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Research Article

Genetic analysis of landscape connectivity in tree populations

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Abstract

Genetic connectivity in plant populations is determined by gene movement within and among populations. When populations become genetically isolated, they are at risk of loss of genetic diversity that is critical to the long-term survival of populations. Anthropogenic landscape change and habitat fragmentation have become so pervasive that they may threaten the genetic connectivity of many plant species. The theoretical consequences of such changes are generally understood, but it is not immediately apparent how concerned we should be for real organisms, distributed across real landscapes. Our goals here are to describe how one can study gene movement of both pollen and seeds in the context of changing landscapes and to explain what we have learned so far. In the first part, we will cover methods of describing pollen movement and then review evidence for the impact of fragmentation in terms of both the level of pollen flow into populations and the genetic diversity of the resulting progeny. In the second part, we will describe methods for contemporary seed movement, and describe findings about gene flow and genetic diversity resulting from seed movement. Evidence for pollen flow suggests high connectivity, but it appears that seed dispersal into fragments may create genetic bottlenecks due to limited seed sources. Future work should address the interaction of pollen and seed flow and attention needs to be paid to both gene flow and the diversity of the incoming gene pool. Moreover, if future work is to model the impact of changing landscapes on propagule movement, with all of its ensuing consequences for genetic connectivity and demographic processes, we will need an effective integration of population genetics and landscape ecology.

Introduction

> 'The genetic consequences of population fragmentation depend critically on gene flow. With restricted gene flow, fragmentation is usually highly deleterious in the long run.' (Frankham et al. 2002: p. 310)

The movement of individuals from one population to another maintains 'connectivity' within species, which – for plant populations – is preserved through the movement of pollen or seeds. Recently, landscape genetics, a research area that combines landscape ecology and population genetics, has focused attention on how landscape features structure

genetic variation within and among populations – through their impact on gene flow, the exchange of genes among populations (Manel et al. 2003). Many of the statistical tools available for application to landscape genetics, e.g., Mantel tests, spatial autocorrelation, Bayesian clustering, and multivariate analyses, have been in use for some time in population genetics (see Epperson (2003) for an extensive review), and newer tools (e.g., GIS) are relatively new in genetic research. These tools help identify the historical impact of landscape features on genetic structure, and biologists are now asking questions about the influence of landscape changes on contemporary gene flow and connectivity. Such questions require new approaches.

What are the motivations for analyzing the genetic component of landscape connectivity? First, fragmentation has become a major topic of concern to landscape ecologists, conservation biologists, and evolutionary biologists. Many populations that were once continuous exist now only as fragments, exhibiting a collection of local populations or a metapopulation (*sensu lato*) structure, with altered degrees of connectivity (Harrison and Hastings 1996). Hamrick (2004) points out that many tree species are likely to be resilient to landscape changes because they already contain high genetic diversity and they have successful mechanisms for extensive propagule flow. The question is whether these genetically isolated, sometimes small, remnant populations are at risk of losing genetic diversity, and potentially adaptive genetic variation (Saunders et al. 1991; Ledig 1992; Ellstrand and Elam 1993; Young et al. 1996; Nason et al. 2000; Young and Clarke 2000; Frankham et al. 2002; Holderegger et al., in press). Second, fragmentation can also change the quality of the landscape within and among fragments through edge effects, invasive species, and changes in species composition. The environmental changes, combined with global environmental change, can influence evolutionary processes in a relatively short time frame and the response to these changes will require genetic variation. Third, many biologists are struggling to follow animal and plant movement across landscapes for their demographic studies, and genetic markers are useful for gauging the impact of real time changes in the landscape.

An important question to address is whether landscape alteration will jeopardize future population fitness and evolutionary potential by disrupting propagule flow (Sork et al. 1999). Does fragmentation alter connectivity? Do fragments experience more or less genetic immigration? What happens when we lose or change pollinators or dispersal agents? To answer these questions, we need an approach that can be deployed over a large enough spatial scale to capture the majority of propagule movements that occur during a single reproductive episode (Sork et al. 1999). One step in understanding genetic movement is to estimate how far both pollen and seed move, providing an estimate of the reproductive neighborhood size of the population (*sensu* Wright 1943, 1946), because the neighborhood size provides a sense of whether populations are at risk of losing genetic diversity. Also needed are estimates of the rate of genetic immigration to provide a measure of the degree of genetic isolation for the fragment.

The goals of this paper are to review methods for examining contemporary propagule movement in plant populations and then to examine the empirical literature to understand the impact of landscape change on the risk of loss of genetic variation. The first, and major, part of this paper will concentrate on pollen flow. We will briefly recapitulate two common genetic methods of measuring real-time pollen movement, as a measure of the impact of landscape change: parentage analysis and TwoGener analysis, both used to translate pollen pool structure into an estimate of the scale of propagule movement. We will then summarize the numerous studies of pollen flow in fragmented landscapes, and propose two elements of risk of loss of genetic variation: (i) the degree of isolation of a fragment, and (ii) the diversity of incoming gene sources. The second part of the paper will address seed flow, which is equally, or perhaps even more critical for demographic and genetic connectivity, but for which available data are fewer. We will summarize methods (with additional detailed discussion in Appendices) and then present available studies on the impact of fragmentation on connectivity. We will conclude

the paper with a brief mention of other methods and their potential contribution to such questions.

Pollen movement across the landscape: methods

For pollen movement, paternity analysis of progeny (e.g., Meagher and Thompson 1987; Devlin and Ellstrand 1990; Adams et al. 1992; Smouse et al. 1999; Burczyk et al. 2002; Gerber et al. 2003) and the more recently developed two-generation pollen pool structure approach, dubbed TwoGener (Austerlitz and Smouse 2001; Smouse et al. 2001), provide alternative ways of describing pollen flow (Smouse and Sork 2004). Both of these approaches can be used to estimate the pollen contribution to neighborhood size, the average distance of pollen dispersal, and the shape of the dispersal kernel. We have compared and contrasted these two methods elsewhere, in *extenso* (see review of both methodologies in Smouse and Sork (2004)), but their differences can be summarized briefly, as follows. Paternity analysis provides a description of the source and location of the pollen that fertilizes the seeds produced by a small number of maternal trees. Within a delimited area, this method can identify the total number of pollen sources, describe the dispersal curve, and estimate the proportion of incoming pollen, i.e., pollen that comes from outside that area. With paternity analysis, it is possible to estimate the proportion of pollen immigration coming from outside the study area. For a larger portion of the landscape, TwoGener can estimate the average effective number of pollen donors, and the effective neighborhood size for a site and use this information to model the effective pollen dispersal curve, assuming a given dispersal function. Both methods are useful for studying landscape change because they measure on-going processes of gene movement. With TwoGener, we can more easily estimate the effective number of pollen donors contributing to the genetic diversity of the gene pool, over a larger area. Below we give an overview of the two methods.

