

## Factors influencing male mating success in bur oak, *Quercus macrocarpa*

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**Application.** Genotyping at DNA microsatellite loci identified pollen donors of a large sample of acorns and saplings in one stand of bur oak. The data were used to test if several characteristics of pollen donors were correlated with reproductive success. Male reproductive success could not be completely explained by any factor tested, including distance from maternal tree, direction of tree relative to maternal tree, size of tree, or genetic relatedness to maternal tree. Managers of seed orchards should consider that pollination contamination from distant sources may be extremely common and may not necessarily be confined to trees at the edge of the orchard. In general, simple models of wind pollination based on distance and direction should be avoided because they will not adequately predict gene flow patterns of male reproductive success.

**Abstract.** Pollen donors of acorns and saplings in a stand of bur oak were identified by paternity exclusion using microsatellite genotype analysis. Here we examine the influence of several factors likely to affect reproductive success of males with wind-dispersed pollen, including distance of pollen donor from maternal tree, genetic relatedness of pollen donor to maternal tree, direction of pollen donor relative to maternal tree, and size of pollen donor (crown volume and trunk diameter). Surprisingly, none of these factors were strongly correlated with fertilization success, although weak but significant correlations with crown volume and distance were found. The slight influence of distance is actually overestimated here, because our analysis necessarily excluded a large proportion of pollinations that were effected by trees outside the stand. The pollen donors outside the stand were not identified, but were more than 150 meters away. Pollination patterns in this stand of bur oak are quite complicated. Seed parents were fertilized by multiple pollen donors within the stand from all directions and as far as 200 m away. These results caution against the use of models of pollination that are based on simple distance or directional parameters because they will not adequately predict gene flow or male reproductive success. Our results further emphasize the importance of genetically identifying successful pollen donors rather than relying on patterns of physical movement of pollen.

### Introduction

Dispersal of genes through pollen and seeds is an extremely important factor in determining the genetic structure of plant populations. Effective dispersal

of pollen resulting in fertilization probably accounts for the largest component of gene flow in outcrossing wind-pollinated plant species (Ennos 1985), and most temperate trees are anemophilous. Therefore the factors influencing reproductive success of plants through male function will in large part determine the effective population size of stands of trees, the number and location of mates of individual trees, and the extent of genetic isolation among stands. Fertilization patterns will influence the genetic composition of open-pollinated families used in progeny testing, where it is often assumed that progeny within families are half-sibs (Squillace 1974, Gregorius 1975, Surles et al. 1990, Adams 1992). Knowledge of factors influencing male reproductive success in forest trees is important for successfully designing and managing seed orchards (Caron and Leblanc 1992), assessing the "genetic efficiency" of seed orchards, and predicting the success of supplemental mass pollination (Wheeler and Jech 1992). Information about patterns of pollen movement will also be useful for predicting the genetic impact of habitat fragmentation on plant populations (Foré et al. 1992, Hall et al. 1994; 1996, Chase et al. 1996b). Finally, design of effective management strategies for endangered plant species requires knowledge of demographics and mating systems, and pollination patterns determined by male reproduction are a key component of both. For simplicity in this paper, we will equate the term "pollination" with "fertilization" throughout, recognizing that the physical movement of pollen or patterns of pollen deposition may be quite distinct from patterns of fertilization.

Despite the fact that pollen dispersal and male mating success are key components of plant mating systems, there is still only limited information regarding these processes in natural plant populations. Most studies of wind-pollinated plants have relied upon indirect measures of fertilization, by following the physical movement of pollen by use of traps or dyes. Such studies have generally concluded that pollen has a leptokurtic distribution around the source, with the highest densities close to the source plant and concentrations of pollen dropping rapidly with distance (Bateman 1947, Griffiths 1950, Wang et al. 1960, Silen 1962, Handel 1976, Faegri and van der Pijl 1979, Whitehead 1983, Peart 1985, Okubo and Levin 1989). These conclusions predict that male reproductive success will be determined in large part by the distance between pollen donor and maternal tree. The underlying assumption here is that pollen-flow distributions are congruent with gene-flow distributions and pollen movement will accurately predict patterns of fertilization. For animal-pollinated plants, paternity studies using allozymes have shown that pollinator flight patterns significantly underestimate pollen gene flow (Schaal 1980, Campbell 1991, Fenster 1991, Adams 1992, Hamrick et al. 1995, Karron et al. 1995, Schnabel and Hamrick 1995). Less direct evidence

using paternity testing is available for anemophilous species, but indirect evidence from patterns of allozyme variation suggest that gene flow is generally higher than predicted by studies of the physical movement of wind-dispersed pollen (reviewed in Hamrick et al. 1995).

Methods that allow pollen donors to be identified by genotypic paternity testing provide a much more accurate assessment of pollen-mediated gene flow and male reproductive success in natural populations. Paternity testing will be most effective when all potential males except a single putative pollen donor can be excluded as fathers (Chakraborty et al. 1988, Adams 1992). This exclusion probability depends upon the number of polymorphic loci analyzed, the number of alleles at each locus, and the evenness of allele frequencies at these loci. Complete exclusion is difficult to achieve with allozyme markers because a sufficient number of highly polymorphic loci is generally not available, and some type of model of probability structure must be incorporated into the analysis such as “most likely” methods (Meagher 1986, Devlin and Ellstrand 1990) or “fractional paternity assignment” (Devlin et al. 1988). Most-likely methods have been shown to potentially underestimate gene flow (Broyles et al. 1994) and bias assignment of paternity to homozygotes (Adams 1992, Devlin et al. 1988). Both methods require a questionable assumption that the likelihood of mating success is equal for all males (Adams 1992).

