Plant Parentage, Pollination, and Dispersal: How DNA microsatellites have altered the landscape

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Abstract

DNA microsatellites provide plant ecologists with molecular markers precise enough to assign parentage to seeds and seedlings. This allows the exact distance and trajectory of successful pollen to be traced to characterize pollination patterns. Parentage assignment of established seedlings also allows researchers to accurately determine how far new recruits have traveled from their seed parent. This paper reviews the history and development of molecular parentage assignment in studies of native plants, as well as the limitations and constraints to this approach. This paper also reviews 53 articles published in the past 15 years that use parentage assignment to study pollination and seed dispersal in native plants. These parentage studies have overturned many common assumptions regarding pollen and seed dispersal patterns. They show that long-distance dispersal of pollen is common in both wind and animal dispersed systems, with average pollination distances commonly being hundreds of meters. The pollination neighborhood is often extremely large, and simple dispersal functions based on distance alone fail to make accurate predictions of pollination. Rather than hindering gene flow, fragmentation and isolation sometimes, and perhaps even commonly, results in increased pollination distances. Studies of seed dispersal using parentage assignment have also yielded some surprises. We now know that it may be erroneous to assume that seeds growing under the crown of a conspecific adult are growing beneath their mother, or that seed dispersal distances are more limited than pollen dispersal distances. Taken together, the studies to date demonstrate that both seed and pollen dispersal are quite complex phenomenon influenced by many ecological processes.

Key Words: parentage assignment, paternity assignment, microsatellites, pollen dispersal, seed dispersal
I. INTRODUCTION

Because plants are stationary as adults, dispersal of gametes and offspring nearly always occurs through the movement of pollen or seeds. Characterizing the distance and direction of pollen and seed movement is therefore of critical importance for many areas of plant science. Pollination patterns will dictate the reproductive neighborhood size for a plant, the connectivity of populations and the impacts of habitat fragmentation. Pollen movement will also determine the level of contamination of seed orchards and the risk of gene flow from genetically modified crops to wild species. Seed dispersal, mediated by gravity, wind, water, or animals, shapes not only gene flow patterns but recruitment and demography of plant populations. The seed dispersal kernel, that is the frequency distribution of the dispersal distances, influences many key demographic and ecological processes, including colonization, population persistence and plant community structure. Seed dispersal patterns will determine how rapidly invasive species may spread and how quickly plants may shift their ranges in response to climate change, both natural and human-induced.

Despite the importance of seed and pollen dispersal in plant ecological and community dynamics, the study of these processes has always been challenging. There have been many attempts to study pollination by tracking the physical movement of pollen using traps (Caron and Leblanc, 1992; Greenwood, 1986) or dyes (Linhart et al., 1987; Waser and Price, 1982). Other studies have followed the movements of pollinators (Levin and Kerster, 1974; Parra et al., 1993; Walther-Hellwig and Frankl, 2000). Problems with these indirect approaches are both logistical and substantive. Logistically, pollen traps will collect whatever pollen the trap intercepts and in nearly all cases will be from multiple individuals. Substantively, for most studies, it is the actual patterns of fertilization and gene flow rather than pollen movement and deposition that are of interest. Identifying sires through paternity assignment is the only direct way to retrace the path of a pollen grain that has resulted in successful fertilization of a seed, that is, a dispersal event that is genetically relevant.

Tracking the movement and fates of seeds has proven to be at least as daunting as tracking pollen movement (Forget and Wenny, 2005; Xiao et al., 2006). As with pollen, both the origin and destination of
seeds must be identified to track seed dispersal, and few techniques are efficient at both ends. Seed traps, for example, document only the endpoint of dispersal, not the origin. Many studies have tagged seeds to track their path from source to destination, and ecologists have been ingenious in devising tagging methods, attaching threads (Forget, 1992; Jansen et al., 2004; Wenny, 2000), wire tin tags (Li and Zhang, 2003, 2007), or magnets (Alverson and Diaz, 1989; Sork, 1984) to seeds, even spraying seeds with fluorescent microspheres that can be recovered from fecal samples of birds (Levey and Sargent, 2000). Seeds have also been labeled with radioactive (Jensen and Nielsen, 1986; Vander Wall and Joyner, 1998) and stable isotopes (Carlo et al., 2009). In such seed tagging studies, predation is often so intense that few if any viable seeds remain at seed stations. The number of dispersed seeds that survive is typically extremely low and the recovery of these few of perhaps thousands of marked seeds is often challenging (Jensen and Nielsen, 1986; Jorge and Howe, 2009; Li and Zhang, 2007; Sork, 1983, 1984; Wenny, 2000). Tagging itself can influence seed removal rates (Xiao et al., 2006). Aside from logistical difficulties, the quandary with seed tagging studies is the complexity of the seed dispersal cycle itself (Wang and Smith, 2002), with processes such as secondary dispersal, seed and seedling predation, and seedling competition jumbling the match between initial seed deposition and the resulting distribution of seedling recruits. Using genetic markers to establish the parentage of established seedlings allows the researcher to assess the end product of the seed dispersal cycle, and thus evaluate how the cumulative effects of these ecological processes shape plant demography and vegetation structure. Given this, advances in genetic marker technology that allow parentage assignment were needed before significant advances in pollen and seed dispersal biology could occur.

II. MICROSATELLITES AND PARENTAGE ASSIGNMENT

It has been about a quarter century since the discovery that short, tandem repeats were a common feature of eukaryotic genomes (Hamada et al., 1982; Tautz and Renz, 1984). These DNA repeats typically have a two to six base pairs sequence repeated a dozen or more times and were dubbed microsatellites, but are also known as simple sequence repeats (SSRs, Jacob et al., 1991) or short tandem repeats (STRs, Craig et al., 1988). While first characterized in mammalian genomes (Stallings et al.,
microsatellites were subsequently found to be ubiquitous and widely dispersed in plant genomes (Akkaya et al., 1992; Condit and Hubbell, 1991; Morgante and Olivieri, 1993). As sequence information from the non-coding regions of genomes accumulated, an important characteristic of microsatellites emerged; microsatellites are highly variable due to mutations involving the number of repeating units (Akkaya et al., 1992; Dow et al., 1995; Tautz, 1989; Weber and May, 1989). This hyper-variability, in conjunction with the codominant inheritance of microsatellite alleles, provides a means of distinguishing individuals, and hence a rich source of neutral genetic markers for mating systems studies, including inference of parentage (Ashley and Dow, 1994). For plant ecologists, this meant that parentage of seeds could be established by exclusion, allowing identification of pollen donors, and in some cases, seed parents. While allozymes have also been applied to parentage studies in plants (Ellstrand, 1984; Nason and Hamrick, 1997; Schuster and Mitton, 2000), they generally lack the levels of variability required for categorical assignment of individual parents for most of the seeds sampled in a study (Chakraborty et al., 1988).

