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A TWO-GENERATION ANALYSIS OF POLLEN POOL GENETIC STRUCTURE IN FLOWERING DOGWOOD, *CORNUS FLORIDA* (CORNACEAE), IN THE MISSOURI OZARKS[1]

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Anthropogenic landscape change can disrupt gene flow. As part of the Missouri Ozark Forest Ecosystem Project, this study examined whether silvicultural practices influence pollen-mediated gene movement in the insect-pollinated species, *Cornus florida* L., by comparing pollen pool structure (Φ_{st}) among clear-cutting, selective cutting, and uncut regimes with the expectation that pollen movement should be least in the uncut regime. Using a sample of 1500 seedlings—10 each from 150 seed parents (43 in clear-cut, 74 in selective, and 33 in control sites) from six sites (each ranging from 266 to 527 ha), eight allozyme loci were analyzed with a pollen pool structure approach known as TWOGENER (Smouse et al., 2001; *Evolution* 55: 260–271). This analysis revealed that pollen pool structure was less in clear-cut ($\hat{\Phi}_C = 0.090$, $P < 0.001$) than in uncut areas ($\hat{\Phi}_U = 0.174$, $P < 0.001$), with selective-cut intermediate ($\hat{\Phi}_S = 0.125$, $P < 0.001$). These estimates translate into more effective pollen donors (N_{ep}) in clear-cut ($N_{ep} = 5.56$) and selective-cut ($N_{ep} = 4.00$) areas than in uncut areas ($N_{ep} = 2.87$). We demonstrate that $\Phi_C \leq \Phi_S \leq \Phi_U$, with $\hat{\Phi}_C$ significantly smaller than $\hat{\Phi}_U$ ($P < 0.034$). The findings imply that, as long as a sufficiently large number of seed parents remain to provide adequate reproduction and to avoid a genetic bottleneck in the effective number of mothers, silvicultural management may not negatively affect the effective number of pollen parents, and hence subsequent genetic diversity in *Cornus florida*.

Key words: California; *Cornaceae*; gene flow; genetic structure; landscape change; pollen movement; silvicultural treatment; TWOGENER.

Landscape alteration can influence the amount and structure of genetic variation within plant populations by reducing the sizes of local populations, changing the level of gene flow among them, or reducing the amount of genetic diversity (Ledig, 1992; Ellstrand and Elam, 1993). Population fragmentation, in particular, has raised much concern (Saunders et al., 1991; Forman, 1995), but less dramatic forms of landscape change may also affect the genetic structure of populations. Increasingly, silviculture is being practiced with a forest ecosystem management approach (Oliver, 1992; Christensen et al., 1996; Carey, 1999), which may also have genetic consequences. Practices such as clear-cutting and stand thinning may change stand structure in ways that influence pollen movement across the landscape by modifying the aerodynamics of wind pattern (e.g., Okuba and Levin, 1989), influencing pollinator behavior of insects (e.g., Handel, 1983; Morris, 1993) or causing changes in the species of animals that pollinate plants (e.g., Herrera, 1995).

Studies of gene flow via wind and insect pollinators in altered landscapes show different patterns of results. For wind-pollinated species, pollen-mediated gene flow among fragmented forest populations (Fore et al., 1992; Young and Merriam, 1994) and within clear-cut vs. thinned forest stands (Robledo-Arnuncio, 2004) seems to be greater when forest cover is reduced. However, we do not know whether fragments or the individual trees with a greater degree of isolation would show the same result. For insect-pollinated species, the impact of landscape change caused by fragmentation or silvicultural practice seems to depend on the idiosyncrasies of the behavior of pollinators and/or the composition of pollinators in response to changes in forest canopy structure. In tropical systems, we can find examples where fragmentation sometimes promotes long-distance pollen movement (e.g., *Dinizia excelsa* in Brazil (Dick et al., 2003) and *Spondias mombin* in Costa Rica (Nason and Hamrick, 1997)). Conversely, we also observe cases where pollen-mediated gene flow is reduced for solitary pasture trees (e.g., *Enterolobium cyclocarpum* (Rocha and Aguilar, 2001) and *Pachira quinata* (Fuchs et al., 2003)). Thus, the key issue is whether and how the specific form of landscape alteration influences the availability of adult plants and the pollinator community.

In this paper, we examine contemporary pollen movement

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in *Cornus florida* L., an understory, insect-pollinated species of North American temperate deciduous forests. *Cornus florida* is pollinated by generalist pollinators, primarily by andrenid and halictid bees (Mayor et al., 1999), but also by beetles, flies, and butterflies (Eyde, 1988). These pollinators might be sensitive to changes in forest canopy and conspecific adult densities. For example, Reese and Barrows (1980) found that the bee, *Andrena erigeniae* (Andrenidae), preferred sunny and partly sunny flower patches on the east-facing slope, but not on the west-facing slope and that foraging females tended to concentrate on plots with the densest flowers. Previous work in the Missouri Ozark study region of this paper indicates that biparental inbreeding in *Cornus florida* is higher on north-facing slopes, where the physical environment is cooler and more mesic, the canopy area is smaller, and the adult density is higher, than on south-facing slopes (Bailey, 2002). This information on both the pollinators and the outcrossing rate of this species indicates the potential impact of silviculture on pollen-mediated gene movement.