Paternity analysis

Once we designate the genotype of the pollen donor for a particular offspring, sampled from a mother of known genotype and location, and if we can pinpoint his location on the landscape, then we can measure the pollen dispersal distance directly. With enough such paternally designated seeds, we can work out the effective pollen dispersal distribution, and from it, both the average and variance of effective pollen dispersal. In practice, we genotype the mother and her offspring, and given those genotypes and standard paternity analysis mathematics, we compute the likelihood of any particular male genotype donating the required pollen gamete (Essen-Moller 1938). With a multiple-locus genetic battery of sufficient resolving power, we can reduce the list of credible paternal genotypes to a small number, all others having been excluded genetically, with (in general) one candidate genotype (of a small set) being much more likely than any of the others. With a genetic battery of limited resolution (the more usual case), we can exclude most paternal candidates for any particular offspring, but cannot compellingly assign paternity to a single candidate genotype.

When we can reasonably assume that the battery of potential pollinators is drawn primarily from the set of local males, say those within a fragment or within a sufficiently small circle of arbitrary radius, often dubbed a 'neighborhood', then we discover that the set of credible fathers for any particular offspring contains a single candidate male, who is designated the father. With enough offspring, we can estimate the 'spectrum' of paternity, even where we cannot designate each and every father exactly. Common approaches will use either maximum likelihood assignment analysis to identify potential fathers for purposes of categorical or fractional assignment (e.g., Roeder et al. 1989; Devlin and Ellstrand 1990; Marshall et al. 1998), or paternity exclusion analysis to identify at least one potential father whose genotype is completely compatible with the progeny (e.g., Ellstrand 1984). The usual result is that some males contribute substantially more offspring to overall recruitment than do others and that male contributions vary by female as well (see Smouse and Sork 2004, and references therein). This information can yield the source pollen donors and their location within a proscribed area, the proportion of progeny who are likely to have been fertilized by pollen donors outside an area, and the shape of the dispersal curve for pollen from within

the area. Connectedness is characterized by the estimate of incoming pollen into the site or fragment. (See Appendix A for more detail.) The details, opportunities and limitations offered by the various paternity approaches are discussed elsewhere (Sork et al. 1999; Burczyk and Chybicki 2004; Smouse and Sork 2004).

A useful demonstration of paternity analysis is a study of pollen movement in a 5.76 ha forest stand with 296 adult trees of *Quercus petraea* and *Quercus robur*, located within a continuous oak forest in France. Sampling 13 maternal trees and 986 of their offspring, and using a six-locus microsatellite battery, Streiff et al. (1999) used paternity exclusion analysis to identify the pollen donors. They identified 310 progeny that were compatible with a single pollen donor within the stand, 27 with multiple potential pollen donors, 17 that were self-fertilized, and 23 that were interspecific hybrids. All others were classified as due to pollen flow (not attributable to any adult within the stand, and thus immigrants). For progeny with pollen sources within the stand, average pollen dispersal distance was about 44 m (SD = 33.2 m). They found that the majority of donor-attributable pollinations resulted from pollen sources near the maternal tree and used a negative exponential dispersal curve to describe the pattern, but >65% of the progeny resulted from pollen donors located more than 100 m away. This study found that the single most frequent pollen source was close to the maternal plant, but the number of pollen sources was high, and the proportion of progeny from fathers outside the plot was also high.

TwoGener analysis

For a great many species, we cannot realistically sample and genotype enough of the candidate males to make paternity analysis a useful approach. The males are spread over too large an area to enumerate effectively, or the number of potential candidates within pollination range is simply too large to genotype effectively. Forest tree species, with relatively low density per hectare, are relatively tractable for the required sampling, but imagine an understory shrub, forb, or grass, where there are potentially thousands of candidates within reasonable sampling distance of the index mothers. Exhaustive enumeration is beyond reach. In these situations, an alternative is provided by TwoGener analysis, the central principle of which is that we can genotype a sample of mothers, a sample of their seeds, and can infer something about the pollen cloud from which a particular mother has drawn, without actually knowing who the contributing males are or where they are to be found. To the same extent that pollination is localized, mothers – spaced out across the landscape – will sample different arrays of pollen donors, so that the pollen clouds fertilizing those mothers will have different allele frequencies (Austerlitz and Smouse 2001; Smouse et al. 2001). We measure the 'genetic structure' of the pollen pool, encapsulated in the intraclass correlation coefficient, measured as the fraction of the total genetic variation of male gametes that is attributable to maternal differences in their sampled pollen pools, specifically $\Phi_{ft} = \sigma_A^2/(\sigma_W^2 + \sigma_A^2)$.

From this measure of pollen pool structure, Φ_{ft}, we can extract two types of information: (1) a measure of the effective number of pollen donors contributing to the average female, N_{ep}, and (2) a measure of the average distance of effective pollen dispersal, δ (see Appendix B for details). Essentially, N_{ep} is the number of *idealized* pollen donors (all contributing equally and randomly) that would yield the pollen structure actually observed. Because pollen sources are not represented equally among progeny, this number is usually somewhat smaller than the total number of contributing pollen donors (Smouse and Sork 2004). If the sampled mothers are spaced far enough apart on the landscape that they draw pollen from completely different males, then we can translate $N_{ep} = (1/2\Phi_{ft})$. To translate Φ_{ft} into an estimate of the average pollination distance (δ), we must assume some particular family of pollen dispersal curves. Austerlitz and Smouse (2001, 2002) gave special cases for normal and exponential functions of pollen dispersal, but accumulating experience suggests that pollen dispersal has a more extended and flatter tail than had been realized (Austerlitz et al. 2004), and the field is now moving toward generic use of the exponential power family of distributions (Clark 1998). See Appendix B for more detail.