Here we apply an alternative genetic method, microsatellite analysis, to identify paternal parents of acorns and saplings in a remnant savanna stand of bur oak (*Quercus macrocarpa* Michx.) in northern Illinois (Ashley and Dow 1994). Microsatellites are short DNA repeats (1–6 bp) that are highly variable for repeat number. The markers are codominant and inherited in a Mendelian fashion. Furthermore, microsatellite analysis can be applied to any individual in a natural population; paternity assignment is not limited to individuals carrying rare marker alleles or to a small subset of individuals that have high exclusion probabilities.

Microsatellite genotyping at four hypervariable loci allowed identification of the pollen donor for 120 acorns and 32 saplings in a stand of 62 adult bur oak (Dow and Ashley 1996; 1997). These individuals were the offspring of five seed parents located in different parts of the stand (Figure 1, Trees 2, 3, 17, 33 and 35). Approximately 10% of the identified within-stand pollinations may have matched by chance, and actually have been fertilized from outside the stand. All adult trees in the stand could be excluded as pollen donors for the remaining 162 acorns and 68 saplings sampled. Therefore, over half of the fertilizations observed in both acorn and saplings were effected by trees outside of the stand, at distances necessarily greater than 150 meters; there were no other bur oaks within 150 meters of our study site. This level of resolution represents a dramatic improvement over allozyme approaches for

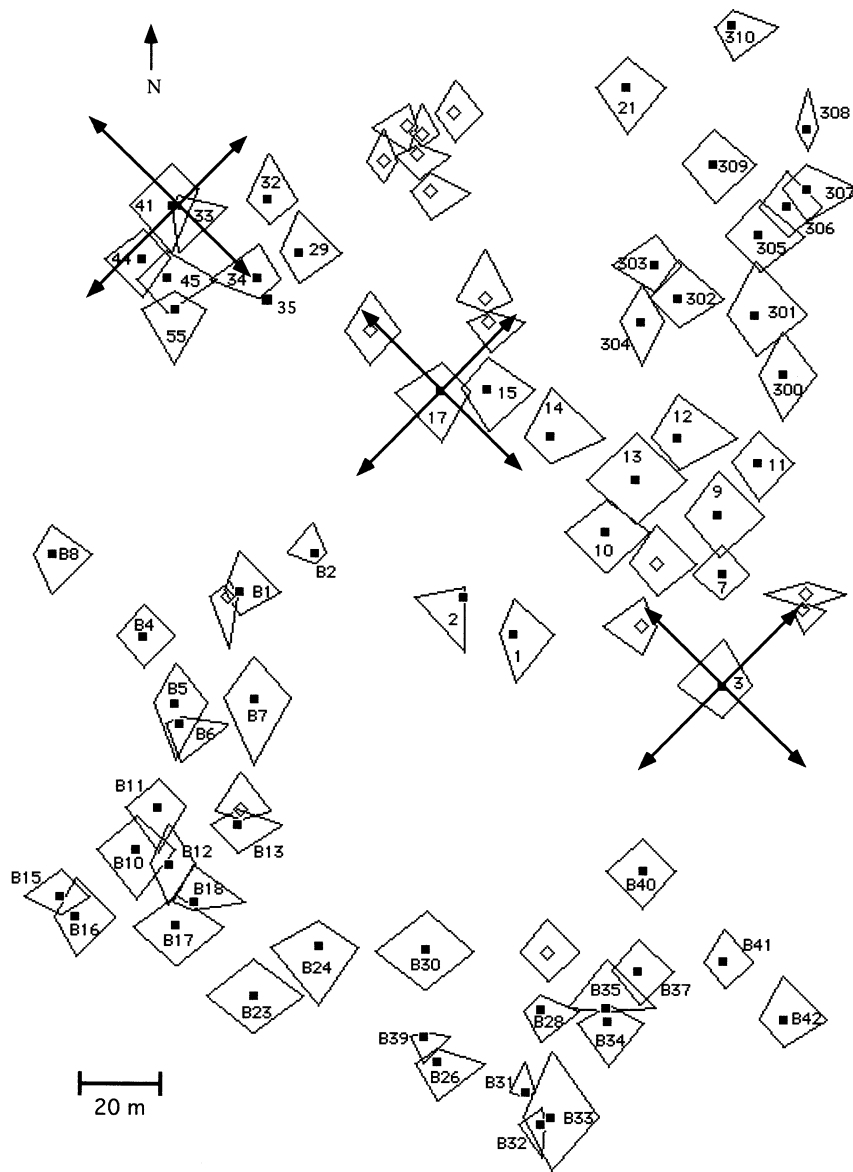


Figure 1. Map of all mature trees in the stand showing approximate quadrants for analysis of directional bias in pollination of Trees 3, 17 and 33. Black squares are mature bur oaks (*Quercus macrocarpa*), open diamonds are mature red oaks (*Q. rubra*). Letters and numbers are used to identify trees. Polygons reflect crown diameters in the north-south and east-west direction. Tree 35, which was the parent of many saplings included in this study, has been dead for several years. The crown could not be measured because there are no branches remaining on the stump.

