2	3	5

Table 2**:** Analysis of molecular variation (AMOVA) for Valley oak, describing the partitioning of male gametic contributions into within- and among-mother components, with extraction of an estimate of the intra-class correlation coefficient, $\hat{\Phi}_{ft} = \hat{\sigma}_f^2 / \left(\hat{\sigma}_f^2 + \hat{\sigma}_w^2\right)$

Source of Variation	*df*	*SS*	*MS*	$\hat{\sigma}^2$	%	Φ_{ft}
Among Mothers	18	55.333	3.074	0.186	0.136	0.136
Within Mothers	176	208.709	1.186	1.186	0.864	
Total	194	264.042	------	1.372	------	

Fig. 1

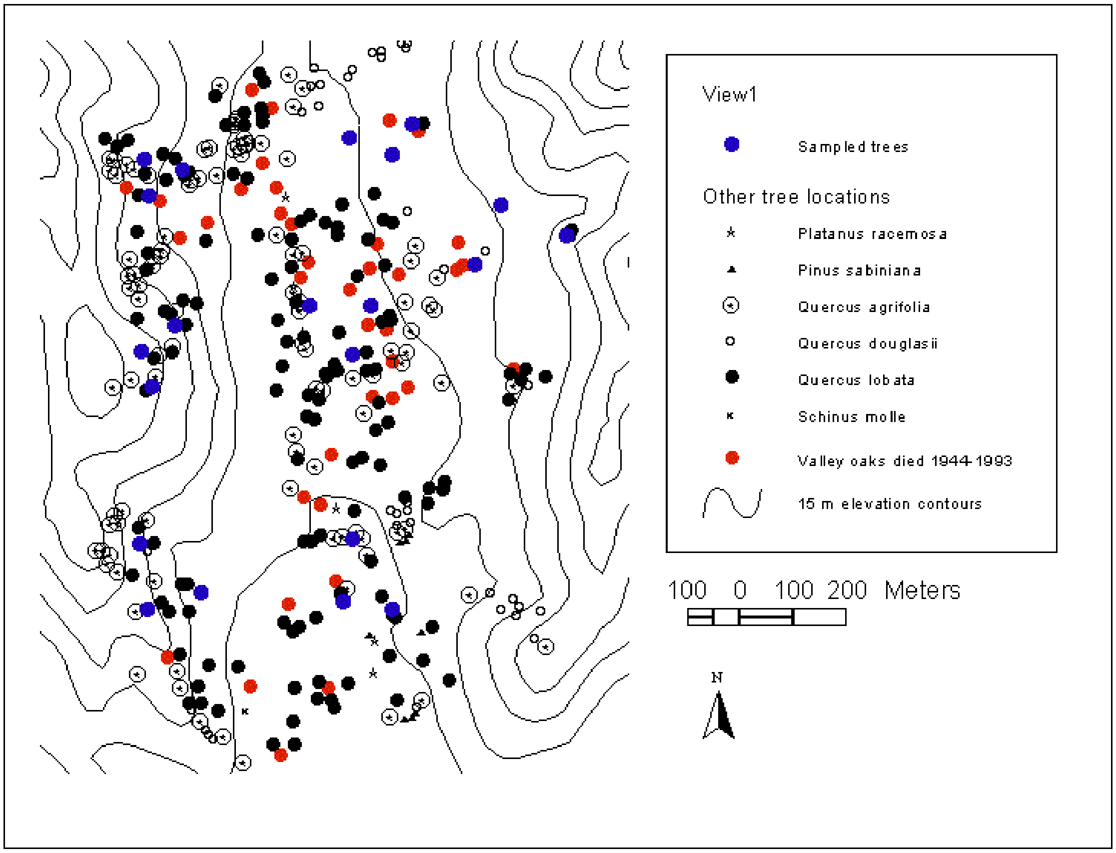

View1
Sampled trees
Other tree locations
Platanus racemosa
Pinus sabiniana
Quercus agrifolia
Quercus douglasii
Quercus lobata
Schinus molle
Valley oaks died 1944-1993
15 m elevation contours
100 0 100 200 Meters
N

Fig. 2.