Our goal here is to examine the impact of forest management on *C. florida*, as part of the Missouri Ozark Forest Ecosystem Project (MOFEP). This landscape-scale experiment was conducted by the Missouri Department of Conservation to test whether selective-cutting or clear-cutting of small areas affects forest dynamics or the species occupying the Ozark forest ecosystem (Brookshire and Hauser, 1993; Brookshire et al., 1997). We have three specific objectives. First, we will test the hypothesis that silvicultural treatment, imposed as part of the MOFEP design, affects pollen pool structure (as measured by the statistic Φ_{st}) and the effective number of pollen parents (N_{ep}). This N_{ep} estimate is equivalent to the pollen movement portion of Wright's (1943) effective neighborhood size that is due to variation in pollen dispersal (Austerlitz and Smouse, 2001a, 2002). Specifically, we predict that removal of trees for stand thinning in the MOFEP silvicultural treatments will promote pollen movement. To test this hypothesis, we introduce a new test for detecting differences among treatments. Second, we test whether our estimates of Φ_{st} are biased by the presence of adult spatial structure, through adult inbreeding, by using the correction for inbreeding derived by Austerlitz and Smouse (2001b). Third, to correct for the effect of adult spatial structure, we apply a new approach developed by Dyer et al. (2004) referred to as StAMOVA, which removes the effects of environmental gradients in adult genotypes from the AMOVA estimate of pollen pool structure.

MATERIALS AND METHODS

Study site—The Missouri Forest Ecosystem Project (MOFEP) study sites are located across Carter, Reynolds, and Shannon counties in southeastern Missouri, USA. The contemporary forest consists of various species of oak (*Quercus alba, Q. coccinea, Q. stellata, Q. velutina*), hickory (*Carya tomentosa, C. glabra*) and shortleaf pine (*Pinus echinata*) (Cunningham and Hauser, 1989). The MOFEP experimental design deploys two treatment types, which they refer to as uneven-age management (selective cutting) and even-age management (clear-cutting), and a third category of no harvest management (uncut control) (Brookshire and Hauser, 1993). All three treatments were done three times across nine sites, ranging in size from 266 to 527 ha (Sheriff and He, 1997). MDC implemented the two cutting regimes in 1996, according to MDC Forest Land Management Guidelines (1986), by harvesting approximately 10% of the standing biomass for the sites receiving a silvicultural treatment and leaving 10% of the area without any timber harvest to represent ''old growth'' forest, with no timber harvest. For the selective cut (uneven-age management), 5% of the timber was harvested by single-tree selection to balance tree size classes and 5% of the timber was harvested by selecting groups of trees to create openings (21–43 m in diameter) that could promote regeneration. For the clear-cut treatment (even-age management), the practice is to harvest all trees within each of six to nine areas of 3–12 ha per treatment site. The MOFEP design reduced overall density of trees in the two cutting treatments so that their total biomass across treated sites was similar, but in a different spatial pattern (Kabrick et al., 2002).

Study species—*Cornus florida* L. (flowering dogwood) is an understory, insect-pollinated, self-incompatible woody plant. The flowering period occurs from late March through April (Radford et al., 1968). The inflorescence consists of four white or cream bracts subtending 15–35 individual, perfect flowers, each with a single ovule. Andrenid and halictid bees are likely to visit *C. florida*, primarily andrenid and halictid bees, as well as beetles, flies, and butterflies (Eyde, 1988). A single *C. florida* inflorescence contains multiple flowers that are not open simultaneously, which can be visited by multiple pollinators. Each inflorescence matures up to eight drupes (V. Apsit and V. Sork, personal observation) that turn bright red as they ripen during late September and October and are dispersed by birds, mammals, and gravity (McLemore, 1990). Previous studies indicate that *Cornus florida* is essentially 100% outcrossed, with about 2% mating among relatives, but with some variation among sites (Apsit et al., 2002; Bailey, 2002).

Field sampling—In 1998, we selected 252 trees on ridge tops and south- to southwest-facing slopes, spread across sites 1–6 in the MOFEP study area (Fig. 1). We chose to sample the northern MOFEP sites only because the southern three sites are sufficiently different among themselves and from the northern sites in stand density and stand history (Brookshire and Shifley, 1997) that those differences would confound differences caused by the silvicultural treatments. Moreover, the sampling design of our study does not require the additional replication. While the MOFEP design works well for taxa, such as birds, for which a very large landscape scale is necessary, that scale is much too large to estimate pollen flow. Our analytical methods emphasize individual mother trees, spread over a spatial scale that is encompassed within a single MOFEP site, and we used two sites per treatment to increase sample size of individual trees (see description of statistical design later).

Within each of the six study sites, we sampled trees in localized clusters of 2–5 trees, to produce sets of trees with relatively close near-neighbors. Each cluster contained individuals with a minimum pairwise distance of 5–10 m and a maximum inter-tree distance of 100 m between trees within a cluster. In the control sites without harvest treatment (sites 1 and 6), we sampled clusters of trees in scattered locations within a site. In the selective-cut treatment, referred to as uneven-age treatment by MOFEP (sites 2 and 4), we sampled trees within and proximal to the small group cuts (~20–40 m in diameter). For the clear-cut treatment, called even-age treatment by MOFEP (sites 3 and 5), we sampled trees adjacent to and no further than 50 m from the clear-cut patch to evaluate the effect of this type of treatment on pollen movement. No *Cornus* adults were present in the clearings themselves. In the selection of trees within each of the sites across all treatments, we chose trees from throughout the site, leaving a buffer area of at least 200 m from adjacent MOFEP sites.

In Fall 1998, we sampled fruit from multiple infructescences, attempting to collect a sufficient number of fruits to ensure 25 germinants for each maternal tree, but germination was highly variable across seed parents (Apsit et al., 2002). During spring 1999, we collected fresh leaf material from all adults, for identification of maternal genotypes. For the analyses presented here, we selected 150 seed parents, for which we completed a battery of genotypic assays on 10 seedlings: 43 parents in clear-cut sites; 74 in selective-cut sites; and 33 in uncut control sites. The total sample size for our analyses was 1500 seedlings and 150 seed parents.