paternity testing, where unambiguous assignment to a single pollen donor is generally confined to a small fraction of sampled individuals in natural populations (Meagher 1986, Schnabel and Hamrick 1995), to experimental systems (Tonsor 1985, Smyth and Hamrick 1987, Ellstrand et al. 1989, Galen 1992, Karron et al. 1995), to individuals carrying a rare marker allele (Yazdani et al. 1989, Eguiarte et al. 1993), or to plants that bear fruit with many seeds pollinated by a single paternal parent ("progeny arrays"; Broyles and Wyatt 1991, Godt and Hamrick 1993, Broyles et al. 1994). These limitations restrict the general utility of allozyme approaches for characterizing pollen gene flow and male reproductive patterns in forest trees, and outcrossing plants generally. The use of microsatellites for paternity assignment and pollen gene flow was also recently demonstrated in a smaller study of the tropical tree, *Pithecellobium elegans* (Chase et al. 1996a, b).

Other studies using genetic markers have suggested that gene flow in anemophilous trees is quite high. For example, more than 50% of the pollen in a loblolly pine (*Pinus taeda*) seed orchard isolated by 400 m was found to be contamination from an outside source (Friedman and Adams 1985). In a black spruce seed orchard contamination was as high as 83% in one year (Caron and Leblanc 1992). Nevertheless, ours is the first study of a natural population of trees which could unambiguously identify pollen donors within the stand. Once pollen donors were identified, we could track individual pollination events, document mating success of individual trees as males, and characterize the dynamics of pollen movement within the stand.

In the study reported here, data on male reproductive patterns for the 62 trees within the stand were used to examine several factors likely to influence the success of trees as pollen donors. These factors were (1) distance of pollen donor from maternal tree; (2) direction of pollen donor relative to maternal tree; (3) size of pollen donor (crown volume and trunk diameter); and (4) genetic relatedness of pollen donor to maternal tree.

Given the prevailing view that wind-dispersed pollen travels from the source tree in a leptokurtic distribution, distance between pollen donor and maternal tree might be considered the single most important determinant of fertilization probability. This model predicts that even if rare long-distance pollen movements occur, the majority of intermatings will occur among neighboring trees, resulting in a strong influence of distance. For wind-borne pollen, local or prevailing wind directions might also be predicted to influence patterns of pollination. We examined two questions regarding the role of direction in male reproductive success. First, was there any directional bias in pollination success, as might occur if there was a prevailing wind? Second, are flowers on one side of a tree more likely to be pollinated by trees on that side of the tree?

The success of a tree as a pollen donor may also be correlated with tree size. A larger crown has more branch tips, and might therefore be expected to produce more male flowers and more pollen. Two measures of tree size, crown volume and diameter at breast height (dbh) were compared to pollination success. The latter measure may be considered a test for a relationship between male mating success and relative age of the tree, since trunk diameter should be correlated with tree age.

Another possible explanation for differential male reproductive success is female choice, that is, some form of active mate selection by the maternal tree. Evidence for female choice in plants is accumulating (Willson 1979, Willson and Burley 1983, Stephenson and Bertin 1983, Waser et al. 1987, Marshall and Folsom 1991, Waser and Price 1991; 1993, Rigney et al. 1993) and the opportunity for mate choice to affect paternal fitness warrants consideration. Selection in oaks may occur by delayed fertilization, selective abortion or embryo competition (Willson and Burley 1983). We tested for mate choice based on nonrandom genetic relatedness for microsatellite genotypes of maternal tree and pollen donor. If the maternal tree can exercise choice over pollinations based on relatedness, we would expect that pollen donors would share fewer alleles (if selection is for outbreeding) or more alleles (if inbreeding were favored) with the maternal tree than would happen by chance. We tested this hypothesis using a Monte Carlo simulation.

## Materials and methods

### *Study system*

The study site consists of a fragment of presettlement oak savanna in McHenry County, Illinois. The stand is part of an abandoned farm that was released from agriculture approximately 20 years ago. The stand is approximately 200 m × 250 m and consists of 62 mature bur oaks, *Quercus macrocarpa* Michx., and 17 mature red oaks, *Quercus rubra* L. (Dow and Ashley 1996). The mean nearest neighbor distance of trees in the stand is 12.4 m ± 6.7 (S.D.). There are seven other small stands of mixed oaks to the north, west and south sides of the study site at distances ranging from 150 m to 750 m. A large corn field was located east of the study area. A detailed study of the flowering phenology was not done, but observations made over three years during leaf collection in the spring and acorn collection in the fall indicated variable reproductive effort among individuals, particularly in acorn production. It was common for most trees to have no observable acorn production some years (e.g., no acorns in two of three years) while a few trees produced large crops of acorns every year. All trees produced male flowers every year, and

although there seemed to be tree-to-tree variation in the number of catkins produced, differences were not quantified.