Consider a typical microsatellite pollination study in a temperate tree. Most temperate trees are diploid, monoecious, and wind-pollinated, and occur in low diversity forests. A study site would be chosen where the density of the study species would low or moderate, or perhaps a forest fragment would be chosen that had a manageable number of reproductive adult trees (< 200). Adults, either all of those in a forest fragment, or within a circumscribed area in the forest, would be sampled (leaves or cambium tissue) and genotyped at a handful (5-10) of microsatellite loci. Seeds would then be collected from a set of ‘focal maternal trees’ and also genotyped at these loci. At every microsatellite locus, a seed would have two alleles, one inherited from the seed parent and one from the pollen donor. Because the seed parent has been genotyped, the maternal allele at each 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. Variability across these loci would likely provide sufficient exclusion probabilities (97-99%) to resolve paternity of seeds with a fair degree of confidence. In this situation, the exclusion probability is the probability of excluding an unrelated male as the father of an offspring, given that the mother is
known. If none of the candidate pollen donors provides a match, the researcher can conclude the pollen source lies somewhere outside the fragment or study site. By genotyping several hundred seeds, a detailed view of the distribution of pollination distances, directions, and paternal reproductive success emerges. This scenario matches quite well some of the earliest application of microsatellite parentage analysis, and involved studies of American and European oaks (Dow and Ashley, 1998a, b; Streiff et al., 1999). Modifications of this study design also was used in an early microsatellite study of insect-mediated pollination in a tropical tree (Chase et al., 1996), with the major difference being the geographical scale of sampling due to the low density of conspecific trees in the tropics.

Seed dispersal presents some additional challenges for parentage assignment studies. In the case of dispersed seeds or seedlings, neither parent is known. Among the sampled adults at a study site, a seed or seedling may have one parent in the stand (pollen or seed parent), two parents (both pollen and seed parent), or no parents (immigrant seed). Most plant species are cosexual, either producing hermaphroditic flowers with both male and female parts, or producing separate, monoecious male and female flowers. For cosexual plants, it is generally impossible to determine whether an assigned parent is the seed parent or the pollen parent when using nuclear microsatellites. This problem is circumvented in dioecious species, because the search will be for a compatible male/female pair (Bittencourt and Sebbenn, 2007; Hardesty et al., 2005; Slavov et al., 2009). Maternal and paternal origins could potentially be distinguished based on organelle DNA (mitochondria or chloroplast) that is uniparentally inherited (Lian et al., 2008; Ouborg et al., 1999), but generally mtDNA and cpDNA are not variable enough for individual identification. A recent and more productive approach has been the use of maternally inherited seed tissue, including endocarp (Garcia et al., 2007; Godoy and Jordano, 2001; Isagi et al., 2007; Jordano et al., 2007; Terakawa et al., 2009) pericarp (Grivet et al., 2005), and seed wing (Jones et al., 2005) tissue, or megagametophyte tissue of conifers (Iwaizumi et al., 2009; Iwaizumi et al., 2007) to identify seed parents. This approach is generally limited to intact seeds; maternal tissues are generally not present in established seedlings.

III. LIMITATIONS AND CONSTRAINTS
It is important to note that microsatellites are not a panacea for plant dispersal studies. There are several limitations of this approach, and while some of these limitations will be overcome with continued improvement in marker technology, others will remain for the foreseeable future. The four major limitations are 1) development of microsatellite markers; 2) accurate genotyping; 3) paternity assignment and 4) species biology.

The first limitation, marker development, has eased in recent years. This is usually the first step in any microsatellite study, and involves constructing and screening a genomic library, and from the library, developing and optimizing a useful suite of microsatellite primer pairs. In the 1990’s this first step could be a daunting obstacle, as ecologists and population biologists often struggled to master techniques of cloning, screening libraries, sequencing and genotyping. As improved protocols emerged, notably the use of enriched libraries where microsatellite-containing DNA fragments are selectively cloned (Glenn and Schable, 2005; Zane et al., 2002), the development step has been reduced from months to weeks. Researchers with sufficient funding can outsource this step; there are currently several companies that will produce microsatellites for a given species for a few thousand dollars. Also, microsatellites are increasing available for diverse species; recent issues of the journal *Molecular Ecology Resources* typically will report new primers for 20-30 species, including many plant species. Since microsatellite loci often are transferable to closely related species (those within the same genus and sometimes families), it is increasingly likely that microsatellite loci are already available for a plant species of interest. Transfer of published microsatellite loci to a new species, even if closely related, usually involves some optimization (adjusted PCR conditions), but by-passes the entire genomic library screening step.

Technical advances in genotyping (actual scoring allele sizes of individuals) have been even more dramatic over the past 20 years. The first plant microsatellite studies used nucleotides labeled with $^{35}$S or other radionucleotides (Dow and Ashley, 1996). This method involved pouring and manipulating large polyacrylamide gel, autoradiography, and handling and disposal of radioactive waste. Silver staining (Streiff *et al.*, 1998) was an alternative but no less laborious option. Scoring of allele sizes was done by
eye, by comparing band sizes to the known sizes of a labeled DNA ladder run on each gel. Fluorescent labeling techniques combined with high-volume capillary DNA sequencing machines has decreased time, improved throughput, and improved accuracy of genotyping, although the expense of genotyping is still relatively high, in some cases prohibitively so. New software, usually developed by the manufacturers of the DNA sequencing hardware, aids in ‘allele calling’ (sizing) and data management, although in our experience this step is not yet completely automated.