Laboratory analysis—After germination, we collected freshly harvested leaf material from young germinants and extracted plant enzymes by grinding the leaf material in 1 mL of a modified phosphate buffer (Alvarez-Buylla and Garay, 1994) with a mortar and pestle, absorbing the exudate onto chroma-

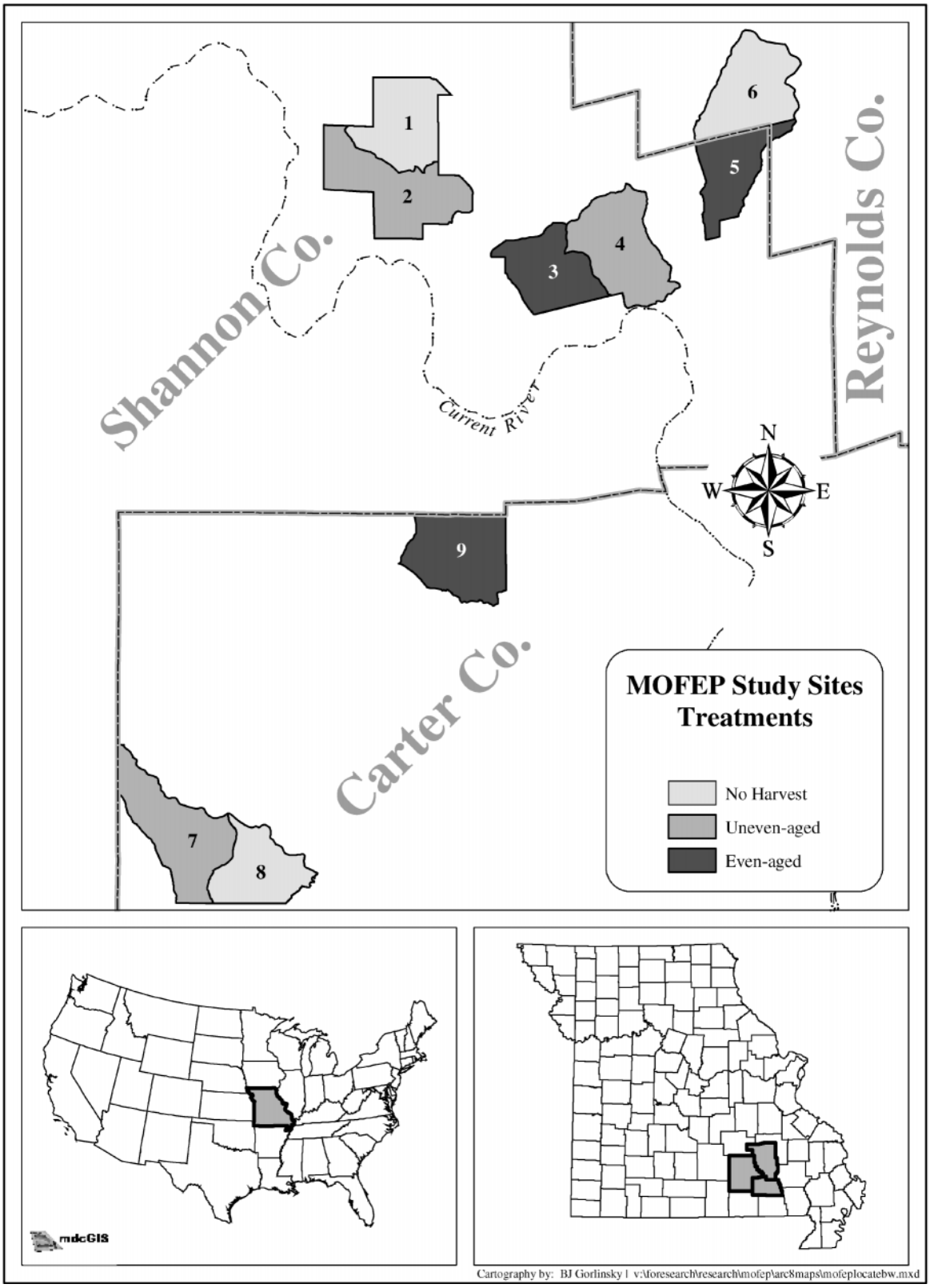

Fig. 1. Map of the treatment sites of the Missouri Ozark Forest Ecosystem Project (MOFEP). Lower left panel indicates location of Missouri within USA. Lower right panel indicates location of MOFEP within state of Missouri.

tography paper wicks, and storing the wicks at −70°C. We identified eight polymorphic allozyme loci using standard techniques for starch gel electrophoresis (Soltis, 1983; Kephart, 1990; Sork et al., 1993). On the Soltis et al. (1983) modified system 8 gel/electrode buffer, we assayed fluorescent esterase (*Fe*, E.C. 3.1.1.1) and triosephosphate isomerase (*Tpi*, E.C. 5.3.1.1). On morpholine citrate pH 8.0 buffer system (Soltis, 1983), we assayed aconitase-1 and −2, (*Aco*, E.C. 4.2.1.3), isocitrate dehydrogenase (*Idh*, E.C. 1.1.1.42), malic enzyme-1 (*Me-1*: E.C. 1.1.1.38), and phosphoglucomutase (*Pgm-2*, E.C. 2.7.5.1). We also determined the maternal genotypes for each of these loci. A complete listing of maternal genotypes, along with their global positioning system (GPS) coordinates is archived on the Missouri Department of Conservation website for the Missouri Ozark Forest Ecosystem Project (http://mofep.mdc.state.mo.us/).