DNA was extracted from frozen leaves of 62 adults, 100 saplings and 288 seedlings (96 germinated acorns from each of 3 trees) using previously published protocols (Dow et al. 1995). A bur oak genomic library was previously screened for microsatellite repeats and PCR primers were designed from the regions flanking those repeats (Dow et al. 1995). The codominant inheritance and high variability (13–21 alleles per locus) of four microsatellite loci in most cases allowed identification of a single matching pollen donor or exclusion of all potential pollen donors in the stand (Dow and Ashley 1996; 1997). In a few instances, two potential pollen donors could not be distinguished because they shared relatively common alleles. Amplification of microsatellite loci was performed using four primer pairs (MSQ3, MSQ4, MSQ13 and MSQ16), and genotypes were scored by PCR product length at each locus as described previously (Dow et al. 1995, Dow and Ashley 1996). PCR products were radiolabelled by direct incorporation of <sup>35</sup>S-dATP and size fractionated by electrophoresis on a denaturing polyacrylamide gel. To determine potential parentage, all possible gamete combinations for all parents and offspring were entered into a database then matched using the “sort” function.

In the analysis of paternity of 96 acorns from each of three trees (Figure 1), referred to as “acorn study” below (Dow and Ashley 1997), six acorns did not yield usable data at one or more loci and were excluded from analysis. For 120 of 282 acorns, we could identify a pollen donor in the stand. Based on allele frequencies, it was calculated that approximately 12 of these acorns may have matched trees within the stand by chance rather than relatedness, and may have actually been pollinated by trees outside the stand (cryptic gene flow). For the remaining 162, all adult trees in the stand were excluded as pollen donors. Because it could not be determined which acorns resulted from cryptic gene flow events, data for all 120 acorns with an identified paternal parent are included in the analyses described below. Parentage of 100 saplings in the stand was also determined using microsatellite analysis (Dow and Ashley 1996), referred to as “sapling study” below. Of these, 32 had both a maternal tree and pollen donor from within the stand and are included. We found no evidence for self-fertilization or clonal reproduction in bur oaks at our site.

### *Distance*

A correlation analysis had previously revealed that pollination success for some trees was weakly related to distance from the maternal tree, but the analysis did not take into consideration that different numbers of potential pollen donors will occur at different distances (Dow and Ashley 1997). A more

detailed analysis was therefore undertaken that compares the distribution of pollen donors around maternal trees to the distribution of all adult trees. If no relationship exists between distance and pollination, i.e., the maternal tree is fertilized by trees in the stand irrespective of their distance, there should be no difference between the distribution of pollinations and the distribution of trees. The distance between each tree in the stand and a particular maternal tree was included in a frequency distribution with distance intervals of 10 m. A similar frequency distribution was made for pollination distances for that maternal tree. In addition to examining each of the three maternal trees in the acorn study individually, a composite frequency distribution for all three trees was made by summing the values in each distance interval.

The sapling study revealed that of the 32 saplings with both parents in the stand, 30 were offspring of four trees (Dow and Ashley 1996). The offspring were generally found in clusters of half-sibs around these four trees, a pattern consistent with limited seed dispersal around the maternal parent. Assuming these trees to be the seed parents of the saplings, frequency distributions of tree distances and pollinations distances were made as described above. Because there were relatively few pollinations per maternal tree in the sapling study, analysis was only performed on the combined data for the four maternal trees (Trees 2, 3, 17 and 35) in the sapling study. It should be noted that Tree 35 died several years before this study was undertaken, and its genotype was inferred from alleles shared by 25 saplings encircling its stump. Two of the three trees used in the acorn study were also maternal trees in the sapling study, so a final combined data set (acorns and saplings) included five maternal trees (2, 3, 17, 33 and 35). All between-tree distance and pollination distance distributions were compared using Kolmogorov-Smirnov tests (Ebdon 1985).

#### *Tree size*

Two measures of tree size, crown volume and diameter at breast height (dbh), were examined for their relationship to male mating success. To estimate crown volume, two axes are needed. The north-south and east-west crown diameters were averaged to produce one axis and the other axis was crown height. The crown volume was calculated using the formula for an oblate spheroid:

$$V = \pi a^2 b / 6 \quad (1)$$

where  $a$  is the smaller axis and  $b$  is the larger axis (Phillips and MacMahon 1981, Wright and Howe 1987). To test for association between crown volume and number of fertilizations, Spearman rank correlation coefficients were calculated for the total number of acorns fertilized by each tree in the stand

and their crown volume. Trunk diameter (dbh) was also analyzed using a Spearman rank test. Tree 35 was excluded from this part of the analysis because its crown size was not known. This exclusion probably did not affect the outcome of the study as Tree 35 could not have donated pollen to any of the acorns and matched only one sapling outside its cluster of half-sib offspring (Dow and Ashley 1996).

### *Direction*

The data from the acorn study were used to ask: (1) is there a directional bias in pollination success? and (2) are female flowers on one side of a maternal tree more likely to be pollinated by trees on that side? Obviously the sapling data could not be included because the relative position of the acorn on the maternal tree was unknown. Using a map of the stand, lines were drawn from each maternal tree at 45°, 135°, 225°, and 315° (North = 0° or 360°), dividing the stand into north, east, south and west quadrants (Figure 1). The numbers of possible pollen donors in each quadrant were counted for each maternal tree. The numbers of pollinations coming from each direction were also tabulated. The proportion of trees in each area was compared to the proportion of pollinations from each area using a Kolmogorov-Smirnov test (Ebdon 1985).

