



*Quercus macrocarpa* group in northern Illinois

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# **Paternity and Pollination in Oaks: Answers Blowin' in the Wind**

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Pollination is an important component of the biology of oaks and other plants. The distance and direction of pollen movement determine the reproductive neighborhood for each plant, and the genetic diversity of each tree's acorn crop will be the result of the diversity of the pollen that reaches that tree. Pollination patterns will also determine impacts of forest fragmentation, because pollination shapes the reproductive connectivity of woodlands and isolated stands across the landscape. In oaks, which have wind-dispersed pollen, pollen is likely to be responsible for more gene flow than the dispersal of relatively large acorns. Despite its importance in many areas of plant science, pollination has been an extremely difficult phenomenon to investigate. Researchers have attempted to track the physical movement of wind-dispersed pollen using traps (Caron and Leblanc 1992; Greenwood 1986) and wind tunnels (Tonsor 1985), but there are substantial logistical problems with such studies. In the field, it is almost impossible to track pollen from source to destination, and pollen traps will intercept pollen from multiple, unknown sources. More importantly, even if the physical movement of pollen can be tracked, it is the actual pattern of fertilization that is usually of interest, rather than pollen movement itself.

Fortunately, oaks have served as a model system for the development of new approaches to the study of pollination. Beginning in the mid-1990s, a new class of genetic markers called DNA microsatellites has been brought to bear on the question of pollination (Ashley 2010). Oaks were among the first plants to be studied using this novel approach (Dow and Ashley 1996, 1998b; Streiff et al. 1999). Microsatellites are short, tandem repeats of one to six nucleotides that are found scattered around the noncoding regions of the genomes of plants and animals. Microsatellite loci are highly variable due to mutations involving the number of repeating units (Akkaya et al. 1992; Ashley and Dow 1994; Weber and May 1989). Microsatellites exhibit straightforward Mendelian inheritance, and this combined with their hypervariability provide a rich source of genetic information for distinguishing individuals genetically and also allows the inference of parentage (Ashley and Dow 1994).

The ability to infer parentage to seeds provides a way to track pollination patterns directly (reviewed in Ashley 2010). Specifically, paternity assignment allows a researcher to identify the sire of a seed, and therefore provides a way to retrace the exact distance and direction of a successful pollination event. At every microsatellite locus, an acorn will have two alleles, one inherited from the seed parent (the maternal allele), and one from the pollen donor (the paternal allele). Because the seed parent has been genotyped, the maternal allele at

each microsatellite locus is identified, and the genotypes of all the adult trees in the stand are examined to find one (and hopefully only one) that could have contributed all the paternal alleles. Alternatively, all the trees in the stand may be excluded as possible sires, and thus the researcher can conclude that pollen came from outside the stand, and represents a pollen immigration event. By sampling all the nearby oaks in a stand, and assigning paternity to a sample of acorns, within-stand pollination patterns can be characterized precisely, and the pollen immigration rate into the stand can also be measured.

This approach was first used to study pollination in a relatively isolated stand of bur oak, *Quercus macrocarpa* Michx., in northeastern Illinois (Dow and Ashley 1996, 1998b). The stand consisted of 67 mature trees, all of which were genotyped at several microsatellite loci, and 300 acorns from three seed trees were also genotyped. The results were surprising and unpredicted. Over half the acorns had no father in the stand, which indicated that the pollen donors were outside the stand. There were no other bur oaks with 150 m of the stand, so the average pollination distance was greater than 150 meters! For the within-stand pollinations, the pollen donors were distributed nearly randomly in the stand, with little or no pollination advantage for neighboring trees. Also, no directional bias to pollination was observed, so prevailing wind direction played at most a minor role in shaping pollination patterns (Dow and Ashley 1998a).

Why were these results so surprising? The classic view of wind-dispersed pollen suggested the pollen from a source tree formed a steep, leptokurtic distribution surrounding the source (Levin and Kerster 1974), with pollen dissipating quickly in the air column. As a result, most pollinations were thought to occur between neighboring individuals (Ehrlich and Raven 1969). The dispersal kernel, that is the frequency distribution of the dispersal distances, was thought to have a thin tail, so pollen immigration from outside the stand would only rarely occur. However, the work of Dow and Ashley (1996; 1998a; 1998b) on *Q. macrocarpa* showed that pollen immigration was extremely common, and within the stand, pollinations occurred almost irrespective of the distance between two trees.

Since these first studies on *Q. macrocarpa*, paternity assignment using microsatellites has been applied to oaks in at least ten different studies involving seven species of oaks (Table 1). With few exceptions, the results from the early studies on bur oak have been supported. Craft and Ashley (2010) extended Dow and Ashley's work on *Q. macrocarpa* in Illinois. They studied pollination at three additional sites, two extremely isolated remnant savanna stands, and one patch in a continuous forest. One of the isolated sites, Burnham Prairie, is in a prairie remnant surrounded by rail yards, industry, and residential areas on the southeast side of Chicago. The second isolated site, Goose Lake Prairie, is comprised of 26 trees isolated in the largest prairie remnant in Illinois, surrounded by a largely agricultural landscape. Although the landscape surrounding each site differs markedly, at both sites just over half of the acorns sampled had no father in the stand, indicating pollination distances of hundreds of meters. At a third site, Cranberry Slough, which is within the Cook County Forest Preserve District, pollination within a larger continuous forest was studied. Outside pollinations were slightly lower at this site, but they were still quite high at 47%. At all sites, correlated paternity was very low; in other words, most acorns collected from a