Pollen pool statistical analyses*—Basic analytical framework*—We are primarily interested in the spatial pattern of genetic variation among pollen genotypes, i.e., we are interested in a ''pollen's eye spatial view'' of the landscape. Given that viewpoint, the seed parents can be considered as conveniently placed biological pollen traps and the pollen parents as spatially distributed pollen point sources. The statistical analyses reported here are variations on the general AMOVA theme of Excoffier et al. (1992), the general point of which is to partition the available genetic variation among various potential factors/sources. The parameter we estimated is Φ_{ft}, a measure of genetic differentiation among females in an analogous way that AMOVA estimates Φ_{st} as a measure of the population differentiation among populations. The essential change from standard analyses of variance is that significance testing is accomplished via permutational shuffling of individuals among strata, computing a nonparametric (empiric) null distribution, rather than by recourse to standard (normal or chi-square) statistical theory and classical tests, because the genetic variables themselves are not normally distributed.

Progeny analysis—The compartments, and the management treatment areas within them, are large enough internally and far enough apart physically that they are effectively drawing pollen from non-overlapping sets of males (Sork et al., 1998; Smouse et al., 2001). We therefore decided to ignore the remote possibility of pollen movement among sites, and our analysis of pollen flow is conducted strictly within a treatment area. For the analysis, we have 10 male gametes nested within each seed parent. We have subsampled the seed-bearing adults, obtaining a collection of 150 adults for a single year, 1998. Because pollen dispersal in a single year does not extend beyond the scale of a single site, we conducted a pooled within-site analysis for each treatment. That amounts to ''centering'' the male gametic genotypes on the pollen pool allele frequencies for each site separately, removing small differences (see Table 1) in the average pollen pool allele frequencies among sites; we are not comparing the pollen pools of mothers in different MOFEP sites. The analysis is strictly ''within-site,'' which is the appropriate scale for the localized pollen flow that characterizes this situation.

Comparison of Φ_{ft} values—We characterize the divergence of the pollen pools of different seed parents, spaced out across the landscape, by estimating the intraclass correlation coefficient for variation in paternal gametic variation within seed parents, formally defined as

$$\hat{\Phi}_{ft} = \frac{\hat{\sigma}^2_A}{\hat{\sigma}^2_W + \hat{\sigma}^2_A}, \tag{1}$$

where $\hat{\sigma}^2_A$ is an estimate of the among-mother (seed parent) genetic variation in male gametes and $\hat{\sigma}^2_W$ is an estimate of the corresponding within-mother (seed parent) variation. We have described previously how to construct an empiric null distribution for the hypothesis, $\Phi_{ft} = 0$, by permuting seedlings among seed parents (Smouse et al., 2001). The analytical challenge here is larger; we are attempting to determine whether different silvicultural regimes yield the same or different levels of pollen pool structure. It is conceivable, for example, that Φ_{ft} would be non-zero for each of the management alternatives but that the values would not differ among them. We need a test of the divergence in the Φ_{ft} coefficients of the three management regimes that does not depend on the average value of the Φ_{ft} coefficient. At this time, no formal statistical test exists that would allow us to do that. Moreover, in this particular case, the alternative hypothesis is one-tailed, because we anticipate that stand thinning should promote pollen movement. The earlier work by Bailey (2002) strongly suggests that we should anticipate that Φ_U (uncut control) $> \Phi_S$ (selective cut) and that Φ_S (selective cut) $> \Phi_C$ (clear-cut), and we need tests that will allow us to evaluate such one-tailed alternative hypotheses.

To motivate the construction of such tests, recall that, for TWOGENER analysis, the estimates of the variance components and of Φ_{ft} are contained within the deviations of the sampled maternal pollen pools from the average pollen pool of the collection of all seed parents under examination. These deviations are encapsulated in the sums of squares (among seed parents, SSA), relative to the deviations of the individual pollen genotypes from the average pollen pool drawn by a single seed parent, which are encapsulated in SSW (within seed parents). For the j^{th} seed parent in the i^{th} site, call those deviations A_{ij} and W_{ij}, respectively, we need a test that preserves those estimated deviations, but which permutes them among management treatments. We are not testing the hypothesis that the deviations among seed parents are zero, but rather the hypothesis that the relative values (however large or small they may be) of the variance among seed parents (V_A) and the variance within seed parents (V_W), are the same for different management treatments.

To extract the A_{ij} and W_{ij} from our $N \times N$ genetic distance matrix, D, we might first convert it into a corresponding covariance matrix, C, as described in Smouse and Peakall (1999), and then manipulate the diagonal elements in various ways. In the current situation, it is easier to compute the A_{ij} and W_{ij} directly, using vector multiplication methods in Irwin et al. (2003). We define the vector of genetic variables for the k^{th} male gamete for the j^{th} seed parent in the i^{th} site as $\underline{Y}_{ijk}$, the mean vector for the $K = 10$ male gametes sampled by that seed parent as $\underline{\bar{Y}}_{ij\cdot}$ and the mean vector for the entire collection of male gametes, for all seed parents in a particular site, as $\underline{\bar{Y}}_{i\cdot\cdot}$. There are 28 alleles for the eight loci (see Table 1), so these vectors are of length 28. We compute the A_{ij} and W_{ij} as:

$$A_{ij} = K(\bar{Y}_{ij\cdot} - \bar{Y}_{i\cdot\cdot})'(\bar{Y}_{ij\cdot} - \bar{Y}_{i\cdot\cdot}) \quad \text{and}$$

$$W_{ij} = \sum_{k=1}^{K} (Y_{ijk} - \bar{Y}_{ij\cdot})'(Y_{ijk} - \bar{Y}_{ij\cdot}). \tag{2}$$

For the j^{th} seed parent in the i^{th} site, we have an estimated A_{ij}, describing its deviation from the overall genetic center of the total pollen pool for that site, in addition to an estimated W_{ij}, describing the sums of squared deviations among pollen genotypes sampled by that j^{th} seed parent. By centering the A_{ij} and W_{ij} on the site-specific averages, we remove the comparison of pollen pools from different sites from consideration, because all measurements are deviations from the site average. We have 33, 74, and 43 (A_{ij}, W_{ij}) pairs for the uncut control, selective-cut, and clear-cut treatments, respectively, with the inter-site differences suppressed.