Data on the quadrant of the maternal tree where each acorn had been collected was used to address the second question. The purpose of this analysis was to assess movement of pollen within the crown of the maternal tree. Unlike the previous test, which compared proportions of adult trees to proportions of actual pollen donors in each quadrant, this analysis examined the spatial relationship of individual pollen donors to acorns they had fertilized case by case. For trees 3 and 17, acorns were collected from the north, south, east and west quadrants (Figure 1). Tree 33 had an unusual crown shape which extended only east and south from the trunk so acorns from this tree were classified into only two groups. The direction of each known pollen donor from the maternal tree was determined for each acorn. To determine if trees in one quadrant of the maternal tree were pollen donors for acorns in that quadrant more than other quadrant, the pollinations for each quadrant of the tree were classified as “same” if, for example, a tree to the east had pollinated an acorn in the east quadrant, or “different” if otherwise. Chi-square was used to test a null hypothesis of equal numbers of pollinations from the same and different quadrants of the tree (if expected number of “same” pollinations in all quadrants = expected number of “different” pollinations in all quadrants, then the expected number in each group = 1/2 total pollinations).

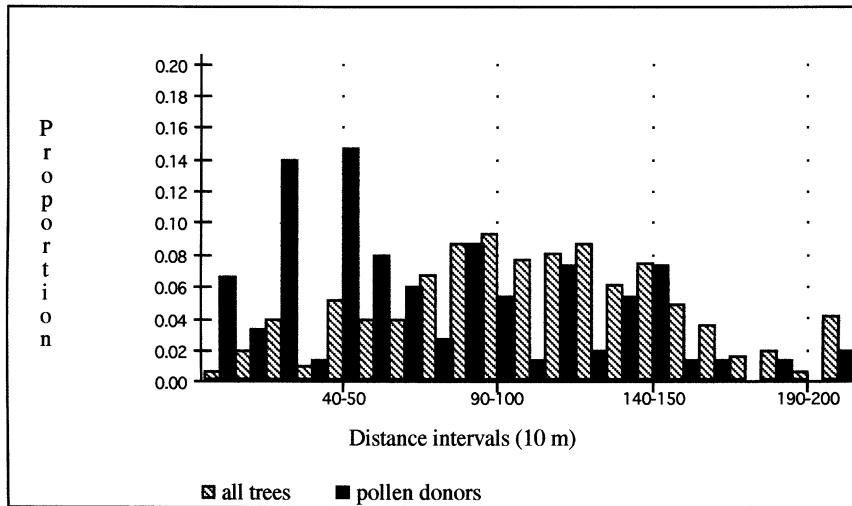


Figure 2. Comparison of the proportion of trees located at 10 m intervals from each seed parent and the proportion of pollinations from 10 m intervals from the seed parent. If there is no effect of distance, the proportion of trees in each interval would equal the proportion of pollinations from each interval. However, the two distributions are significantly different (Kolmogorov-Smirnov test,  $p < 0.01$ ), indicating the near trees effect a higher proportion of pollinations than far trees.

### Female Choice

Through female choice, maternal trees may have some mechanism whereby they selectively “choose” pollen from one source over another for fertilization. As discussed above, if this choice is based on outbreeding or inbreeding, pollen donors would share fewer or more alleles with the maternal tree than would happen by chance. To test this hypothesis, assuming the loci were independent, a Monte Carlo simulation was done using SAS (SAS Institute 1989) to generate random numbers between 0 and 1. For each maternal tree, the probability of sharing its alleles with a pollen donor was set at the frequency of each allele in the adult population. If the random number was less than or equal to the allele frequency, it was assigned a weight of 1 (a match), otherwise, it was 0. Each pollen donor within the stand could potentially share 0 to 8 alleles (2 at each of 4 loci) with the maternal tree. The number of shared alleles was then summed over all 8 alleles. The simulation was repeated 10,000 times, and expected numbers of matches calculated by multiplying the frequency of 0, 1, 2 ... n matches with the total number of within-stand pollinations for each tree. The results of the Monte Carlo simulations were compared to the actual numbers of matching alleles in each parent pair using a Chi-square test.

## Results

### *Distance*

A total of 48 of the 62 adult trees in the stand fertilized one or more of the 152 acorns and saplings that had an identified pollen donor in the stand (Dow and Ashley 1997). The distance between each maternal tree (acorns and saplings) and every other tree in the stand was tabulated by 10 m intervals, then converted to a proportion of all trees. Similarly, every pollination distance was tabulated by 10 m intervals and converted to a proportion of all pollinations. A histogram of the proportions of between-tree distances and pollination distances at 10 m intervals for the combined acorn and sapling data is shown in Figure 2. A Kolmogorov-Smirnov test showed a significant difference between the two distributions ( $p < 0.01$ ), with the proportion of pollinations (54% total) exceeding the proportion of trees (20% total) in all distance intervals to 70 m. The summed distributions for all acorn pollinations and between-tree distances were similar, and significantly different ( $p < 0.01$ ), and are not shown. These results indicate that trees closer to a maternal tree have a reproductive advantage in male function.