We will illustrate TwoGener with one of our own studies that shows the impact of landscape context on Φ_{ft} and N_{ep} (Sork et al. 2005). In this

case, we were examining the question of whether tree-harvesting methods implemented by the Missouri Ozark Forest Ecosystem Project (MOFEP) might influence patterns of pollen flow. The MOFEP experimental design deploys two treatment types, referred to as uneven-aged management (selective cutting, *S*) and even-aged management (clear-cutting, *C*, with scattered clearings of 3–12 ha each), and a third category of no harvest management (uncut, *U*), used as a control (Brookshire and Hauser 1993). Both cutting regimes were achieved by harvesting approximately 10% of the standing biomass for the sites receiving a silvicultural treatment, which reduces the overall vegetative structure of the forest (Kabrick et al. 2002). In a study of *Cornus florida*, an insect-pollinated, mostly understory tree species, we found that removal of forest vegetation increased pollen movement for trees sample adjacent to the clear-cut areas and, to a lesser extent, for trees in the selective cut areas (Sork et al. 2005). Using TwoGener, we found that $\Phi_C \leq \Phi_S \leq \Phi_U$, so that pollen pool structure was less in clear-cut than in uncut areas, with selective-cut intermediate, which translates into more effective pollen donors in clear-cut ($N_{ep} = 5.56$) and selective-cut ($N_{ep} = 4.00$) areas than in uncut areas ($N_{ep} = 2.87$). This study illustrates how landscape change alters pollen movement and how we might test for differences among sites.

Pollen movement in landscapes: empirical findings

We will now integrate results based on these two approaches, as well as others, to address the critical issue of how anthropogenically-derived population fragmentation might affect genetic connectivity. Here, we will present studies of pollen flow and diversity of pollen/seed sources, the two key factors that affect the risk of loss of genetic variation.

Many landscape scale studies have examined pollen flow into forest fragments (see Smouse and Sork 2004). These studies, mostly tropical, identify a focal area that includes one or more fragments and then estimate proportion of progeny fertilized from pollen donors outside the fragment. Two striking results have emerged so far. First, many tropical canopy species have immigrant pollen dispersal distances from other areas greater than 100–200 m away (see papers cited in Hamrick and Nason 2000), and, in the case of species with specialized pollinators, such as tropical figs (*Ficus* spp.), the pollen immigration is over distances greater than 1 km, resulting in an extremely large neighborhood area (Nason et al. 1996). Second, many populations in small fragments receive moderate to high levels of pollen immigration (see Table 6.2 in Hamrick and Nason (2000)). These results suggest that, for many tropical plant species, especially those with specialized pollinators, fragmentation and physical isolation do not impede pollen flow resulting in fertilization.

It should be pointed out that one reason that trees in small fragments receive a high proportion of immigrant pollen is the paucity of local trees available as pollen sources. This explanation is illustrated by two studies: (a) an insect-pollinated species (*Swietenia humilis*), studied in tropical dry forest fragments in Honduras (White et al. 2002), and (b) a wind-pollinated oak species (*Quercus humboldtii*), in montane tropical forest fragments in Colombia (data from Fernández-M and Sork, 2005). In both cases, the inter-fragment distance is on the order of a kilometer. The two species exhibited similar patterns of decreased proportion pollen flow (*m*) into fragments, as numbers of trees within fragments increased (Figure 1a, b), although the shape of the relationship was a negative power function for *S. humilis* (Figure 1a) and a negative exponential for *Q. humboldtii* (Figure 1b). Thus, it appears that proportion of gene flow is high, because the abundance of local adults is low.

One cause of high pollen flow into fragments is the removal of intervening vegetative structure may enhance pollen movement. In a study of wind-pollinated *Pinus echinata*, (Dyer 2002), conducted in the same three MOFEP treatments describe Sork et al. 2005, pollen movement was higher for trees left standing in clearings than for trees in sites with the other treatments. This study and that of *Cornus florida* indicate that gene movement was enhanced by reductions in the vegetative structure of the forest. When we compare mean distance of effective pollen movement in savanna populations of *Quercus lobata* (Sork et al. 2002) with that of closed forest populations of *Quercus alba* (Smouse et al. 1999), we find that pollen movement was farther in the savanna, although the effective number of pollen donors in

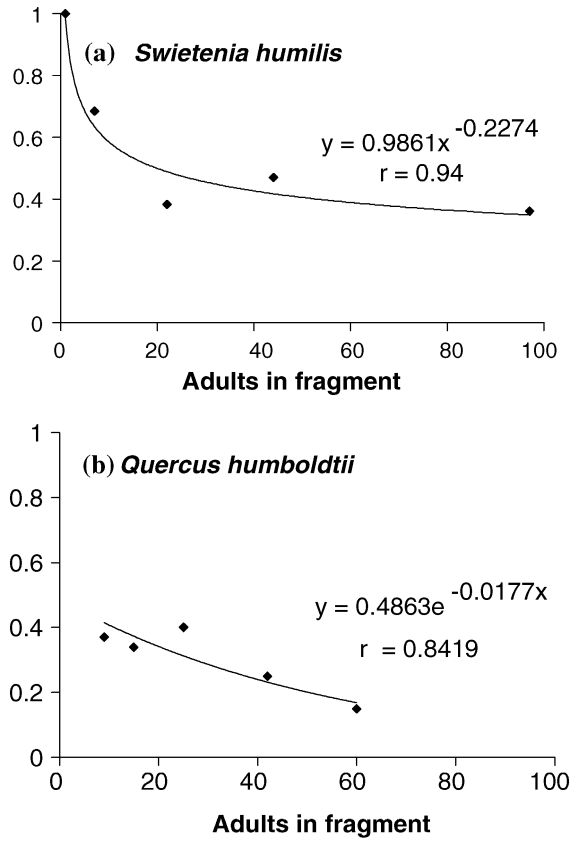

Figure 1. Relationship between proportion of pollen flow into fragments with varying number of local adults for two tropical tree species, showing the functional relationship with the highest correlation value. (a) Wind-pollinated *Quercus humboldtii* in fragmented montane forests of Colombia, South America (data from Fernández-M and Sork, 2005). (b) Insect-pollinated *Swietenii humilis* in fragmented tropical forests in Honduras, Central America (White et al. 2002).