The sums of squares within and among seed parents (within treatments) can be rewritten as the sums of the W_{ij}'s and A_{ij}'s for a particular treatment,

$$\text{SS(within seed parents)} = \sum_{j=1}^{33} W_{ij}, \quad \sum_{j=1}^{74} W_{ij}, \quad \text{and} \quad \sum_{j=1}^{43} W_{ij} \quad \text{and} \tag{3a}$$

$$\text{SS(among seed parents)} = \sum_{j=1}^{33} A_{ij}, \quad \sum_{j=1}^{74} A_{ij}, \quad \text{and} \quad \sum_{j=1}^{43} A_{ij}, \tag{3b}$$

respectively, for the uncut control, selective-cut, and clear-cut treatments. Our estimates of the pooled sums of squares are obtained by adding across treatments,

$$\text{SS(pooled, within seed parents)} = \sum_{j=1}^{33} W_{ij} + \sum_{j=1}^{74} W_{ij} + \sum_{j=1}^{43} W_{ij}, \quad \text{and} \tag{4a}$$

$$\text{SS(pooled, among seed parents)} = \sum_{j=1}^{33} A_{ij} + \sum_{j=1}^{74} A_{ij} + \sum_{j=1}^{43} A_{ij}, \tag{4b}$$

yielding pooled estimates that are fed into the usual AMOVA formula, yield-

ing a pooled estimate, $\bar{\Phi}_{ft}$, that does not depend on which mothers are randomly assigned to which treatment set because we have suppressed the site-to-site differences.

To obtain an empiric null distribution for the null hypotheses ($\Phi_U = \Phi_S$), ($\Phi_S = \Phi_C$), and ($\Phi_U = \Phi_C$), against which to compare our directional (one-tailed) alternative hypotheses ($\Phi_U > \Phi_S$), ($\Phi_S > \Phi_C$), and ($\Phi_U > \Phi_C$), we shuffle the 150 W_{ij}'s into three sets, of sizes 33, 74, and 43, respectively, without replacement. Independently, we shuffle the 150 A_{ij}'s into those same three sets, again without replacement. Now, $\bar{\Phi}_{ft}$ is invariant with respect to permutation of the W_{ij}'s and A_{ij}'s among treatments, so our test of treatment differences holds the general level of divergence among seed parents constant at the observed value. We repeat this process 999 times, using the actual partition as the 1000th replicate, on the premise that if the null hypothesis were correct, the data would constitute just another random shuffle. For each of the 1000 replicates, we compute $\hat{\Omega}_{US} = (\hat{\Phi}_U - \hat{\Phi}_S)$, $\hat{\Omega}_{SC} = (\hat{\Phi}_S - \hat{\Phi}_C)$ and $\hat{\Omega}_{UC} = (\hat{\Phi}_U - \hat{\Phi}_C)$, thereby constructing an empiric null distribution for each of these criteria. We have pointed out elsewhere (Smouse et al., 2001) that the Φ_{ft} values have variances that are inversely proportional to the number of mothers, everything else being equal. Given our sample size differences ($J_S = 74$, $J_C = 43$, $J_U = 33$), we anticipate that the null-hypothesis confidence intervals for the $\hat{\Omega}$- criteria will be smallest for the test of $\hat{\Omega}_{SC} = (\hat{\Phi}_S - \hat{\Phi}_C)$, largest for the test of $\hat{\Omega}_{UC} = (\hat{\Phi}_U - \hat{\Phi}_C)$, and intermediate in size for the test of $\hat{\Omega}_{SC} = (\hat{\Phi}_S - \hat{\Phi}_C)$. Each of the test criteria is compared with its own, empirically determined confidence interval. By construction, $\hat{\Omega}_{UC} = \hat{\Omega}_{US} + \hat{\Omega}_{SC}$, which means that the three tests are not independent. The standard Bonferroni adjustment procedure assumes independence, but by evaluating all three criteria simultaneously under random permutation of the A_{ij}'s and W_{ij}'s, we were able to establish an empiric ''experimentwise α value'' (the probability that at least one of the tests would achieve its indicated α level). We will refer to this test of differences in Φ values across groups as the A&W test.

Correcting for adult spatial structure—From a ''pollen's vantage point,'' we have no abiding interest in the adults themselves, but having said that, it is important to note that the pattern of pollen variation across the landscape could be a function of the spatial genetic patterns among the adults, so that the two signals might be confounded. We need to determine whether spatial genetic gradients exist for the adults that are confounded with the pollen patterns—the focus of our primary concern—and if so, to correct for the adult effects.

The presence of spatially arrayed ''genetic structure'' among the adults, reflecting both recent (human-dominated) and long-term evolutionary history of the population, is somewhat confounded by the pollen pool structure we are attempting to elucidate. Any spatial structure among the adults can inflate our estimate of Φ_{ft}. We proceed by examining the extent to which spatial coordinates for each maternal individual are predictive of the observed distribution of pollen donor haplotypes. This approach, dubbed StAMOVA (or stepwise analysis of molecular variance; Dyer et al., 2004) , seeks to remove the effects of external variables before decomposing the total observed genetic variation into components, representing within- and among-mother components. The external variables used for the *C. florida* study are the spatial coordinates (north and west), as well as the elevation. We standardize these variables to mean zero and rotate them via a principal components rotation (Johnson and Wichern, 1992), prior to entering them into the model.