On first principles, parentage assignment is quite straightforward; in practice it can be quite thorny. A detailed review of methods of parentage analysis is beyond the scope of this paper, and I refer readers to several recent reviews on the subject (Blouin, 2003; Jones and Ardren, 2003; Jones et al., 2010). Some considerations are of particular relevance to pollination and seed dispersal studies. In pollination studies, if seeds are collected directly from a maternal tree, the maternal genotype can be evaluated and the paternal parent (with a matching paternal allele at each locus) can be identified among the pool of candidate plants. This approach, called strict exclusion, is often used in pollination studies. However genotyping errors, null alleles, and (rarely) mutations can lead to false exclusions (Slavov et al., 2005). Likelihood-based parentage techniques have become even more popular than exclusion, with the software CERVUS (Kalinowski et al., 2007; Marshall et al., 1998) the most widely used. These approaches generally accommodate some degree of mistyping. Programs like CERVUS calculate a logarithm of the likelihood ratio (LOD score) and assign parentage to the adult with the highest LOD score. Confidence of assignments is estimated by simulation. While popular, CERVUS can be problematic for plant parentage studies. For example, it requires the user to input the estimated proportion of candidate fathers, a value that is often unknown (and of primary interest) in studies of pollen and seed dispersal. Fractional paternity assignment methods are also available. These approaches split an offspring among all compatible mates (Devlin et al., 1988; Neff et al., 2001; Nielsen et al., 2001), and can be used when exclusion power is too low for categorical assignments. While fractional techniques have some statistical advantages over categorical assignment for estimating population parameters, I do not include them in this review because my focus is on precise tracking of pollen and seed dispersal events.
The larger the pool of candidate parents, the higher the likelihood of Type I and Type II errors in parentage assignment. Type I errors, also referred to as cryptic gene flow, occurs when a seed matches a candidate parent by chance, when the true parent is a plant that was not sampled (false-positive). The likelihood of Type I errors can be estimated, and rates of pollen immigration can be corrected (Dow and Ashley, 1996; Slavov et al., 2005). Type II errors are trickier to accommodate. They occur when a true parent is excluded due to mistyping at one or more loci, or even due to mutation. Microsatellite genotyping errors will occur, either in the laboratory processing, scoring, or data entry. Technical problems such as null alleles and allelic drop-out will occur in many microsatellites studies. Fortunately, simulation studies have shown that parentage assignment approaches such as CERVUS are quite robust to Type II errors (Oddou-Muratorio et al., 2003). While rates of genotyping errors can be estimated (for example, by the frequency of mismatches between a known seed parent and her seeds), larger numbers of candidate parents will require more microsatellite loci to be used to provide the needed resolution, which in turn increases the likelihood of ‘false mismatches’. Likelihood methods of parentage assignment are better than strict exclusion at accommodating Type II errors.

Paternity assignment is most straightforward on diploid species. An offspring (seed) inherits a single paternal allele from the father, and thus assignment or exclusion of a putative father is uncomplicated. Another advantage of diploid plants is that simplicity of scoring genotypes; each individual should have only one (if homozygote) or two (if heterozygote) alleles at each microsatellite locus. Extra ‘bands’ or ‘peaks’ are suspect and signal technical or scoring issues that can be addressed through further optimization. Because an estimated 30-50% of plant species are polyploid (Grant, 1971; Stebbins, 1971), limiting studies to diploids represents a serious drawback. Unfortunately, inheritance of microsatellites in polyploidy plants is far from straightforward. Some polyploid plant genomes are described as ‘highly diploidized’, because the polyploidy events are ancient, and chromosomes now form bivalents at meiosis. In such species, microsatellites might exhibit ‘diploid’ inheritance, as in the case of the octaploid strawberry, *Fragaria virginiana* (Ashley et al., 2003). In many polyploid species, scoring
an unknown number of alleles and inferring polysomic transmission patterns will greatly complicate parentage assignment (Hanson et al., 2007; Hanson et al., 2008).

Microsatellite parentage assignment studies are also limited by the abundance and density of candidate parents. One needs to sample and genotype most if not all of the candidate parents in an area relevant to pollen or seed dispersal distances, which will simply not be possible for plants that occur in densities of tens to hundreds per square meter or thousands per hectare. The costs and time involved in genotyping are prohibitive and even microsatellite markers do not provide enough resolution to distinguish among thousands of candidate parents. Therefore, most studies conducted to date have involved forest trees or other larger plants that occur at low densities (Table 1 and 2).

IV. OVERVIEW OF MICROSATELLITE PARENTAGE STUDIES

To evaluate what conclusions could be drawn from microsatellite parentage studies to date, I conducted a literature. I compiled studies that combine microsatellite genotyping and parentage analysis to directly track either pollen or seed dispersal events, or both (Tables 1 and 2) in native plants. I searched using Google scholar™ and ISI Web of Science™ to search for articles and tracked references within those articles. I only included studies that were able to assign parentage to a sizeable sample of genotyped offspring. I did not include studies that use microsatellite genotyping with indirect methods such as mating models, spatial genetic structure or pollen pool heterogeneity (Smouse et al., 2001) unless they also included parentage assignment. I also did not include studies involving cultivated trees or crops. This literature review is not exhaustive, but I am confident that it is broadly inclusive and representative. There are a total of 53 articles listed between both Table 1 and Table 2. The first papers were published in 1996, but only four papers total appeared in the 1990s. Fifteen papers were published in the period from 2000-2005 and 33 from 2006-2009, showing an acceleration in publication rate using these approaches. These studies employed an average of seven microsatellite loci (range 3-11); I did not include an early study (Dawson et al., 1997) that used a single locus because paternities could not be assigned. I found 41 papers that measured pollen dispersal by microsatellite paternity assignment in a total of 36 different species (Table 1). Of the 36 species studied, 17 were wind-pollinated trees. Among these, oaks (genus
Quercus) were well represented with ten papers on seven oak species. There were 15 studies on 15 different species of insect-pollinated trees. The remainder included a bat-pollinated tree, a bird-pollinated tree, a bird-pollinated shrub, an insect-pollinated shrub, and a columnar cactus pollinated by insects and birds. The list includes only two conifers, and studies of temperate trees outnumbered studies of tropical trees. Studies of seed dispersal were fewer; I identified 15 papers that examined seed dispersal in 13 species (Table 2). All were trees, including one conifer. They included species with a range of seed dispersal agents, including wind, autochory, and different frugivores. A few studies are found in both Table 1 and Table 2 because they characterized both pollen and seed dispersal as part of the same study.