Nevertheless, the distance advantage was weak with numerous counter examples. In three cases (acorns from Tree 3, acorns of Tree 17 and the saplings, see Table 1), the proportion of trees in the 10–20 m category exceeded the proportion of pollinations. If near trees had a strong pollination advantage, the proportion of pollinations in the closest intervals would be greater than the proportion of trees located there. Trees 3 and 17 were not pollinated at all by their closest neighbor. The distance between pairs of parents of saplings (i.e., the distance of pollen movement) was always 20 m or greater, but 5 trees were closer than 20 m to the maternal tree. Finally, Tree 17 has a high proportion of pollinations from quite distant trees (26% in the 130–150 m interval) relative to the proportion of trees at this distance (16%).

### *Tree size*

Crown volumes ranged from 314.48 m<sup>3</sup> to 5438.43 m<sup>3</sup> with a mean of 2094.74 m<sup>3</sup> (S.D.= 1081.58). Because the number of acorns pollinated by any of 62 trees only ranged from 0 to 9, there were many tied ranks, and the correlation coefficient had to be corrected for ties. After this correction, Spearman rank correlation coefficient was weak but significant ( $r_s = 0.32$ ,  $p = 0.01$ ). The coefficient is positive, indicating that large trees fertilize more acorns. One possible explanation is that large trees fertilize more acorns simply because they produce more pollen, but this hypothesis cannot be addressed without more detailed knowledge of individual pollen production.

*Table 1.* Percent of trees (tree) and pollen donors (pollen) within the stand at 10 m distance intervals from each maternal tree (Trees 3, 17 and 33) of the acorn study and all maternal trees of saplings. N is the sample size in each group. Distributions of trees and pollen donors were significantly different in each case (Kolmogorov-Smirnov test); p values are shown at the bottom of the table

Distance (m)	Tree 3		Tree 17		Tree 33		saplings	
	tree	pollen	tree	pollen	tree	pollen	tree	pollen
0–10	0%	0%	0%	0%	2%	25%	0%	0%
10–20	2%	0%	2%	0%	3%	12%	2%	0%
20–30	0%	0%	2%	14%	8%	25%	3%	20%
30–40	2%	2%	0%	0%	0%	0%	1%	3%
40–50	5%	18%	13%	29%	0%	0%	7%	13%
50–60	5%	20%	8%	6%	0%	0%	5%	3%
60–70	6%	18%	5%	0%	0%	0%	5%	3%
70–80	5%	0%	13%	0%	2%	2%	8%	10%
80–90	10%	11%	8%	9%	6%	5%	9%	10%
90–100	6%	9%	10%	0%	2%	0%	11%	13%
100–110	11%	4%	5%	0%	3%	0%	9%	0%
110–120	10%	11%	3%	9%	11%	5%	7%	3%
120–130	6%	2%	8%	3%	8%	0%	9%	3%
130–140	14%	2%	2%	3%	6%	8%	6%	10%
140–150	2%	0%	14%	23%	13%	5%	6%	3%
150–160	11%	2%	2%	0%	6%	0%	4%	3%
160–170	5%	0%	6%	6%	5%	0%	3%	0%
170–180	0%	0%	0%	0%	3%	0%	1%	0%
180–190	0%	0%	0%	0%	3%	5%	2%	0%
190–200	0%	0%	0%	0%	2%	0%	0%	0%
200+	0%	0%	0%	0%	16%	8%	2%	0%
N	62	45	62	35	62	40	248	30
P	< 0.01		< 0.05		< 0.01		< 0.05	

Trunk diameter (dbh) ranged from 40.7 cm to 125.7 cm, with a mean of 88.7 cm (S.D. = 15.0). The Spearman correlation coefficient, corrected for ties, for dbh and total number of acorns fertilized per tree was not significant ( $r_s = 0.19$ ,  $p = 0.15$ ), indicating that trunk diameter is not an important factor in pollination success. Interestingly, the two measures of tree size were only weakly correlated to each other ( $r^2 = 0.34$ ). This lack of association may be due to variable tree density in the stand, leading to proportionately larger crowns on open-grown trees compared to trees growing in clusters. Also, many trees had obviously lost large branches to storms or decay, which would decrease crown volume without changing the trunk diameter.

*Table 2.* Percent of trees (tree) and pollinations (pollen) within the stand from different directions around three maternal trees (Trees 3, 17 and 33). N is the sample size in each group

Direction	Tree 3		Tree 17		Tree 33	
	tree	pollen	tree	pollen	tree	pollen
North	37%	49%	7%	11%	—	—
South	43%	31%	51%	51%	59%	60%
East	—	—	29%	29%	39%	18%
West	20%	20%	13%	9%	2%	22%
N	61	45	61	35	61	40