the savanna was smaller. For insect-pollinated species, the dynamics may depend on whether the pollinators are generalists or specialists. Specialized pollinators that pollinate species in low abundance, such as the wasps that pollinate tropical figs (Nason and Hamrick 1997), have evolved the ability to find conspecific adults spaced far apart. For the Amazonian tree species, *Dinizia excelsa*, native pollinators move pollen between close neighbors in continuous forest, but invasive Africanized honeybees move pollen great distances between isolated trees (Dick et al. 2003). Thus, in spite of concerns that fragmentation will isolate populations, many tree species – representing diverse pollen vectors – experience enhanced pollen immigration for isolated trees.

The other consequence of pollen movement in fragments is a change in the diversity of pollen sources. Is the immigrant pollen pool derived from few sources or from many pollen sources? High immigration rates alone do not prevent genetic bottlenecks. One way to measure the genetic diversity is to estimate the effective number of pollen donors (N_{ep}). In the aforementioned study of *Quercus humboldtii* (Fernández-M and Sork, submitted), the genetic diversity of seedlings within fragments was less than that in continuous forest, suggesting that adults within fragments are mating with fewer pollen donors and receiving less pollen diversity than adults in continuous forest. Dunphy et al. (2004) also reported a paradoxical pattern for *Hymenea courbaril*, a tropical dry forest tree often found in small stands, relatively high rates of pollen immigration (m) but low effective numbers (N_{ep}) of pollen donors.

One process that can occur in fragments, even in the presence of high gene flow, is an increase in the level of inbreeding. For example, the tropical tree *Spondias mombin* exhibited significant reduction of germination and fruit production in small fragments, when compared with continuous forest populations, despite high rates of pollen inflow (Nason and Hamrick 1997). In the Costa Rican dry forest species *Enterolobium cyclocarpum*, pollen deposition, fruit production, seeds per fruit, and progeny vigor were lower for trees in pastures than for those in continuous forest (Rocha and Aguilar 2001). Similar reductions in reproductive success were observed for solitary pasture trees in the dry tropical forest species, *Pachira quinata* (Fuchs et al. 2003). Collectively, these results suggest that high levels of inbreeding and/or loss of adaptive genetic variation may be occurring in fragments and for isolated trees, in spite of the amount of gene flow. However, few studies have measured this factor specifically.

Studies that estimate N_{ep} for tree populations, either through TwoGener analysis or similar methods (e.g., MLTR mating system analysis, Ritland 1989, 1990), report a range of N_{ep} from 2 to 200 for the average seed tree (see Smouse and Sork 2004, Table 3). Scattered and insect-pollinated tree species such as *Jacaranda copaia* in Costa Rican dry forests and *Albizia julibrissin* in an urban setting exhibit values of $N_{ep} < 2$ and < 3, respectively (James et al. 1998; Irwin et al. 2003). We also see that fragmented populations of wind-pollinated

Quercus humboldtii exhibit estimates of N_{ep} ($\sim$ 6 individuals; Fernández-M and Sork, 2005), which are only slightly smaller than populations in closed forests of *Quercus alba* ($N_{ep} \sim$ 8 individuals, Smouse et al. 2001) and of *Quercus velutina* ($N_{ep} \sim$ 6–8 individuals, Fernández-M, Idol, & Sork, under review). By way of contrast, several examples show high values of N_{ep} in continuous forest populations (e.g., $N_{ep} > 70$ in *Pinus sylvestris*, Robledo-Annucio et al. 2004; see also Smouse and Sork 2004, Table 3). There are evidently exceptions, however, because *Enterolobium cyclocarpum* has higher N_{ep} in pasture populations than in the closed forest populations (Rocha and Aquilar 2001). There seems to be a tendency for insect-pollinated species to have lower values of N_{ep} than wind-pollinated species, suggesting that these species might be much more vulnerable to fragmentation. However, one might ask whether the high pollen movement by wasps for tropical figs (Nason et al. 1996) or African honey bee pollen dispersal for a Brazilian rainforest tree (Dick et al. 2003) are the exceptions or rule.

As we integrate the findings of these various studies, we can see that the degree of risk of loss of genetic variation for a given fragment will depend on (i) the degree of isolation, and (ii) the diversity of pollen (or seed) sources that come into the fragment (see Table 1). Fragments that have high isolation and low diversity of contributing pollen sources, such as found in isolated, small fragments, will have a high risk of loss of genetic variation. Fragments that are not isolated and receive a low number of incoming pollen sources will have high gene flow but a moderate risk of loss of genetic variation. This pattern was illustrated by the study described above of *Hymenea courbaril* (Dunphy et al. 2004). A third pattern occurs in fragments with high isolation and high number of pollen sources, which would result in low risk of loss of genetic variation. So far, no examples exist, probably because the combination of high isolation and many pollen sources is unlikely. Finally, the category with the lowest risk applies to fragments with low isolation and high number of pollen sources. An example here is the study of high gene flow and high N_{ep} observed for *Dinizia excelsa* that occurs in Brazilian fragments, and whose pollen is dispersed by Africanized honeybees (Dick et al. 2003). In sum, risk of loss of genetic diversity through pollen flow is determined by the extent of connectivity for a given fragment or set of fragments and the effectiveness of the dispersal process in delivering pollen to these fragments. As we look at the continuum of high to low risk of loss of genetic variation, it becomes apparent that the diversity of the incoming pollen (or seed) influences overall gene pool diversity more than simply having a high level of gene flow (Table 1). The maintenance of genetic variation requires gene flow, but especially gene flow from diverse sources.

Seed movement across the landscape: methods

The methods for genetic assessment of contemporary seed dispersal have been somewhat refractory, because it is often difficult to recover seeds that have already been dispersed. Moreover, it is more difficult to determine the genotypes of both parents from an already-dispersed seedling than it is to determine the paternal genotype from a seedling sampled directly from a mother of known

Table 1. Summary of two components of landscape connectivity that affect the risk of loss of genetic variation for a given population fragment: (i) high vs. low degree of isolation; (ii) few vs. many sources contributing to incoming pollen/seed pool.