V. POLLEN DISPERSAL IN WIND-POLLINATED SPECIES

As mentioned above, the first studies that used microsatellites and paternity assignment to study pollination were wind-dispersed temperate oaks. Results from these early studies were surprising, and provided the first indication that much of what we thought we knew about pollen dispersal, we didn’t know. The classic view of wind-dispersed pollen envisioned a steep, leptokurtic distribution (Levin and Kerster, 1974). Because pollen was thought to dissipate quickly in the air column, most pollinations were expected to be between neighboring individuals, with the implication that most plants would be completely pollinated with pollen from nearby sources (Ehrlich and Raven, 1969). The dispersal kernel was thought to have a thin tail, so pollen immigration rates would be relatively low. For this reason, the results of the first studies of wind pollination, conducted in bur oaks, Quercus macrocarpa (Dow and Ashley, 1996, 1998a, b; Streiff et al., 1999) came as quite a surprise. In a relatively isolated remnant stand of bur oaks (Quercus macrocarpa), over half the pollinations came from trees outside the stand, at distances of greater than 150 meters (Dow and Ashley, 1996, 1998b). Within the stand, pollinations occurred nearly at random, with no or only a slight advantage for nearest neighbors (Dow and Ashley, 1998b). Similar results were soon reported in the European species Q. robur and Q. petraea, with again over half the pollen immigrating from outside the stand, and mean pollination distances well above mean nearest neighbor distances (Streiff et al., 1999). These early results in oaks have generally been confirmed as representative of pollination patterns in many other tree species.
As mentioned above, parentage studies can only document dispersal within a circumscribed study site. Table 2 lists pollen immigration rates as a percentage of the sampled seeds, seedlings or juveniles that had all sampled adults excluded as the parent, or for dioecious species, that had all males excluded. Most of these studies sampled all adults within a fragment or isolated remnant, or within a circumscribed area. For wind pollinated species, pollen immigration is generally high, with 18 of 20 studies finding values of 20% or more, with an overall average of about 44% across studies. These levels of gene flow suggest that populations of wind-pollinated trees will be effectively panmictic over large spatial scales, and that even small, isolated fragments or remnants may not be reproductively isolated. There are two notable exceptions. A study of an extreme isolated stand of *Pinus sylvestris* in central Spain (Robledo-Arnuncio and Gil, 2005) and a study of *Fagus crenata* in northern Japan (Hanaoka et al., 2007) found 4.3% and 8% immigrant pollen, respectively. The *Pinus sylvestris* population was tens of kilometers from the nearest populations, which makes any level of pollen immigration impressive. Hanaoka et al. (2007) suggest that the large size of *Fagus crenata* pollen may be hinder long-distance dispersal; pollen size was not mentioned as a factor in the other studies. Interestingly, there has been little or no evidence for directionality in pollen dispersed by wind; prevailing wind direction does not seem to influence mating patterns in these species (Dow and Ashley, 1998a, b; Pluess et al., 2009). This counterintuitive result indicates that plausible assumptions should not be taken at face value, and need to be empirically tested.

What is the record for the longest pollination distance documented in a wind-pollinated plant? The answer depends on whether one requires that the true father be identified, or simply inferred. If identification is required, several studies have recorded mating events between trees separated by 600-700m (Hanaoka et al., 2007; Pakkad et al., 2008; Robledo-Arnuncio and Gil, 2005). Indeed, in most studies, the maximum within stand pollination distance simply reflect the maximum potential distances between trees within the study site. Black cottonwood, *Populus trichocarpa*, would hold this particular record (Slavov et al., 2009). The Vinson study site studied in western Oregon covered an area of > 300 km², made possible because of the low density of trees in an arid landscape. The mean pollination
distance at Vinson was 7.6 km, with many recorded matings over 10 km. If, on the other hand, the father need not be identified, the Vinson population of *Populus trichocarpa* is even more remarkable, because approximately one-third of the pollinations at this site were from immigrant pollen traveling more than 16 km. By this relaxed criteria, however, the record may go to the extremely isolated remnant stand of *Pinus sylvestris* in central Spain mentioned above (Robledo-Arnuncio and Gil, 2005). Although this population only received 4.3% immigrant pollen, that pollen necessarily traveled at least 30 km from the nearest conspecific population.

In the case of pollen immigration from outside the study site, only the minimum distances for immigrant pollen can be inferred. Actual distances can only be measured for pollinations that occurred within the study site. Because such a large fraction of the pollinations are from unknown distances, microsatellite paternity studies have generally not been all that useful for describing the tail of the pollen dispersal kernel, except for the obvious implication that the dispersal kernel is ‘fat-tailed.’ The *Populus trichocarpa* study (Slavov et al., 2009) is an interesting exception, because sampling at the Vinson study site covered such a large geographic area that the long-distance component of the dispersal curve could be characterized. Results indicated that exponential, exponential power, and Weibull functions (commonly used to model pollen dispersal) are poor predictors of observed pollinations, especially the long-distance component. Instead, a model with a two-component process, with local dispersal described by an exponential distribution and long-distance dispersal described by a uniform distribution, provided a much better fit to the paternity data. A biological interpretation of this may be that there exists a portion of wind-dispersed pollen that follows traditional predictions of exponential declines from the source and accounts for local pollinations, but a second, often substantial component may be caught in updrafts and remain airborne for some time, producing a regional pollen cloud that effects pollinations over large distances. Paternity studies have clearly shown that the ‘tail’ of the pollen dispersal curve is a critical component that is difficult to measure and challenging to model. Additional studies over large spatial scales are needed to determine if pollen dispersal should indeed be modeled with mixed dispersal
functions; simple one- and two-parameter functions are likely too simplistic for what now appears to be a rather complex process.

VI. POLLEN DISPERSAL IN ANIMAL-POLLINATED SPECIES

Like the first studies in wind-pollinated trees, the first for an insect pollinated tree revealed surprisingly high pollination distances. This was work on *Pithecellobium elegans*, a large neotropical canopy tree pollinated by strong-flying hawkmoths. At their study site in Costa Rica, Chase et al. (1996) found that the average pollination distance was 142 m, whereas the average distance of nearest neighbors was only 27 m, and “a typical leptokurtic distribution of pollination events was not evident in this population.”