The StAMOVA approach follows the univariate stepwise regression procedure, whereby the most correlated predictor variable is entered into the model first and tested for significance. Then, subsequent predictor variables are entered into the model until the additional sums of squares associated with the predictor variable (e.g., the type III sums of squares) is no longer significant. After fitting all significant spatial predictor variables, the residual variation is then decomposed following the usual AMOVA variance decomposition, with the exception that the degrees of freedom must be adjusted for the spatial variables used for the regression. As explained in Dyer et al. (2004), significance is evaluated via permutation.

Correcting for adult inbreeding—Austerlitz and Smouse (2001b) have shown that inbreeding among adults will exacerbate the natural tendency for homogenization of a localized pollen draw for any given female, effectively reducing the number of pollen parents and inflating Φ_{ft}. Having observed significant biparental inbreeding in *Cornus florida* (Apsit et al., 2002; Bailey, 2002) that could create local genetic clustering in the adult population we then used the seed parent genotypes to estimate adult inbreeding (F_{IS}) for each treatment, based on two sites per treatment, using the Weir and Cockerham (1984) calculations in Arlequin software (Schneider et al., 1999). We used the AMOVA partition of the residuals to gauge the impact of local adult inbreeding (F_{IS}) on our estimates of Φ_{ft}. Austerlitz and Smouse (2001b) show that:

$$\hat{\Phi}_{ft} = \frac{[Q_0 - Q(\bar{z})](1 + F)}{2 - Q(\bar{z})(1 + F)}, \tag{5}$$

where Q_0 is the probability that two random male gametes, sampled from the same seed tree, are from the same pollen parent, and $Q(\bar{z})$ is the probability that two random male gametes, sampled from two different seed parents, an average distance of $\bar{z}$ apart, are from the same pollen parent, and where F is the inbreeding coefficient, estimated here by F_{IS}. Using data-generated estimates of $\bar{z}$ and F_{IS}, we extracted an adjusted estimate of pollen structure, $\tilde{\Phi}_{ft} = Q_0/2$, an estimate of the probability of identity by descent from two random male gametes, drawn from the same female, adjusted for inbreeding in the adults. What we were ultimately pursuing is $Q_0 = N^{-1}_{ep}$, which conveys information about the effective number of pollen donors for the average seed parent.

RESULTS

Allele frequencies—The allele frequencies for male gametes vary slightly from site to site (Table 1). Some of this variation is undoubtedly due to sampling variation, but an element of the variation is likely due to subtle gradients in allele frequencies across this extended landscape (Sork, unpublished result). These site-specific allele frequencies are used to ''center'' the A_{ij} values of the 150 seed parents to the genetic averages of the separate sites, for purposes of the A&W analyses.

Pollen structure analysis—*Management effects*—We first estimated the values of Φ_{ft} for each of the treatments and for the control (Table 2). The management regimes influence pollen structure in these populations. The estimate for the uncut control ($\hat{\Phi}_U = 0.174$, $P < 0.001$) is larger than that for the selective-cut regime ($\hat{\Phi}_S = 0.125$, $P < 0.001$), which, in turn, is larger than that for the clear- cut regime ($\hat{\Phi}_C = 0.090$, $P < 0.001$). After removing the inter-site variation, the average (across silvicultural treatments) is $\Phi_{ft} = 0.128$, $P < 0.001$. Converting to effective number of pollen donors, via the relationship $N_{ep} \cong (2 \cdot \Phi_{ft})^{-1}$ as described in Austerlitz and Smouse (2001a), we obtained first-approximation estimates of $N_{ep} \cong 2.87$, 4.00, and 5.56 for control, selective-cut, and clear-cut treatments, respectively, basically doubling the effective number of pollen donors under very open (as opposed to closed) canopy conditions.

We then evaluated the statistical differences among treatments. The formal A&W tests yield $\hat{\Omega}_{US} = 0.049$ ($p \leq 0.124$), $\hat{\Omega}_{SC} = 0.035$ ($p \leq 0.164$), and $\hat{\Omega}_{UC} = 0.084$ ($p \leq 0.034$). The directions of this result are those anticipated in advance, though only the control vs. clear-cut difference (between the silvicultural extremes) is formally significant at the $P = 0.05$ level. These are single-comparison p values, of course, and the ''experimentwise α value'' (the empiric probability of obtaining at least one difference of the size encountered) was $\alpha = 0.283$. The ''experiment'' (as a whole) is not formally signif-

TABLE 1. Allele frequencies for eight allozyme loci (*Aco-1, Aco-2, Fe-1, Fe-3, Idh, Me-1, Pgm-2, Tpi*) in flowering dogwood (*Cornus florida* L.) from the Missouri Ozarks.