### *Direction*

Tree 3, on the east side of the stand, had no potential pollen donors to the east. The proportion of trees compared to pollinations from the north, south and west side of Tree 3 were not significantly different in the Kolmogorov-Smirnov test, indicating no directional bias in pollination success (Table 2). Similarly, no significant differences were found between the proportion of trees and pollinations on the east, north, south and west side of Tree 17 (Table 2). A significant difference was observed for Tree 33, which may be due in part to the higher density and spatial clustering of neighboring trees. The proportion of trees and pollinations on the south side were similar (0.59 and 0.60, respectively), but these proportions on the east and west side differed (Table 2). Tree 33 had only one tree (2%) to the west, and this tree was responsible for 9 pollinations (22%). The exceptional fertilization success of this tree may be due to the fact that its crown was in physical contact with the crown of Tree 33 (Figure 1). Except for this tree, there is no evidence that effective pollination has any overall directional bias as might be expected from a local or prevailing wind. Interestingly, even though most of the within-stand pollen donors of Tree 33 were from the dense cluster of trees around it, the proportion of pollinations from outside the stand was not significantly different from the other two trees with neighbors farther away (Dow and Ashley 1997). Densely spaced trees, therefore, can effect a higher proportion of fertilizations but do not appear to inhibit long-distance pollinations.

The Chi-square analysis of acorns from different sides of the tree did not reveal directional bias in the expected direction. It was expected that a tree on a given side of the maternal tree might have an advantage in fertilizing acorns on that side. As shown in Table 3, same side fertilizations were generally less numerous than different side fertilizations. The number of fertilizations from

Table 3. Number of acorns pollinated by trees on the same or different side of three maternal trees (Trees 3, 17 and 33)

Direction	Tree 3			Tree 17			Tree 33		
	same	different	total	same	different	total	same	different	total
North	7	4	11	4	9	13	—	—	—
South	5	6	11	1	5	6	13	8	21
East	—	—	—	3	4	7	2	17	19
West	1	14	15	2	7	9	—	—	—

the same and different sides were significantly different from equal for Tree 3 and Tree 33. In both cases, the total number of different-side fertilizations exceeded the total number of same-side fertilizations. Because the number of within-stand pollinations for Tree 17 was less than 10 on three of four sides, a Chi-square test was not valid using all four directions. When the Chi-square test was repeated using collapsed categories of east + south and north + west, the numbers of same- and different-side pollinations were not significantly different from equal. We conclude that pollen is well distributed throughout the crown of a tree, and not restricted to the side of the tree where the pollen donor is located.

#### *Female choice*

Mate choice might be based on the number of shared alleles, which increases with increasing relatedness. If a tree can recognize a related individual and reject its pollen on the stigma or selectively abort embryos fertilized by its pollen, complete outcrossing will be ensured. Such a system would result in fewer than expected matching alleles among pollen donors and maternal trees. When the observed number of shared alleles was compared to the random expectations generated by the Monte Carlo simulations for independently assorting alleles, however, the opposite trend was observed. For all three trees, there was an excess of shared alleles between the maternal tree and paternal trees, and for Tree 3 and Tree 33 these differences were significant ( $p < 0.05$ ,  $p < 0.001$ , respectively). Of course, each pollen grain contains a haplotype from an individual rather than a random assortment of alleles from the gene pool, thus it was important to assess if the population as a whole shared more alleles with the maternal trees than would be expected by chance. By repeating the test using all adult trees rather than just the pollen donors, highly significant ( $p < 0.005$ ) excesses of matching alleles were found for all three maternal trees. A likely explanation is that adult trees are not completely unrelated.

Since the parent population did not represent a random assortment of alleles, the Monte Carlo simulation was modified to better represent a situation in which gametes were drawn from the actual gene pool in the stand, rather than from all possible gamete combinations. The modified simulation used the observed frequencies of 0, 1, 2, 3 and 4 matches (no two trees in the stand shared more than 4 alleles) between all adult trees in the stand and each maternal tree. As in the first simulation, selection for unrelated individuals would result in fewer matching alleles between the pollen donor and the maternal tree than are present in the population as a whole. There was no significant difference between the expected number of matches from the simulation and that observed for Tree 3 or 17, suggesting that pollen donors did not differ genetically from a random sample of adults. There was a significant excess of matches ( $p < 0.05$ ) for Tree 33, suggesting nonrandom mating for this tree, which is consistent with previous findings, discussed above, that this tree had a high proportion of matings with neighboring trees (Dow and Ashley 1997).

## Discussion

We used genetically-determined paternity to examine several factors that may influence the reproductive success of male function in bur oak. Our study was limited to a single year of acorn production, and thus cannot assess year-to-year variations in pollination. However, the sapling study included young trees established over several years, and the pollen dispersal distances, spatial distributions of pollen donors and proportion of outside pollinations were highly consistent with the results of the acorn study. Nevertheless, additional years of data would be required to assess if the results we observed were typical.

Two of the factors we tested, distance from pollen donor to maternal tree and crown volume, were significantly associated with pollination success. However, the relationships were weak, and neither of these factors alone or in combination adequately explained fertilization patterns in the stand. Although near trees had a reproductive advantage, the number of effective pollinations did not decline precipitously over distance, as would be predicted from a simple model of pollen diffusion from a single source. Many adult trees in the stand were identified as pollen donors, and in some cases these trees successfully pollinated distant trees while failing to pollinate their nearest neighbor. For example, Tree 32 and Tree 15 each pollinated an acorn on Tree 3, but of the acorns sampled, Tree 32 did not pollinate Tree 33 and Tree 15 did not pollinate Tree 17 (Dow and Ashley 1997). Furthermore, the data presented here were necessarily limited to the pollinations that occurred

within the stand where the pollen donor could be identified. Within-stand pollinations represented a minority (43%) of total pollinations (Dow and Ashley 1996; 1997). Therefore, while near trees appear to have an advantage over distant trees within the stand (54% of pollinations coming from the 20% of within-stand trees 70 m away or closer) the effect is overestimated because the most distant pollinations coming from outside the stand could not be included in the analysis as these trees were not identified. Proximity to a maternal tree is not sufficient to explain the observed patterns of pollination success.