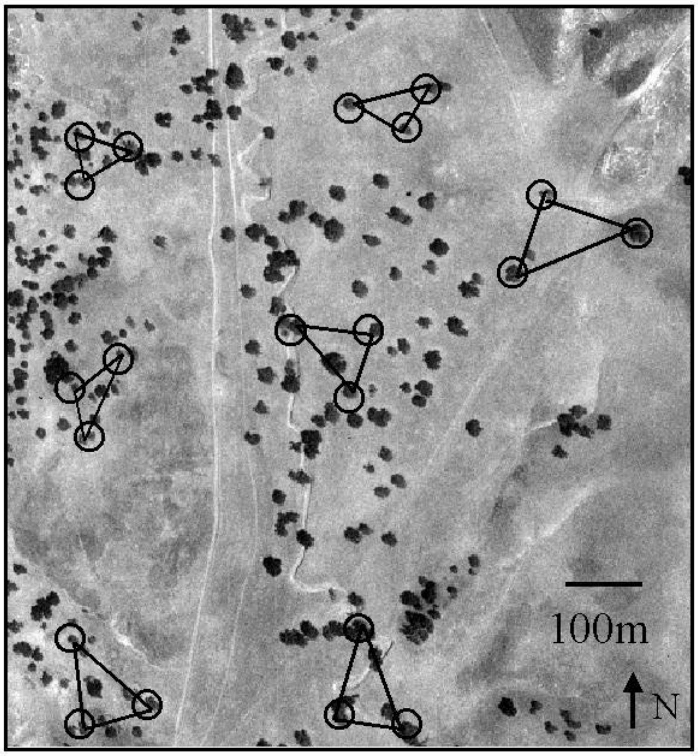


Fig. 3

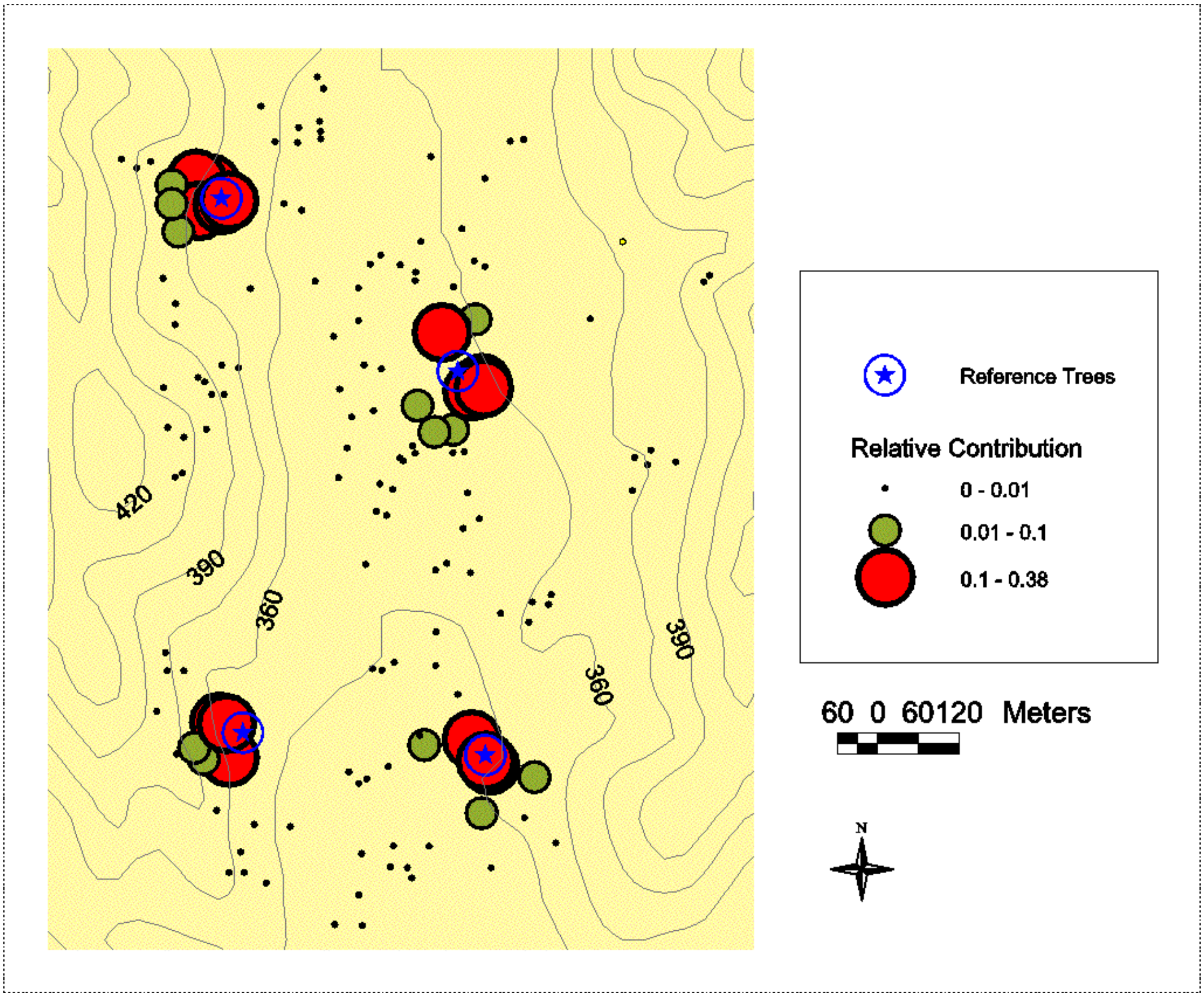

420
390
360
360
390
Reference Trees
Relative Contribution
0 - 0.01
0.01 - 0.1
0.1 - 0.38
60 0 60120 Meters
N