Using microsatellites and parentage assignment, several studies in tropical trees have tested and rejected Janzen’s ‘living dead’ hypothesis (Janzen, 1986) which posits that remnant trees left standing in pastures have no reproductive potential and are of little conservation value. An important study by Dick (2001) looked at both seed production and pollen donor diversity in a prominent Amazonian tree, *Dinizia excelsa*, in remnant forest, forest fragments, and isolated pasture trees. Contrary to the ‘living dead’ hypothesis, trees in pasture and forest fragments produced more than three times as many seeds as trees in continuous forest. Even isolated pasture trees received pollen from multiple pollen donors at distances of hundreds of meters, thanks to exotic African honeybees that were more common pollinators than native bees in disturbed habitats. A similar finding was reported for *Swietenia humilis* in disturbed habitats in Honduras (White et al., 2002). Reduction in fragment size resulted in increased proportions of long-distance pollinations, with one isolated tree having most of its pollen delivered from > 4.5 km. Pollen dispersal in *Dipteryx panamensis* in fragmented Costa Rican forests also followed this pattern, with increased dispersal distances in fragments and pastures compared to continuous forest (Hanson et al., 2008). The conclusion from these studies is that physically isolated trees are usually not reproductively isolated. This conclusion holds in quite different landscapes and for species with a variety of pollinator syndromes. In the vast fragmented agricultural landscapes of southern Australia, Byrne et al. (2007) report that the bird-pollinated shrub, *Calothamnus quadrifidus*, received up to 43% of its pollen from
other populations up to 5 km away. Extremely isolated stands may sometimes experience low levels of pollen immigration, as shown for *Prunus mahaleb* (Garcia *et al.*, 2005; but see Herrera, 2009; Hoebee *et al.*, 2007) and *Sorbus torminalis* (Hoebee *et al.*, 2007). However, these cases seem to be an exception, with the general pattern being that physically isolated trees are not reproductively isolated from conspecifics.

There have been a number of striking examples of long distance pollen movement for insect pollinated tropical trees, including species with pollinators not expected to be strong fliers. Hardesty *et al.* (2006) studied the dioecious *Simarouba amara* on Barro Colorado Island, Panama, a species pollinated by small generalists insects. They found an average pollination distance of 334 m, more than six fold greater than the mean distances between nearest male-female pairs (54 m). Notable mean pollination distances of hundreds of meters have been reported for both temperate (Hoebee *et al.*, 2007; Isagi *et al.*, 2000; Isagi *et al.*, 2007) and tropical insect pollinated trees (Chase *et al.*, 1996; Hanson *et al.*, 2008; Hardesty *et al.*, 2006). *Dinizia excelsa* briefly held the record for the most distant documented insect pollination event, 3.2 km (Dick, 2001), until 4.5 km was reported for *Swietenia humilis* study the following year (White *et al.*, 2002). All records were shattered, however, in a remarkable recent study of the African fig tree *Ficus sycomorus* that was sampled along 253 km of the lower Ugab River in Namibia (Ahmed *et al.*, 2009). Paternity assignment of 40 seeds revealed a mean pollination distance of 88 km, with a new record of 160 km. Remarkably, *Ficus sycomorus* is pollinated by a small host-specific fig wasp, *Ceratosolen arabicus*, that lives only 48 hours and must be carried by wind. Unlike the findings for wind-pollinated trees, *Ficus sycomorus* shows a marked east-to-west directionality of pollination flow matching the predominantly easterly winds.

Taken together, the results of paternity studies of pollination offer solutions to at least two biological paradoxes. The first, dubbed “Slatkin’s paradox”, is the contrast between direct and indirect estimates of gene flow, where direct estimates suggest very low levels of migration and gene flow but indirect measures such as Wright’s $F_{ST}$ indicate high levels of gene flow and little population subdivision (Ahmed *et al.*, 2009; Slatkin, 1987). The studies reviewed here suggest that solution to the paradox lies in
underestimated dispersal by “direct methods,” which until parentage assignment, including what really amounts to indirect approaches, those that track pollen or pollinator movements by behavioral observations or the use of pollen dyes of pollen traps. These approaches fail to adequately characterize the extent of long distance dispersal. The second is the paradox of forest fragmentation genetics (Kramer et al., 2008). This paradox involves the contradiction between population genetic theory, which predicts genetic declines due to drift and inbreeding in forest fragments, and the lack of empirical evidence supporting these predictions. Again, the paradox is solved by the frequency of long distance pollination revealed by parentage studies. Gene flow occurs across large spatial scales and reproductive neighborhoods will generally extend beyond the boundaries of fragments. Furthermore, a large number of the studies cited in Table 1 involve trees in fragmented and disturbed habitats, and in most cases the results indicate that pollen-mediated gene flow is enhanced, not inhibited, by fragmentation.

**VII. PARENTAGE ASSIGNMENT AND SEED DISPERAL**

There are fewer microsatellite parentage studies of seed dispersal than pollination, largely because of the challenges outlined above for identifying seed parents, and they vary more broadly in their design and the type of data they have produced. Therefore, generalizations from the studies listed in Table 2 are a bit murkier than those of pollination studies, but similarly suggest that seed dispersal is a complicated and multi-faceted process. The first study using microsatellites to study seed dispersal involved genotypes of established saplings of *Quercus macrocarpa* (Dow and Ashley, 1996). Seed dispersal into the study stand was 6%, far lower than pollen-mediated gene flow. Within the stand, approximately half of the saplings had been dispersed beyond the crown of the maternal tree, the rest were growing under or near their seed parent. Several subsequent studies listed in Table 2 have also found fairly low levels of seed immigration (Bittencourt and Sebbenn, 2007; Jones et al., 2005; Sato et al., 2005). In marked contrast, a study of *Fraxinus excelsior* in Scotland reported very high seed mediated gene flow (about 50% seed immigration), levels that exceeded gene flow by pollen, for these wind-dispersed seeds (Bacles et al., 2006). Impressive seed dispersal distances (hundreds of meters) are
reported in most studies, usually spanning the length of the study site, but means were generally fairly low (tens of meters, Table 2).

A study of *Simarouba amara* on Barro Colorado Island, Panama, has important implications for tropical ecologists (Hardesty *et al.*, 2006). These authors found that germinated seedlings were seldom the offspring of the nearest or nearby reproductive adults. They also showed that modeling approaches based on seed arrival data into seed traps underestimated seedling establishment distances by more than 10-fold. In their study, seed dispersal distances were similar to pollen dispersal distances. If these results are typical, they suggest that ecological studies of seed establishment in the absence of genetic parentage studies may result in quite inaccurate estimates of fecundity, dispersal, and seedling competition.

One interesting study compared both pollen and seed movement across two life stages, seeds and saplings, in a deciduous Japanese tree *Aesculus turbinata* (Isagi *et al.*, 2007). Selection was observed against seedlings that had resulted from self-pollinated and crosses between related trees (that were physically closer than unrelated trees). As a result, the effective pollen movement increased from about 180 to 290 meters between these two stages. This finding suggests that reproductive performance, and effective pollen and seed dispersal, should be assessed at later growth stages. Another innovative study examined seed dispersal in *Prunus mahaleb* by different classes of animals. This was done by collecting seeds from feces, regurgitation pellets, and seed traps in different microhabitats (Jordano *et al.*, 2007). The *P. mahaleb* seed dispersal kernel is comprised of at least two components: short-distance dispersal by a diverse array of small frugivores, and long-distance dispersal by medium-sized birds and carnivorous mammals. Thus the multimodal nature of dispersal kernels may hold for both pollen and seed.