	Degree of isolation	
	High	Low
Few sources of incoming pollen/seed	Low gene flow from few sources HIGH risk	High gene flow from few sources MODERATE risk
Many sources of incoming pollen/seed	Low gene flow from many sources LOW risk	High gene flow from many sources VERY LOW risk

Gene flow is defined as the proportion of progeny whose parental donor came from outside the fragment. The number of incoming pollen sources l can be estimated by N_{ep}, effective number of pollen donors per mother, or genetic diversity of pollen pool. The number of effective incoming contributors to seed pool can be estimated by N_{em}, effective number of seed sources per patch, or maternal genetic diversity of seed/seedling pool.

genotype (Smouse and Sork 2004). The very recent innovation of using maternally inherited seed coat tissue to identify maternal genotype directly overcomes this latter problem (Godoy and Jordano 2001). With this genetic assay, it is now possible to do maternity analysis of seeds rather than parentage analysis. Frequently, the tail of the seed dispersal distribution often extends beyond the area that can be sampled effectively, and we must sometimes extrapolate far beyond the range of sampling. Under such circumstances, we can use a seed-pool structure approach, which allows one to sample a larger portion of the landscape to identify the scale and extent of seed movement (e.g., Grivet et al. 2005). However, this method is also limited in its sensitivity to the tail of the distribution. Nonetheless, methods are now available that allow estimation of the scale of seed dispersal, the effect of seed dispersal on the genetic diversity of the seed pool, and the fraction of the local gene pool that is due to immigration. These seed pool methods are less easily applied than are their pollen pool analogs and the results lag behind those for pollen, but the role of seed dispersal in seedling recruitment, migration among populations, and colonization of new sites is so pivotal that we continue to study it. At a minimum, we need information on the proportion of seedlings that come from outside a fragment, as well as whether the immigrant seedlings are derived from few or many maternal trees. Below we discuss two methods for describing contemporary seed-mediated gene movement.

Maternity analysis

We can use the same genetic and statistical principles to assess maternity as we have for paternity, but, with dispersed seed, we have to infer the genotypes of both parents. We have entered the realm of the 'two parent problem' (Meagher and Thompson 1987), an extremely difficult genetic and statistical challenge that has (until recently) impeded forward progress on the genetic analysis of seed flow. The determination of the maternal genotype from maternally inherited seed tissue, however, places us in the enviable position of being able to determine the diploid maternal genotype exactly and without reference to the paternal contribution. All we need to do is determine where that mother is to be found. If seed movement is strictly local, as it seems to be in many species, intensive adult sampling over an area of manageable size will yield an immediate characterization of the seed-dispersal kernel (Godoy and Jordano 2001). If seed-dispersal is more extended, we can still genotype all of the adults within a neighborhood of the recovered seed, and can estimate the dispersal function for seeds inside the neighborhood.

Seed pool structure analysis

For many plant species, seeds are widely dispersed in a patchy manner (e.g. beneath nesting sites of spider monkeys (*Ateles paniscus*) in Amazonian forests (Russo and Augspurger 2004)) and it is not feasible to genotype the full range of maternal candidates. Or, in some experimental situations, seeds are collected from seed traps (e.g. Dalling et al. 2002), and it is also difficult to sample all maternal candidates. Under these two circumstances, if we can determine the maternal genotype from the seed tissue *a la* Godoy and Jordano (2001), we can deploy a seed pool structure approach by conducting a classical AMOVA of the genetic composition of groups of seeds in patches or seed traps. We treat the groups of seeds as strata and the diploid seed coats as replication within strata. Here, we are trying to characterize the local seed pool, not the pollen pool. Using similar logic, we can estimate the effective number of seed sources per seed pool and we can estimate the scale of seed dispersal. Using AMOVA is a simple and accessible way to conduct this analysis (for details see Appendix B).

To illustrate this approach we will describe our study (Grivet et al., 2005) with *Quercus lobata* and a major seed-vector, the acorn woodpecker (*Melanerpes formicivorous*). In this study, we quantify the seed pool structure of the storage granaries used by the birds to store acorns, and assess whether woodpeckers, which are capable of flying many kilometers in a day, actually do so in the course of storing acorns. In an initial study, we found that the average granary has very few seed donors and that the principal seed donors are closely proximal to the granary, each contained within the territory of a familial foraging group. Effective seed movement is quite localized. We are

aware of the jay studies in eastern North America and Europe that report long distance seed transport (e.g., Bossema 1979; Darley-Hill and Johnson 1981), and the particulars will obviously depend on the species in question, but the important point here is that the seed pool structure approach offers a general method of measuring seed dispersal distance (δ), as well as the impact of dispersal on seed pool diversity.

Seed movement across the landscape: empirical studies

For seed dispersal, we have few genetic studies that describe the mean dispersal distance and shape of the curve (but see review by Schnabel 1998). Like pollen movement, to understand genetic connectivity within a landscape through seed dispersal, we should assess both the degree of isolation of a fragment and the number of incoming seeds sources (Table 1).

Unless a fragment is extremely isolated, we might expect moderate to high levels of seed inflow. In an early parentage study based on bi-parental exclusion analysis of seedlings, Dow and Ashley (1996) inferred that 6–14% of 100 sampled *Quercus macrocarpa* saplings appear to have immigrated into a 4-ha forest fragment. In a study of the animal-dispersed *Prunus mahaleb*, from an isolated location, Godoy and Jordano (2001) estimated 18% seed-inflow, and, in a study of seed movement across a fragmented landscape, Aldrich and Hamrick (1998) found that 68% of the seedlings of the tropical tree species *Symphonia globulifera* found in remnant forest stands were dispersed from outside the stands by bats.