Fig. 4

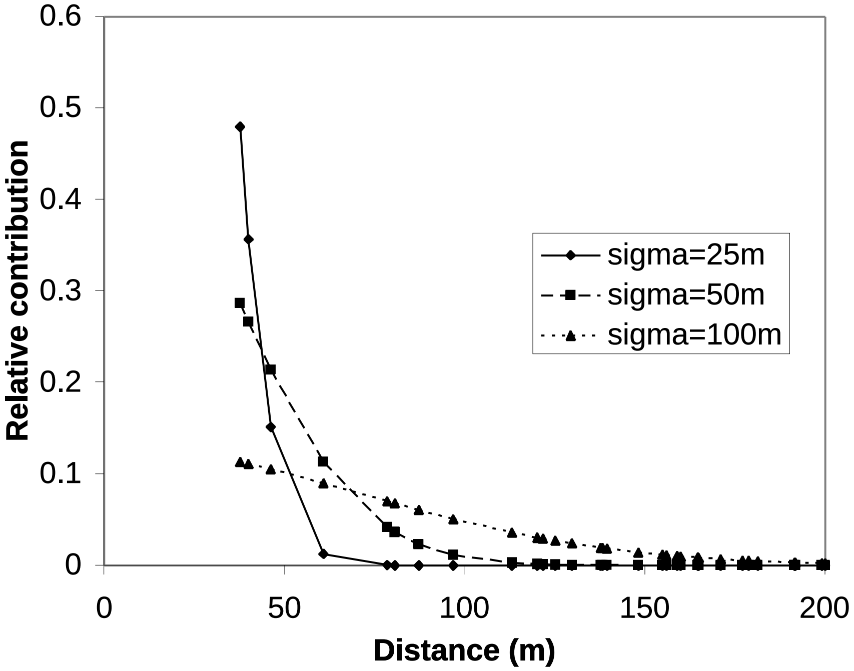
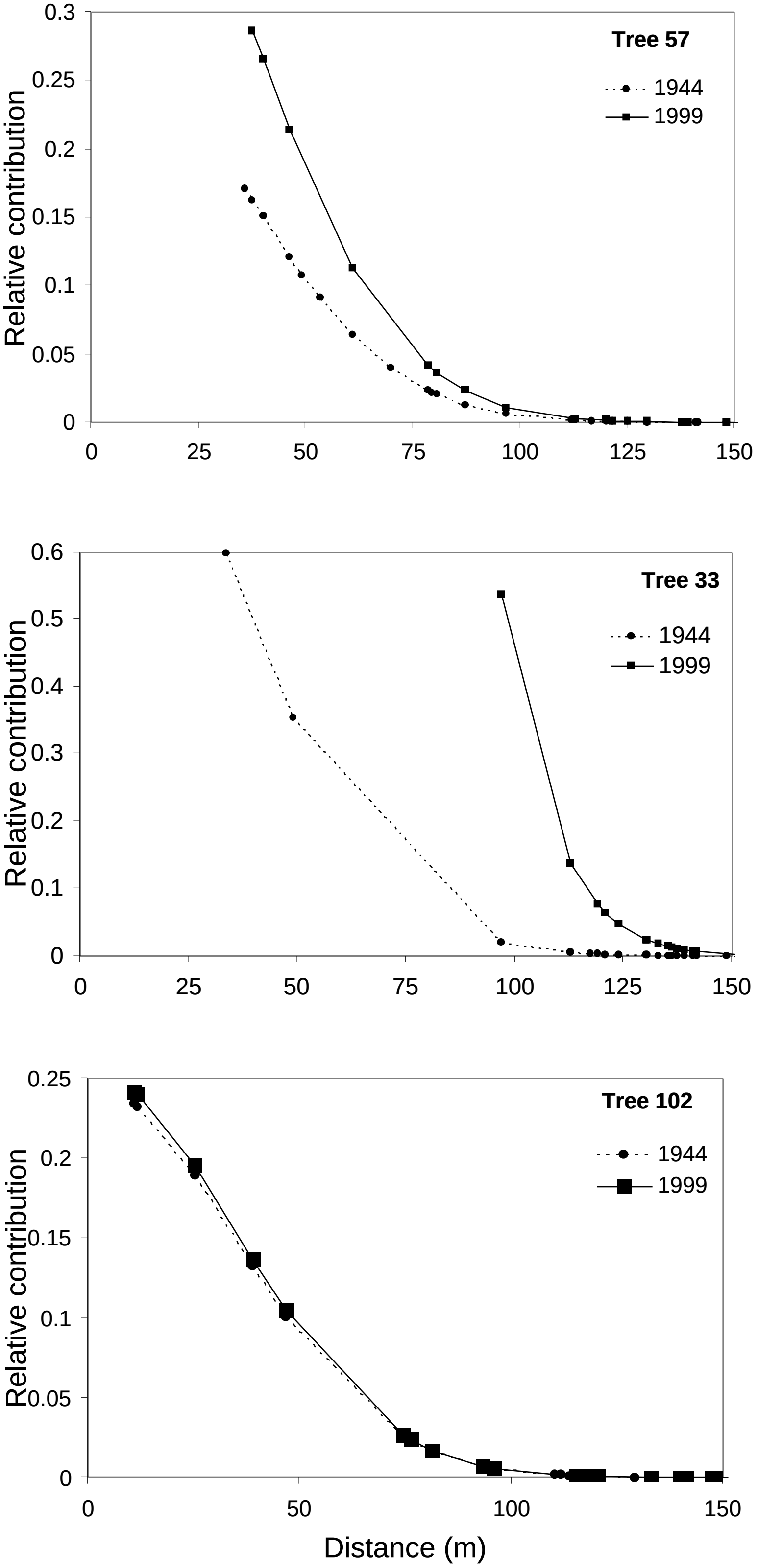

0.6
0.5
0.4
0.3
0.2
0.1
0
Relative contribution
0
50
100
150
200
Distance (m)
sigma=25m
sigma=50m
sigma=100m

Fig. 5


Tree 57
1944
1999
Relative contribution
0.3
0.25
0.2
0.15
0.1
0.05
0
0
25
50
75
100
125
150
Tree 33
1944
1999
Relative contribution
0.6
0.5
0.4
0.3
0.2
0.1
0
0
25
50
75
100
125
150
Tree 102
1944
1999
Relative contribution
0.25
0.2
0.15
0.1
0.05
0
0
50
100
150
Distance (m)