Locus and Allele	Uncut Control			Selective-Cutting Regime			Clear-Cutting Regime		
	Site 1	Site 6	Mean	Site 2	Site 4	Mean	Site 3	Site 5	Mean
Aco-1									
3	0.012	0.006	0.009	0.000	0.012	0.007	0.000	0.000	0.000
4	0.000	0.013	0.006	0.000	0.000	0.000	0.000	0.004	0.002
5	0.988	0.981	0.985	0.997	0.976	0.985	1.000	0.992	0.995
7	0.000	0.000	0.000	0.003	0.012	0.008	0.000	0.004	0.002
Aco-2									
3	0.065	0.281	0.170	0.000	0.056	0.033	0.000	0.026	0.016
4	0.012	0.000	0.006	0.000	0.002	0.001	0.000	0.000	0.000
5	0.923	0.719	0.824	1.000	0.942	0.966	1.000	0.974	0.984
Fe-1									
2	0.006	0.000	0.003	0.000	0.000	0.000	0.000	0.004	0.002
3	0.994	1.000	0.997	1.000	1.000	1.000	1.000	0.996	0.998
Fe-3									
1	0.018	0.019	0.018	0.000	0.000	0.000	0.000	0.000	0.000
3	0.298	0.335	0.316	0.252	0.379	0.326	0.343	0.356	0.351
5	0.684	0.627	0.656	0.748	0.619	0.673	0.657	0.644	0.649
7	0.000	0.019	0.010	0.000	0.002	0.001	0.000	0.000	0.000
Idh									
1	0.000	0.006	0.003	0.000	0.002	0.001	0.000	0.000	0.000
2	0.018	0.006	0.012	0.000	0.005	0.003	0.000	0.004	0.002
3	0.953	0.975	0.964	0.945	0.963	0.955	0.994	0.955	0.970
5	0.029	0.013	0.021	0.055	0.030	0.041	0.006	0.041	0.028
Me-1									
2	0.006	0.025	0.015	0.032	0.016	0.023	0.025	0.019	0.021
4	0.953	0.849	0.903	0.891	0.898	0.895	0.855	0.879	0.870
5	0.023	0.088	0.055	0.045	0.057	0.052	0.046	0.063	0.057
6	0.018	0.038	0.027	0.032	0.029	0.030	0.044	0.039	0.052
Pgm-2									
1	0.018	0.000	0.010	0.013	0.009	0.011	0.044	0.015	0.026
2	0.078	0.052	0.065	0.110	0.107	0.108	0.050	0.058	0.055
3	0.904	0.904	0.904	0.870	0.868	0.869	0.894	0.923	0.913
5	0.000	0.044	0.021	0.007	0.016	0.012	0.012	0.004	0.006
Tpi									
3	0.483	0.517	0.500	0.498	0.451	0.471	0.415	0.488	0.460
5	0.505	0.470	0.488	0.495	0.530	0.515	0.579	0.500	0.530
7	0.012	0.013	0.012	0.007	0.019	0.014	0.006	0.012	0.010

icant, but we are nevertheless encouraged by a pollen structure pattern that matches exactly what the stand structure had led us to anticipate. We suspect that with larger numbers of maternal trees per treatment, the differences encountered would have been statistically compelling. We will return to the issue of how to increase the power of this new test in the Discussion.

Correcting for adult spatial structure—We employed the StAMOVA analysis to gauge the extent to which the spatial location of the maternal individual explained the observed differentiation among pollen donors. The three-dimensional coordinates for each of the 150 seed parents (north, west, elevation) were standardized and rotated via a principal components rotation. The three rotated axes accounted for 79.86, 20.10, and 0.04% of the spatial variation. The first rotated axis (i.e., that accounting for the largest amount of spatial variation) results in 2.22% reduction in the among-mother component of genetic variation. The additional sums of squares associated with this first axis, 5.005, is not significant, so we did not pursue subsequent axes. The adjustments in Φ_{ft} would have been in the third decimal place in any case. There is no convincing evidence of carryover spatial genetic pattern from the adults to the pollen.

Correcting for adult inbreeding—The inbreeding of adult populations was not credibly positive for any of the treatments, ranging from $\hat{F}_U = 0.0242$ and $\hat{F}_C = -0.0153$ (neither significant) for uncut and clear-cut, respectively, to $\hat{F}_S = -0.1046$ ($P \leq 0.05$, selective cut). These values, taken at face value, would yield adjusted estimates, via Eq. 5, of $\hat{\Phi}_U = 0.17$ (uncut); $\hat{\Phi}_S = 0.14$ (selective cut); $\hat{\Phi}_C = 0.091$ (clear-cut). Thus, only the selective-cut estimate required much adjustment for adult structure. In this case, the adult structure created an underestimate rather than overestimate of Φ_{ft}, and the correction does not change our conclusions.

DISCUSSION

Silvicultural practice—These findings clearly show that forest management can modify pollen movement in a species pollinated by generalist pollinators. Based on our estimates of pollen pool structure, we observe that pollen movement is

TABLE 2. TWOGENER partition of the pollen variation in *Cornus florida* L. from the Missouri Ozarks, where $\hat{\Phi}_*$ is the pollen structure measure, and $\alpha \leq 0.001$ is indicated by (***); (a) uncut control; (b) selective cut; (c) clear cut; (d) comparison of Φ estimates.

Uncut Control Treatment					
Sources of Variation	Degrees of Freedom	Sums of Squares	Estimated Mean Squares	Estimated Variance Component	Estimated Φ_{ft} Coefficient
Trees/Replicates	31	60.328	1.946	$V_A = 0.132$	$\hat{\Phi}_U = 0.174$***
Gametes/Trees	297	186.244	0.627	$V_W = 0.627$	
Treatment					
Sources of Variation	Degrees of Freedom	Sums of Squares	Estimated Mean Squares	Estimated Variance Component	Estimated Φ_{ft} Coefficient
Trees/Replicates	72	101.870	1.415	$V_A = 0.083$	$\hat{\Phi}_S = 0.125$***
Gametes/Trees	666	387.765	0.582	$V_W = 0.582$	
Clear-Cut Treatment					
Sources of Variation	Degrees of Freedom	Sums of Squares	Estimated Mean Squares	Estimated Variance Components	Estimated Φ_{ft} Coefficient
Trees/Replicates	41	45.911	1.120	$V_A = 0.056$	$\hat{\Phi}_C = 0.090$***
Gametes/Trees	387	217.770	0.563	$V_W = 0.563$	
Comparative Tests					
Null Hypothesis	Alternative Hypothesis		Test Criterion	Estimated Value	Tail Probability
$\Phi_U = \Phi_S$	$\Phi_U > \Phi_S$		$\Omega_{US} = (\Phi_U - \Phi_S)$	0.049	$P \leq 0.124$
$\Phi_S = \Phi_C$	$\Phi_S > \Phi_C$		$\Omega_{SC} = (\Phi_S - \Phi_C)$	0.035	$P \leq 0.164$
$\Phi_U = \Phi_C$	$\Phi_U > \Phi_C$		$\Omega_{UC} = (\Phi_U - \Phi_C)$	0.084	$P \leq 0.034$