**VIII. CONCLUSIONS**

The major surprise coming from of microsatellite paternity studies that track pollination events is *not* that distance is associated with siring success; it usually is. As we have always assumed, plants that are closer to each other are more likely to be mating partners that plants than are more distant (although at local scales even this generalization doesn’t always hold, Chase *et al.*, 1996; Dow and Ashley, 1996, 1998b). The major surprise is that distance explains only a portion of the variation in mating patterns,
often a relatively modest portion, and this holds for both wind and animal pollinated plants. The traditional view of pollination biology, and the assumption of all plant dispersal models, is that distance is paramount; it is usually the only parameter considered. The findings reviewed here, however, suggest that models of seed and pollen dispersal based simply on distance will provide poor predictions of plant gene flow and dispersal patterns for many plants. While parentage studies have certainly made the dispersal scene more complex, the emerging paradigm of dispersal is much richer and multifaceted. There are intriguing and underappreciated ecological processes influencing dispersal patterns that we can now begin to explore. We should begin to look more carefully at processes such as flowering phenology, wind column dynamics, pollen properties, pollinator behaviors, and even mate choice to help explain mating patterns in plants. Perhaps pollen has a maturation process, providing a fertility advantage for pollen that has traveled greater distances. The distribution and density of plants across the landscape certainly needs to be incorporated into future models of pollen dispersal because several studies reviewed here suggest that the pollination landscape changes along with plant distributions. The ecology of seed dispersal, including behaviors of multiple dispersers, secondary dispersal, and density dependent effects has been considered and investigated previously, but more parentage studies of seedlings will improve our understanding of how ecological processes contribute to resulting recruitment patterns. More studies in the future should combine molecular parentage assignment with the study of ecological processes in the field.

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*Heredity* 84: 348-361.


Table 1. Studies that use microsatellites and parentage assignment to track effective pollen movement.