Crown volume was weakly correlated to pollination success ( $r_s = 0.32$ ), suggesting that trees with larger crowns may have more male flowers or may disperse their pollen more effectively. To our knowledge, the relationship between crown volume and pollen production has not been investigated in this species. The finding that crown volume but not dbh, our other measure of tree size, was correlated with male success suggests that size rather than age of tree is more important for pollination success. Schnabel and Hamrick (1995) studied pollination success in *Gleditsia triacanthos*, a dioecious, insect pollinated, leguminous tree using allozyme data and maximum-likelihood estimates of paternity. Their findings were similar to ours, with male fertilization success negatively correlated with distance and positively correlated with size, but together these factors only explained 16% of the variation in pollination success.

The complex pollination patterns found here could be explained several ways. First, the movement of bur oak pollen may be much more complicated than simple physical models predict. For example, clustering of pollen grains may create a platykurtic distribution (Tonsor 1985) or air currents may produce laminar flow, creating "rivers" of pollen flowing from one stand to the next. Nevertheless, pollen will become diluted in air as it travels from its source and we think it unlikely that pollination success can be explained solely by the complexities of pollen movement.

Secondly, the timing of pollen release could influence mating success. On each bur oak, male flowers mature before female flowers, and flowering times are staggered among individuals throughout the population. The pollen of one tree is released before its stigmas are receptive but while another tree is receptive. This system promotes outcrossing and minimizes selfing. The observed pollen donors in this study may have been the only trees releasing pollen when the maternal trees had receptive stigmas. We do not have sufficiently accurate field observations of flowering times to determine if timing was indeed a factor in the observed pollination patterns. However, we would still expect pollen produced within the stand to be present at higher densities than pollen produced elsewhere, as discussed above, so timing could

not completely account for the high proportion of pollinations from outside the stand.

A third possibility is some form of nonrandom mating such as mate choice. Marshall and Folsom (1991) define mate choice as the “differential reception and use of pollen by plants acting as seed parents”, and infer some mechanism under the control of the maternal plant to choose among available pollen. Several events during fertilization and embryonic development may be related to a selective process, which can occur at the fertilization stage, based on pollen genotype, or later in development, based on the fitness of the embryo (Willson and Burley 1983, Stephenson and Bertin 1983). A female may somehow influence which pollen grains germinate on the stigma, delay fertilization or selectively abort embryos. The female plant may be able to control which embryos are aborted with hormones or by resource allocation (Kress 1981). White oak acorns do not begin to develop until about a month after pollination and fertilization may be delayed for more than a year in some species of *Quercus* (Krugman et al. 1974), which may allow the female flower to acquire more mates and perhaps even compare the current year’s pollen to last year’s if fertilization is delayed that long (Willson and Burley 1983). Oaks typically have six ovules per flower, of which five are aborted, even though some of the ovules that fail to develop appear to be normal and fertilized (Mogensen 1975). There is, therefore, an opportunity for selection to occur, although it has not been conclusively proven that it does. We did not find any evidence that trees select mates based on the number of shared alleles at the four microsatellite loci examined. It may be that mate choice, if it occurs, is controlled by a distinct part of the genome, and that the markers used in this study were not linked to it.

Our results have important implications for forest geneticists. First, the common assumption in tree breeding experiments that individuals from open-pollinated families are half-sibs is largely supported by our data. We found no evidence for highly skewed pollination success among trees in the stand or frequent mating among nearest neighbors. Although each tree had groups of 2 to 9 full sibs, most acorns on the maternal trees in our study were half-sibs, and there was no evidence for self-fertilization (Dow and Ashley 1997). These findings are in contrast to studies of two leguminous tree species (Surles et al. 1990) and of white spruce (Schoen and Steward 1987) that found the assumption of half-sib relationships among progeny was violated. Second, our results suggest that pollen is well-mixed both within the stand and within the crowns of individual trees tested. If pollen dynamics in seed orchards of wind-pollinated trees are similar to those observed in this natural stand, seed crops will approximate a panmictic orchard parental mix (Wheeler and Jech 1992). However, we also confirm previous studies that indicate that long

distance pollen flow is very common and may actually exceed within-stand pollinations. Our study extends these previous findings of long distance gene flow to further suggest that pollen contamination may not be confined to the trees at the edge of the seed orchard but will affect trees in all parts of the orchard. Pollinations from nearby trees within the stand were higher for a tree that had densely-spaced neighbors, although pollinations from outside the stand were not affected (Dow and Ashley 1997). The effect of density on pollen movement warrants further investigation, particularly for stands which have uniformly high density. Finally, our findings indicate that methods that measure the physical deposition of pollen in and around seed orchards (Caron and Leblanc 1992) may not accurately predict pollination patterns within seed orchards or contamination from outside sources.

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