Unlike pollen flow, the level of genetic diversity in the seed pool may be a significant issue. Schnabel et al. (1998) used a likelihood-based maternity analysis model to identify maternal trees for a large number of naturally established seedlings and saplings in two populations of *Gleditsia triacanthos* L. They estimated that 58% of the 313 progeny at one site and 46% of the 651 progeny at a second site could be attributed to just three females. In the same study of seed movement described above, Aldrich and Hamrick (1998) found that the immigrant seedlings were from a small number of isolated pasture trees. While the rate of seed flow into the fragments was high, the genetic diversity of the derivative saplings was low, due to the small number of contributing seed donors. Sezen et al. (2005) obtained this same result from a study of the neotropical canopy palm species, *Iriartea deltoidea*. They found that all the seedlings in second growth forest sites were derived from old growth forest, but that 56% of the seedlings came from just two adults in old growth forest.

In short, we still have little information about seed-mediated gene flow in a landscape-scale context. From a genetic perspective, it is not clear how isolation and number of seed sources will influence the risk of loss of genetic variation in fragments (Table 1). Many studies have shown that anthropogenic disturbance results in a loss of vertebrate dispersal agents (e.g., Wright and Duber 2001), which may reduce the connectivity among fragments if it increases isolation (Table 1). For wind-dispersed species, the crucial factors will be the aerodynamics of dispersal (e.g., Soons et al. 2004). For animal-dispersed seeds, the effect of landscape fragmentation on propagule flow might be much more complex. It is true that some animals are minimally constrained by landscape features (e.g., bats, as described by Aldrich and Hamrick (1998)), but the dispersed seeds may be derived from only a few females, putting those populations at moderate risk of loss of genetic variation. We have described some examples of severe bottleneck effects, but we are far from having any reliable generalities about the causal conditions, and we still have almost no ability to predict which species are in real jeopardy.

Other approaches to seed flow

Phylogeographical studies

Phylogeographic analyses shed light on the propensity of certain species to exhibit long-distance propagule flow and at what rate. For example, the post-glacial migration rates of European trees from southern refugia may be as high as 500 m per year for oaks (Le Corre et al. 1997), and this post-glacial range expansion seems to be best explained by long-distance seed dispersal (Davis 1981; Clark 1998). Austerlitz and Granier-Gere (2003) modeled various scenarios of seed dispersal and concluded that intermediate (20 km) and long-distance (50 km) dispersal events can both account for the coloniza-

tion rate in Europe, but the shorter values are much closer to the maximum observed distance of flight of 8 km reported for jays, dispersing European oak. In eastern North America, the paleo-ecological record has been interpreted as showing that migration of tree species can be as rapid as 500–1000 m per year, and a similarly high rate of spread has been proposed, based on pollen records (Davis 1981; Clark 1998). More recent analysis, using maternally inherited chloroplast DNA, suggests that the colonization rates may be closer to 100 m per year (McLachen et al. 2005), much closer to contemporary genetically based estimates of seed dispersal. Thus, the analysis of historical genetics may provide a good indication of the rate at which populations can recolonize an area, something that landscape ecologists would like to know.

Paleo-ecological observations might provide an estimate of the rate of migration through seed dispersal, but it is hard to know whether these rates reflect diffusion-dispersal processes like those we observe in contemporary populations, or whether historical long-distance dispersal may best explain colonization through 'jump dispersal' models (*sensu* Pielou 1979). Currently, the genetic approaches discussed in this paper apply to diffusion processes and leptokurtic dispersion kernels. If phylogeographic patterns are the result of 'jump dispersal' events, then paleo and contemporary studies may not be comparable. In fact, sporadic 'jump dispersal' events may be very difficult to detect in contemporary studies, because they will be rare and sporadic events, seldom encountered. Phylogeographical studies may provide insight about long-distance dispersal that is difficult to detect in contemporary populations, but before generalizing from the historical patterns, we need to think carefully about how to extrapolate these findings to contemporary populations in fragmented landscapes or how to extrapolate contemporary processes to historical conditions.

Source population assignment

An important concern for biologists interested in landscape conncectivity is the actual source of incoming propagules. In theory, assignment techniques seem ideally suited for this purpose (e.g., Rannala and Mountain 1997; Cornuet et al. 1999; Pritchard et al. 2000; Paetkau et al. 2004; Manel et al 2005). However, their applicability across a broad range of tree species may be limited by a variety of considerations (Berry et al. 2004; He et al. 2004). First, the typical microsatellite study, based on natural populations, uses less than 13 loci (Berry et al. 2004), which might not provide sufficient genetic resolution to identify source populations. Second, genetic composition of the set of populations being considered needs to differ sufficiently from each other to uniquely identify the source population (Smouse and Chevillon 1998), but recently derived fragments may not be sufficiently different, being remnants of the same parental population. Third, accurate assignment requires enumeration and sampling of all source populations within a prescribed area, and that can be a large challenge. In spite of its limitations, we mention this approach before closing, because many landscape ecologists who work on large spatial scales may benefit from this genetic method, particularly where our interest is in long-distance dispersal.

One illustrative example is the case of dispersal in a metapopulation ($\sim$ 12 km^2 area) of *Banksia hookeriana*, a fire-adapted shrub in southwestern Australia (He et al. 2004). Based on 221 individuals from 21 neighboring dune populations, and using 175 AFLP markers, they assigned 77% of the individuals to their local populations, 3% to one of the other populations, 4% to a population outside the metapopulation (at least >1.6 km away), with 16% unassigned. This example illustrates the important role of inter-population seed dispersal in providing connectivity within the framework of an extensively distributed metapopulation, as it relates to a fire-prone habitat. This method, when applied to study systems that meet the assumptions of these models and succeed in accurately assigning individuals to a population, could provide significant detail on the shape and tail of the seed dispersal curve. In fact, the combination of the assignment methods with parentage or TwoGener approaches could provide excellent information on local and long-distance dispersal, but the cost in terms of sampling effort may be large.

Conclusions

Landscape connectivity is an important concept for landscape ecologists and population geneticists.

A genetic analysis of landscape connectivity for plants includes both pollen and seed movement. Significant work has been done on pollen flow across a variety of landscapes. We agree with Hamrick (2004), who emphasizes the resilience of tree populations to being able to maintain diversity in the face of environmental change, because of (a) existing genetic diversity within populations, and (b) evidence that pollen-mediated gene flow among populations is high in many tree species. We should stress, however, that the extent of resilience to fragmentation is determined by both the amount of gene flow and the diversity of the pollen pool from which immigration is drawn. Thus, as populations become isolated, they are at future risk for loss of diversity, but to date, empirical studies of pollen flow into fragments are encouraging.