<table>
<thead>
<tr>
<th>Species</th>
<th>Distribution//Study site</th>
<th>Breeding System</th>
<th>Pollen Immigration</th>
<th>Distance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fraxinus excelsior</em></td>
<td>Temperate/deforested landscape in Scotland</td>
<td>mixed</td>
<td>43-68%</td>
<td>80% &lt; 100m, up to 2.9 km</td>
<td>Bacles and Ennos, 2008</td>
</tr>
<tr>
<td><em>Juglans mandshurica</em></td>
<td>Temperate /northern China</td>
<td>monoecious, heterodichogamous, self-compatible</td>
<td>28.5%</td>
<td>Median 11-14.9m, up to 140m within stand</td>
<td>Bai et al., 2007</td>
</tr>
<tr>
<td><em>Araucaria angustifolia</em></td>
<td>Tropical conifer/Brazilian forest fragment</td>
<td>dioecious</td>
<td>10-47%</td>
<td>Mean 83m within fragment, up to 2km from outside</td>
<td>Bittencourt and Sebbenn, 2007</td>
</tr>
<tr>
<td><em>Araucaria angustifolia</em></td>
<td>Tropical conifer/Brazilian continuous forest</td>
<td>dioecious</td>
<td>54-91%</td>
<td>Mean 102m within transect, up to 350m within transect</td>
<td>Bittencourt and Sebbenn, 2008</td>
</tr>
<tr>
<td><em>Quercus macrocarpa</em></td>
<td>Temperate/Midwestern US fragments</td>
<td>monoecious</td>
<td>47-58%</td>
<td>Mean 42 to 70m within stand, &gt;&gt;100 m from outside</td>
<td>Craft and Ashley, submitted</td>
</tr>
<tr>
<td><em>Quercus macrocarpa</em></td>
<td>Temperate/Midwestern US fragments</td>
<td>monoecious</td>
<td>71% from &gt;150m</td>
<td>Mean 77m within stand</td>
<td>Dow and Ashley, 1996</td>
</tr>
<tr>
<td><em>Quercus macrocarpa</em></td>
<td>Temperate/Midwestern US fragments</td>
<td>monoecious</td>
<td>57% from &gt;150m</td>
<td>Mean 76m within stand</td>
<td>Dow and Ashley, 1998a</td>
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<tr>
<td><em>Quercus petraea</em></td>
<td>Temperate/Mixed stand in continuous forest, France</td>
<td>monoecious</td>
<td>69%</td>
<td>Means 22-58m within stand for individual trees</td>
<td>Streiff et al., 1999</td>
</tr>
<tr>
<td><em>Quercus petraea</em></td>
<td>Temperate/Mixed stand in central Spain</td>
<td>monoecious</td>
<td>38%</td>
<td>Mean 92m within stand</td>
<td>Valbuena-Carabaña et al.,</td>
</tr>
<tr>
<td>Species</td>
<td>Habitat</td>
<td>Sexuality</td>
<td>Percent</td>
<td>Mean (m)</td>
<td>Reference</td>
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<tr>
<td><em>Quercus pyrenaica</em></td>
<td>Temperate/Mixed stand in central Spain</td>
<td>monoecious</td>
<td>34%</td>
<td>270</td>
<td>Valbuena-Carabaña et al., 2005</td>
</tr>
<tr>
<td><em>Quercus robur</em></td>
<td>Temperate/Mixed stand in continuous forest, France</td>
<td>monoecious</td>
<td>65%</td>
<td>18-64</td>
<td>Streiff et al., 1999</td>
</tr>
<tr>
<td><em>Quercus salicina</em></td>
<td>Temperate/southern Japan reserve</td>
<td>monoecious</td>
<td>52.2%</td>
<td>66.7</td>
<td>Nakanishi et al., 2004</td>
</tr>
<tr>
<td><em>Quercus salicina</em></td>
<td>Temperate/southern Japan reserve</td>
<td>monoecious</td>
<td>52.1%</td>
<td>69.2</td>
<td>Nakanishi et al., 2009</td>
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<td><em>Quercus lobata</em></td>
<td>Temperate/California</td>
<td>monoecious</td>
<td>~20%</td>
<td>114</td>
<td>Pluess et al., 2009</td>
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<tr>
<td><em>Quercus semiserrata</em></td>
<td>Temperate/Forest fragment in Thailand</td>
<td>monoecious</td>
<td>~30%</td>
<td>52</td>
<td>Pakkad et al., 2008</td>
</tr>
<tr>
<td><em>Fagus crenata</em></td>
<td>Temperate/northern Japan fragment</td>
<td>monoecious</td>
<td>8%</td>
<td>37</td>
<td>Hanaoka et al., 2007</td>
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<tr>
<td><em>Pinus densiflora</em></td>
<td>Temperate/northern Japan</td>
<td>monoecious</td>
<td>67%</td>
<td>&gt;100</td>
<td>Iwaizumi et al., 2009</td>
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<tr>
<td><em>Pinus sylvestris</em></td>
<td>Temperate conifer/central Spain isolated relict</td>
<td>monoecious</td>
<td>4.3%</td>
<td>47</td>
<td>Robledo-Arnuncio and Gil, 2005</td>
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<tr>
<td><em>Cercidiphyllum japonicum</em></td>
<td>Temperate/riparian forest in Japan</td>
<td>dioecious</td>
<td>30%</td>
<td>129</td>
<td>Sato et al., 2005</td>
</tr>
<tr>
<td><em>Populus trichocarpa</em></td>
<td>Temperate/Two contrasting sites in Oregon, US</td>
<td>dioecious</td>
<td>32-54%</td>
<td>7.6</td>
<td>Slavov et al., 2009</td>
</tr>
<tr>
<td><em>Populus nigra</em></td>
<td>Temperate/Czech</td>
<td>dioecious</td>
<td>23%</td>
<td>10-230</td>
<td>Pospišková and</td>
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<tr>
<td>Insect pollinated Trees</td>
<td>Republic</td>
<td>Pollination</td>
<td>Mean</td>
<td>Study Site</td>
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<tr>
<td><strong>Ficus sycomorus</strong></td>
<td>Arid/ Ugab River, Namibia</td>
<td>monoecious</td>
<td>50%</td>
<td>Mean 88.6km, up to 160km</td>
<td>Ahmed et al., 2009</td>
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<tr>
<td><strong>Eucalyptus wandoo</strong></td>
<td>Arid/Fragmented landscape, Western Australia</td>
<td>hermaphroditic, self-compatible</td>
<td>55.3% from &gt;350m</td>
<td>Mean 30-50m within stands</td>
<td>Byrne et al., 2008</td>
</tr>
<tr>
<td><strong>Pithecellobium elegans</strong></td>
<td>Tropical/Costa Rican forest fragment</td>
<td>hermaphroditic</td>
<td>28-41%</td>
<td>Mean 142m within study site, up to 350m within study site</td>
<td>Chase et al., 1996</td>
</tr>
<tr>
<td><strong>Dinizia excelsa</strong></td>
<td>Tropical/Amazonian forest fragments and pasture</td>
<td>hermaphroditic</td>
<td>68%</td>
<td>Mean 1288m for pasture trees, 417m for fragment trees, up to 3.2km within study site</td>
<td>Dick, 2001</td>
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<tr>
<td><strong>Prunus mahaleb</strong></td>
<td>Arid/Southeastern Spain</td>
<td>gynodioecious</td>
<td>9.5% from &gt;1.5km</td>
<td>Mean ~100m for outcross pollen within study site</td>
<td>Garcia et al., 2005</td>
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<tr>
<td><strong>Dipteryx panamensis</strong></td>
<td>Tropical/Costa Rican intact forest, fragments and pasture</td>
<td>monoecious</td>
<td>~59%</td>
<td>Means 240-557m per habitat, up to 1 km within study site</td>
<td>Hanson et al., 2008</td>
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<tr>
<td><strong>Simarouba amara</strong></td>
<td>Tropical/Barro Colorado Island, Panama</td>
<td>dioecious</td>
<td>na</td>
<td>Mean 345m, up to 740m within study site</td>
<td>Hardesty et al., 2006</td>
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<tr>
<td><strong>Centaurea corymbosa</strong></td>
<td>Arid/ French Mediterranean</td>
<td>monoecious, self incompatible, monocarpic</td>
<td>17%</td>
<td>Mean 21m within site, up to 100m within study site</td>
<td>Hardy et al., 2004</td>
</tr>
<tr>
<td><strong>Sorbus terminalis</strong></td>
<td>Temperate/two fragmented European forest sites</td>
<td>hermaphroditic</td>
<td>48% and 4%</td>
<td>Means 134 and 59m, up to 528m within site</td>
<td>Hoebee et al., 2007</td>
</tr>
<tr>
<td><strong>Aesculus turbinata</strong></td>
<td>Temperate/Japan, riparian</td>
<td>hermaphroditic</td>
<td>49%</td>
<td>Mean 180m within site, up to 700m</td>
<td>Isagi et al., 2007</td>
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<tr>
<td>Species</td>
<td>Location/Origin</td>
<td>Pollination Type</td>
<td>Pollination Degree</td>
<td>Distance within Site</td>
<td>Reference</td>
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<tr>
<td>Magnolia obovata</td>
<td>Temperate/Japan</td>
<td>Hermaphroditic,</td>
<td>40-57%</td>
<td>40-57%</td>
<td>Mean 131m within site, up to 121 m</td>
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<tr>
<td>Dipterocarpus tempehes</td>
<td>Tropical, Southeast Asia</td>
<td>Hermaphroditic</td>
<td>~11%</td>
<td>Mean 223 within site</td>
<td>Kenta et al., 2004</td>
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<tr>
<td>Malus sylvestris</td>
<td>Temperate/Denmark fragmented forest</td>
<td>Monoecious</td>
<td>74% not assigned</td>
<td>Mean 60m, up to 300 m within site</td>
<td>Larsen and Kjær</td>
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<tr>
<td>Entandrophragma cylindricum</td>
<td>Tropical/Cameroon logged forests</td>
<td>Monoecious</td>
<td>~70%</td>
<td>Mean ~330 m within site, up to 2095 m</td>
<td>Lourmas et al., 2007</td>
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<tr>
<td>Swietenia humilis</td>
<td>Tropical/Honduran fragmented forest</td>
<td>Monoecious</td>
<td>36-100%</td>
<td>Mean 1.6 to &gt;4.5 km</td>
<td>White et al., 2002</td>
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<tr>
<td>Others</td>
<td></td>
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<tr>
<td>Calothamnus quadrifidus</td>
<td>Bird pollinated Australian shrub</td>
<td>Hermaphroditic</td>
<td>10-33%</td>
<td>Up to 5km between sites</td>
<td>Byrne et al., 2007</td>
</tr>
<tr>
<td>Symponia globulifera</td>
<td>Tropical/bird pollinated</td>
<td>Hermaphroditic</td>
<td>35%</td>
<td>Mean ~900 m within site</td>
<td>Carneiro et al., 2009</td>
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<tr>
<td>Hymenaea courbaril</td>
<td>Tropical/Bat pollinated Amazonian tree</td>
<td>Hermaphroditic</td>
<td>55%</td>
<td>Mean 827m within site</td>
<td>Lacerda et al., 2008</td>
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<tr>
<td>Rhododendron metternichii</td>
<td>Temperate/Bee pollinated shrub, Japan</td>
<td>Hermaphroditic</td>
<td>20-30%</td>
<td>Not reported</td>
<td>Kameyama et al., 2000</td>
</tr>
<tr>
<td>Rhododendron metternichii</td>
<td>Temperate/Bee pollinated shrub, Japan</td>
<td>Hermaphroditic</td>
<td>27-53%</td>
<td>Not reported</td>
<td>Kameyama et al., 2001</td>
</tr>
<tr>
<td>Polaskia chichihe</td>
<td>Insect and bird pollinated columnar cactus, Mexico</td>
<td>Hermaphroditic</td>
<td>27%</td>
<td>Overall mean 113m, for 3 mothers &gt;1000m</td>
<td>Otero-Arnaiz et al., 2005</td>
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</tbody>
</table>
Table 2. Studies that use microsatellites and parentage assignment to track seed dispersal