Table 1. Studies that use microsatellites and parentage assignment to study pollination in oaks

<b>Species</b>	<b>Pollen Immigration</b>	<b>Distance</b>	<b>Reference</b>
<i>Quercus macrocarpa</i>	47-58%	Mean 42 to 70m within stand, >>100 m from outside	Craft and Ashley, in press
<i>Q. macrocarpa</i>	71% from >150m	Mean 77m within stand	Dow and Ashley, 1996
<i>Q. macrocarpa</i>	57% from >150m	Mean 76m within stand	Dow and Ashley, 1998a
<i>Q. petraea</i>	69%	Means 22-58m within stand for individual trees	Streiff et al., 1999
<i>Q. petraea</i>	38%	Mean 92m within stand	Valbuena-Carabaña et al., 2005
<i>Q. pyrenaica</i>	34%	Mean 270m within stand	Valbuena-Carabaña et al., 2005
<i>Q. robur</i>	65%	Means 18-64m within stand for individual trees	Streiff et al., 1999
<i>Q. salicina</i>	52.2%	Mean 66.7m within stand	Nakanishi et al., 2004
<i>Q. salicina</i>	52.1%	Mean 69.2m within stand	Nakanishi et al., 2009
<i>Q. lobata</i>	~20%	Mean 114m within study site	Pluess et al., 2009
<i>Q. lobata</i>	70%	Mean 112m within study site, >200 m from outside	Abraham et al., submitted
<i>Q. semiserrata</i>	~30%	Mean 52 within stand, up to 570 within stand	Pakkad et al., 2008

maternal tree had a different father. Thus, the genetic diversity of acorn crops of trees is very high, even for trees in isolated stands.

Another early study examined pollination by paternity assignment in two European oaks. Streiff et al. (1999) studied a mixed stand of *Quercus petraea* (Matt.) Liebl. and *Q. robur* L. in northwest France. Acorns collected from seven of thirteen trees at their study site showed an excess of nearby matings. However, of the 984 acorns sampled, 69% of those from *Q. petraea* and 65% of those from *Q. robur* had no father in the stand, again demonstrating very high levels of pollen immigration over substantial distances. The stand was part of larger forest, suggesting that high levels of pollen immigration are a common feature for both isolated stands and in continuous woodlands. A more recent study looked at pollination and hybridization in a mixed stand of *Q. pyrenaica* Willd. and *Q. petraea* in Spain, near the southernmost limit of the latter species' range (Valbuena-Carabaña et al. 2005). Somewhat lower, but still substantial, levels of pollen immigration were found, 38% and 34% for *Q. petraea* and *Q. pyrenaica*, respectively. Within the stand, pollination distances of up to 381 m were documented.

Two parentage studies have recently been conducted on *Quercus lobata* Née, one of the largest oaks and an important component of many California oak woodlands (Abraham et al. 2010; Pluess et al. 2009). Pluess et al. (2009) studied *Q. lobata* at a site in Santa Barbara County, near the southern limit of the species range, where trees were widely dispersed in the landscape. These authors report that 81.5% of pollen donors were found within 250 m of maternal trees, with an average pollination distance of 114.1 m. Somewhat different results were found in a study of *Q. lobata* at Hastings Reservation in central coastal California, a site where oaks grow at higher densities (Abraham et al. 2010). At this site, only 30% of pollen donors were identified within 200 m of maternal trees. Together these results suggest that within a species, the densities and distributions of conspecific trees may greatly influence pollination distances and patterns.

Paternity studies on Asian oaks have also been conducted. Nakanishi et al. (2004; 2009) studied pollen dispersal in *Quercus salicina* Blume in the Tatera Forest Reserve, Japan. Average pollination distances within their study site were approximately 68 m, somewhat less than other studies of oaks. However, the estimated pollen immigration rate, 52%, was similar to that reported in European and American oaks. The pollen immigration rate reported for *Quercus semiserrata* Roxb. for a study site in Thailand was approximately 30%, with a mean pollination distances within the stand of 52m (Pakkad et al. 2008). These authors also documented an impressive pollination event of 570 m within their study site.

The relatively high rates of long distance pollination documented by paternity studies in oaks provide an explanation for the high inferred rates of gene flow that are typically found in population genetic studies of oaks. Pollen-mediated gene flow over large spatial scales likely prevents populations from becoming genetically differentiated, even for highly fragmented populations (Craft and Ashley 2007) or populations on the periphery of the species range (Marsico et al. 2009; Muir et al. 2004). Wind-pollinated oaks may be quite resilient to the negative genetic consequences of habitat fragmentation.

In conclusion, two nearby oaks may be more likely to mate than two more distant oaks, but microsatellite paternity studies have shown that distance alone explains very little about pollination. Pollination in oaks and other trees appears to be a complex phenomenon that may involve processes including flowering phenology, pollen properties, or perhaps even mate choice (Craft et al. 2009). Long distance pollination over hundreds of meters is common in oaks, and often over half the acorns have fathers from outside the stand. Wind pollination in oaks seems to be extraordinarily efficient at producing highly outbred individuals, yielding diverse acorn crops, and ensuring high rates of gene flow. The detailed but fascinating picture of pollination that is emerging could only be obtained using new tools of molecular genetics.

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