The state of the art on seed-mediated gene movement in contemporary populations lags far behind that for pollen movement, because it is more difficult to find dispersed seeds than dispersed pollen, and the logistics of distinguishing maternal plants are challenging. Notwithstanding the difficulties, seed dispersal is essential for colonization of new sites, and we need to know a great deal more than we do now. The existing evidence of seed dispersal in fragmented landscapes indicates seed dispersal can go beyond local sites (e.g., Aldrich and Hamrick 1998, Sezen et al. 2005), but genetic bottlenecks may result from limited numbers of seed sources in the colonizing pool. We need many more studies on movement of dispersal vectors, the effect of landscape characteristics, landscape change, and landscape fragmentation on them, and the consequences of losing native dispersal agents.

We have learned a great deal about pollen movement and a little about seed movement across extended landscapes, but we know little about the combined effects of the two processes. If seed dispersal creates bottlenecks at the time of colonization of fragments, subsequent high pollen flow might eventually mitigate the low genetic diversity, if the number of available pollen sources is not constrained. If pollen flow is extensive and results in seeds and seedlings, then seed dispersal from elsewhere that comes from just a few sources will not create a genetic bottleneck in the seedling pool. Beyond the interaction of pollen and seed flow is the interaction of pollen and seed flow with the landscape itself. So far, most of the work has neglected the landscape quality within and among fragments. Landscape quality may affect gene flow processes dramatically. Thus, we are still far from an effective integration of population genetics and landscape ecology. We will need that integration if we are to model the impact of changing landscapes on propagule movement, with its all of its ensuing consequences for genetic connectivity and demographic processes.

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Appendix A: The neighborhood model of parentage analysis

Here we provide a brief recap of the formal logic behind the 'Neighborhood' model of paternity analysis. Consider the ith focal female, whose genotype is designated F_i, her jth offspring, whose genotype is designated O_{ij}, and the kth candidate male, whose genotype is designated M_k. We sample and genotype all of the candidate males ($k = 1, \ldots, K$) within a circle of chosen radius (say 100 m) of the index female, and sample and measure her distance to each of those males (z_{ik}). The candidate males outside the circle are not exhaustively genotyped, though we sometimes collect a sample of adults for population characterization.

The standard strategy is to build a complex likelihood function that includes differential male reproductive contributions (usually dependent on inter-mate distances), a provision for the rate of selfing (s), and an immigration rate (m) that describes the fraction of male parentage that comes from outside the circle. A typical likelihood function for the jth seed of the ith mother, allowing for all males (both the specifically sampled males from inside the neighborhood and those from outside), takes the form:

$$\begin{aligned}&\Pr(O_{ij}|M_i,\text{all possible males})\\&=s\cdot\Pr(O_{ij}|M_i\equiv F_i)\\&+(1-m-s)\cdot\sum_{K=1}^{K}\lambda_{ki}\cdot\Pr(O_{ij}|M_i\neq F_k)\\&+m\cdot\Pr(O_{ij}|M_i,\text{outsidepollenpool}).\end{aligned}\qquad(A1)$$

Now, λ_{ki} is the relative contribution of the kth male to the ith female, a measure of his overall mating success, as well as a function of the inter-mate distance. The $\Pr(O_{ij}|M_i\equiv F_i)$,$\Pr\ (O_{ij}|M_i\neq F_k)$, and $\Pr(O_{ij}|\ M_i$,outside pollen pool) are the mendelian probabilities of obtaining the genotype O_{ij} from the maternal genotype M_i, given a male gamete provided by selfing, by outcrossing from within the neighborhood, and by drawing a pollen grain from an external (Hardy–Weinberg) gene pool, respectively. The joint probability of all N progeny is

$$\begin{aligned}&\Pr(\text{of all } O_{ij},\ j=1,\ldots,N)\\&\quad=\prod_{N}^{j=1}\Pr(O_{ij},\text{all possible males}),\end{aligned}\qquad(A2)$$

since each offspring is an independent sampling event. We optimize (A2) for the choice of all the parameters simultaneously. This approach is called the 'neighborhood model' (see reviews in Burczyk et al. 2004; Smouse and Sork 2004). For our purpose of measuring connectedness, the immigration parameter m is the primary target, but we must simultaneously estimate the selfing rate, the variation in male mating success rates, and the distance function as well.

We can use similar methods to model the likelihood of maternal contributions to a seed pool collection. Suppose we locate and genotype the jth seed at some reference position on the landscape (a patch of recruits, a seed cache, or a forest fragment), and can genotype and map all of the potential mothers (seed donors) in the neighborhood (or fragment). We can replace Eq. (A1) with the likelihood that the jth seed has the ith maternal genotype as

$$\begin{aligned}&\Pr(O_j|\text{ all possible females})\\&=(1-m)\cdot\sum_{i=1}^{I}\lambda_i\cdot\Pr(z_{ij})\\&\quad+m\cdot\Pr(O_j=M_i|\text{outside maternal pool}).\end{aligned}\qquad(A3)$$

The λ_i are the relative seed-fecundities of the respective females, and $\Pr(z_{ij})$ describes the declining probability of seed transport with increasing distance. Here, the interest is in m, but we must jointly estimate the λ's and the form of $\Pr(z_{ij})$, typically a declining exponential function of distance. The likelihood equation is proportional to the product of Eq. (A3), over all sampled seeds within the neighborhood. Because we have poor knowledge of the 'outside' maternal candidates and no information on their precise locations, we assume that immigrant seed is drawn at random from a Hardy–Weinberg gene pool.

Appendix B: Twogener analysis of pollen structure

Here we develop an analysis of 'pollen structure' within a population, a measure of the divergence of pollen pools sampled by different mothers, without attempting to determine the male parentage of any particular offspring. We sample a series of seed-donor (maternal) individuals, spread out across the landscape, far enough apart that they should be sampling different arrays of pollen donors. From each mother, we sample as set of offspring (seed), and we genotype all the mothers and seed.