<table>
<thead>
<tr>
<th>Species</th>
<th>Distribution/Study Site</th>
<th>Dispersal Agent</th>
<th>Sample, dispersal results and distances</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fraxinus excelsior</em></td>
<td>Temperate/deforested landscape in Scotland</td>
<td>wind</td>
<td>Established seedlings, ~50% from outside at &gt; 900 ha, seed mediated gene flow exceeded pollen gene flow</td>
<td>Bacles et al., 2006</td>
</tr>
<tr>
<td><em>Araucaria angustifolia</em></td>
<td>Tropical conifer/Brazilian forest fragment</td>
<td>autochory</td>
<td>Established seedlings/juveniles, no seed immigration observed, mean dispersal 83m, up to 291m</td>
<td>Bittencourt and Sebbenn, 2007</td>
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<tr>
<td><em>Quercus macrocarpa</em></td>
<td>Temperate/Midwestern US fragments</td>
<td>gravity and vertebrates</td>
<td>Saplings, 6-14% from outside at &gt;150m, mean dispersal within stand 22.8m, up to 160m</td>
<td>Dow and Ashley, 1996</td>
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<tr>
<td><em>Prunus mahaleb</em></td>
<td>Arid/southeastern Spain</td>
<td>frugivorous birds and other vertebrates</td>
<td>Seed traps, 18% seed immigration, mean dispersal within stand 6.1 m up to 320m</td>
<td>Godoy and Jordano, 2001</td>
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<td><em>Prunus mahaleb</em></td>
<td>Arid/southeastern Spain</td>
<td>frugivorous birds and other vertebrates</td>
<td>Seed traps, 20% seed immigration, median dispersal within stand 14m, up to 990m</td>
<td>Garcia et al., 2007</td>
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<td><em>Prunus mahaleb</em></td>
<td>Arid/southeastern Spain</td>
<td>frugivorous birds and other vertebrates</td>
<td>Seed traps and fecal samples, up to 990m, show differential dispersal patterns by birds and mammals</td>
<td>Jordano et al., 2007</td>
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<tr>
<td><em>Dipteryx panamensis</em></td>
<td>Tropical/Costa Rican intact forest and fragments</td>
<td>frugivorous birds and mammals</td>
<td>Seed transects, only 7% genotyping success, 14 seeds assigned that were dispersed 63- 853m</td>
<td>Hanson et al., 2007</td>
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<tr>
<td><em>Simarouba amara</em></td>
<td>Tropical/Barro Colorado Island, Panama</td>
<td>frugivorous birds and mammals</td>
<td>Established seedlings, mean dispersal distance 392m, up to 1km</td>
<td>Hardesty et al., 2006</td>
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<tr>
<td><em>Aesculus turbinata</em></td>
<td>Temperate/Japan, riparian forest</td>
<td>small mammals</td>
<td>Established seedlings, mean dispersal distance 2.3m, up to 218m</td>
<td>Isagi et al., 2007</td>
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<td>Species</td>
<td>Habitat</td>
<td>Dispersal Mode</td>
<td>Dispersal Details</td>
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<tr>
<td><em>Pinus densiflora</em></td>
<td>Temperate/northern</td>
<td>wind</td>
<td>Seed traps, 19% seed immigration from &gt;100m, Median ~15m within stand</td>
<td>Iwaizumi <em>et al.</em>, 2009</td>
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<td>Japan</td>
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<tr>
<td><em>Jacaranda copaia</em></td>
<td>Tropical/Barro Colorado</td>
<td>wind</td>
<td>Seed traps, 16% seed immigration, mean dispersal distance 40-59m, up to 711 within site</td>
<td>Jones <em>et al.</em>, 2005</td>
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<td>Island, Panama</td>
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<tr>
<td><em>Abies sachalinensis</em></td>
<td>Subboreal conifer/ Japan</td>
<td>wind</td>
<td>Recruitment on fallen logs, 19% seed immigration, mean 24m within stand, up to 237m</td>
<td>Lian <em>et al.</em>, 2008</td>
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<td>Japan</td>
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<td><em>Prunus nigra</em></td>
<td>Temperate/Czech</td>
<td>wind and water</td>
<td>Established seedlings, dispersed up to 370m</td>
<td>Pospíšková and Šálková, 2006</td>
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<td>Republic</td>
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<tr>
<td><em>Cercidiphyllum japonicum</em></td>
<td>Temperate/riparian</td>
<td>wind</td>
<td>7% seed immigration, up to 302m within stand</td>
<td>Sato <em>et al.</em>, 2005</td>
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<td></td>
<td>forest in Japan</td>
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<td><em>Myrica rubra</em></td>
<td>Temperate/Japan</td>
<td>frugivorous</td>
<td>Feces of macaques (<em>Macaca fuscata</em>), mean 270m, up to 634m within site</td>
<td>Terakawa <em>et al.</em>, 2009</td>
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<td></td>
<td></td>
<td>vertebrate</td>
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