The first task is to infer the male gamete from the diploid genotypes of the mother and the seed. For L genes, each male gamete has an L-locus gametic genotype. For any pair of male gametes, whether they are sampled from the same mother or different mothers, we compute a squared intergametic distance. For the lth gene, the squared distance between the hth and jth male gametes is '0' if they have the same allele and '1' otherwise. The squared genetic distance between any two male gametes is simply the sum over all L genes of the '0's and '1's, so $0 \le {d_{hj}}^2 \le L$. For example, two male gametes of genotypes $A_1B_3C_2D_4E_5$ and $A_1B_2C_4D_4E_1$, respectively, have a squared intergametic distance of ${d_{hj}}^2 = (0 + 1 + 1 + 0 + 1) = 3$, a Manhattan (city block) metric that is Euclidean. There are cases where the mother and seedling are of identical heterozygous genotype for some locus (for example, A_1A_3), and we cannot determine which parent contributed which allele. The genetic distance is between '0' and '1' for that locus (see Smouse et al. (2001) for more detail), a

nuance that requires attention but that does not alter the logic to follow. Taking the $N\cdot(N-1)$ pairs of male gametes in the study, we construct an N-dimensional square matrix of genetic distances, **D**, organized into G maternal sibships, thusly

$$\boldsymbol{D} = \begin{vmatrix} \mathbf{D}_{11} & \mathbf{D}_{12} & \mathbf{D}_{13} & \dots & \mathbf{D}_{1G} \\ \mathbf{D}_{21} & \mathbf{D}_{22} & \mathbf{D}_{23} & \dots & \mathbf{D}_{2G} \\ \mathbf{D}_{31} & \mathbf{D}_{32} & \mathbf{D}_{33} & \dots & \mathbf{D}_{3G} \\ \dots & \dots & \dots & \dots & \dots \\ \mathbf{D}_{G1} & \mathbf{D}_{G2} & \mathbf{D}_{G3} & \dots & \mathbf{D}_{GG} \end{vmatrix} \quad \text{(B1)}$$

where the diagonal block $\mathbf{D}_{33}$ (for example) contains the genetic distances between male gametes sampled from the 3rd mother, and where the off-diagonal block $\mathbf{D}_{1G}$ (for example) contains the genetic distances between male gametes sampled from the 1st mother and those from the Gth mother. The point of the exercise is to partition the total pollen pool variation into separate components for variation within mothers and variation among mothers. Our distance measure is Euclidean, by construction, and the sum of all the elements within the matrix, without regard to sibship, yields the sum of squared deviations for the total collection of male gametes in the study, barring a sample size constant. Formally, we have $\mathrm{SSD(Total)} = \mathrm{sum}(\mathbf{D}) \div 2N\cdot(N-1)$. If there are J_i offspring in the ith maternal sibship, then the within-maternal sibship (within-mother) sum of squared deviations is

$$\mathrm{SSD(Withiin\ Sibship)} = \sum_{i=1}^{G} \frac{\mathrm{sum}(D_{ii})}{J_i\cdot(J_i-1)}, \quad \text{(B2)}$$

and the among-sibship (among-mother) sum of squared deviations is measured as the difference,

$$\mathrm{SSD(Among\ sibship)} = \mathrm{SSD(Total)} - \mathrm{SSD(Within\ Sibship)}. \quad \text{(B3)}$$

We have exactly what we need for an Analysis of Molecular Variance (Excoffier et al. 1992). This method is standard in population genetics, and sums of squared deviations are converted into mean squared deviations, and from there into estimated variance components within and among maternal sibships, ${\sigma_\mathrm{W}}^2$ and ${\sigma_\mathrm{A}}^2$, respectively. The final step is to compute the fraction of the inter-male-gamete variation that is accounted for by differences in the maternal draw of pollen, specifically $\Phi_\mathrm{ft} = {\sigma_\mathrm{A}}^2/({\sigma_\mathrm{W}}^2 + {\sigma_\mathrm{A}}^2)$, an analog of the 'population structure' measure of population divergence, F_st (see Cockerham and Weir 1993).

Using population genetic theory, we can extract two types of information from estimates of Φ_ft, a measure of the 'effective number of pollen donors' pollinating the average female (N_ep), and a measure of the average distance of effective pollen dispersal (δ). Essentially, (N_ep) is the effective number of pollen donors that would comprise a randomly mating population. Because pollen sources are not represented equally among progeny, this number is often much smaller than the absolute number of contributing pollen donors (Smouse and Sork 2004). If the sampled mothers are spaced far enough apart on the landscape that they draw pollen from completely different males, then we can translate $(N_\mathrm{ep}) \approx [\,1/2\ \Phi_\mathrm{ft}]$. If they are closer than that, we need small adjustments in the estimate (Austerlitz and Smouse 2001). To translate Φ_ft into an estimate of pollination distance, we must assume some family of pollen dispersal curves. The field is now moving toward generic use of the exponential power family of distributions, characterized by a scale (α) and a shape (β) parameter (Clark 1998)

$$\Pr(z_{ik}|\alpha,\beta) = \frac{\beta}{2\pi\alpha^2\Gamma(2/\beta)}\cdot\exp\left\{-\left(\frac{z_{ik}}{\alpha}\right)^{\beta}\right\} \quad \text{(B4)}$$

where z_{ik} is the inter-mate distance, and $\Gamma(\cdot)$ classic gamma function (Abramowitz and Stegun 1964). To estimate the α and β parameters, we compare the separation of all pairs of maternal sibships, and use numerical integration to solve for the estimates (Austerlitz et al. 2004). The estimates of α and β are converted to an estimate of the average pollination distance is $\delta = \alpha\cdot\Gamma(3/\beta)\div\Gamma(2/\beta)$ (Abramowicz and Stegun 1964).

An extension to seed structure requires only two small changes: (i) a change in the definition of sampling strata, and (ii) a change from haploid scoring of pollen genotypes to diploid scoring of maternal (seed coat) genotypes. Here, the sampling strata are the spatial sampling units from which seeds are collected, typically seed patches, seed caches, or seed traps. The individual seeds represent the replication within strata. Relative to genotypic scoring, Peakall et al. (1995) present a diploid scoring convention that is completely

analogous to that we have described above for haploid pollen genotypes.

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