most restricted in the uncut sites and highest in the clear-cut sites, which is similar to what we found for MOFEP populations of wind-pollinated *Pinus echinata* (Dyer, 2002). Thus, we now have two species at the same study site that show that the clear-cut treatment is associated with reduced pollen pool structure, increased number of effective pollen donors and, thus, increased pollen flow. However, the explanation for the two sets of results must not be the same, because not only does the mode of pollination differ, but the pattern of local conspecific density across treatments also varies between the two studies. In the case of *P. echinata*, the seed parents had the same density across treatments, because MOFEP loggers did not remove this species from the clear-cut treatments. Thus, the trend across treatments is not due to conspecific density; it is best explained by an aerodynamic hypothesis that removal of substantial canopy promotes pollen movement. The explanation for this trend in *C. florida* is a bit more complicated.

Initially, we hypothesized that the stand-thinning treatments would enhance pollen movement, because the reduction in forest structure should promote pollinator movement. In the MOFEP, the sites receiving silvicultural treatments have 9% less canopy cover after treatment than the control site (Kabrick et al., 2002). However, while the canopy cover was similar among harvested sites, the spatial array of the remaining trees was quite different, especially with respect to the location of focal trees, relative to harvested areas. In the selective-cut treatment, the stand was thinned throughout, with occasional small openings. In the clear-cut treatment, our focal trees were adjacent to the clearings. Thus, the presence of the clearing seems to have promoted greater pollinator movement than in the uncut forest and slightly, but not quite significantly, more movement than in the selective-cut sites, where trees were thinned throughout. Because we did not observe the pollinators, we can only speculate on the causes of differences in pollen movement across treatments. One experimental study of bee behavior has shown that the spatial array of plants affects bee movement and that increased interplant distance can increase the average distance of pollen transfer (Morris, 1993). In a study of adrenid bees, one of the taxa that pollinate *Cornus*, the bees were attracted by sunlight and density of flowers (Reese and Burrows, 1980). Herrera (1995) showed that variation in microclimate can influence the composition of pollinators, because some insects prefer sites with high irradiance. Thus, in this study, it is possible that the stand thinning in the selective-cut treatment may have affected pollinators or composition of pollinators in one way, while the presence of clearings in the clear-cut treatment could have affected both the behavior and the species of pollinators due to the more dramatic local treatment effect on microclimate.

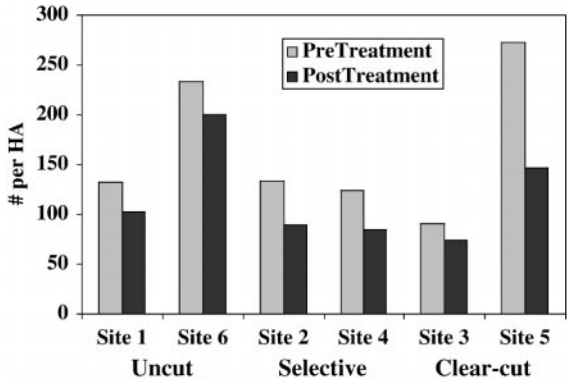

Fig. 2. Mean pre- and post-treatment density of *Cornus florida* (stems > 1 m tall) for six study sites as measured in Missouri Ozark Forest Ecosystem Project (MOFEP) plots distributed randomly across the six study sites. (Unpublished MOFEP data provided by J. Kabrick, formerly Missouri Department of Conservation; for sampling methods, see Kabrick et al., 2002).

We can probably rule out the explanation that the reduction in conspecific stand density in the treated sites is responsible for the decline in pollen pool structure at those sites. In actuality, the density of *C. florida* was lowest at the selective treatment and intermediate in the clear-cut treatments (Fig. 2). The reason that adult *C. florida* density was not similar in sites of the silvicultural treatments, as should have occurred given the nature of the MOFEP design, is due to the site-specific, preharvest demography of *C. florida*. By coincidence, the pretreatment densities were not equal among sites, the two selec-

tive-cut sites, on average, having the lowest density (Fig. 2). Thus, the extent of pollen pool structure does not correspond to the density of *C. florida* adults. As an aside, we note that the *C. florida* density has been declining across all the sites, even the control sites, where trees were not harvested. *Cornus florida* at the control sites had, on average, 69% of the pre-experiment biomass, and sites with harvesting had about 60% of the original *C. florida* biomass. The general decline in *Cornus* biomass is part of a regional decline observed across most species across all sites (Kabrick et al., 2002). In spite of the overall reduction in biomass and heterogeneity in *C. florida* density among the MOFEP sites, the trend in Φ_{ft} across treatments supports the initial hypothesis that stand thinning promotes pollen movement.

The fact that density varied across the different treatment sites complicates our interpretation of the effective number of pollinators, because $N_{ep} \cong d\sigma^2$ where d is adult density and σ^2 is the variance in dispersal. Our estimates of Φ_{ft} and the varying densities yield effective number of pollinators $N_{ep} =$ 2.87, 4.00, and 5.56, respectively, for the uncut, selective-cut, and clear-cut treatments. The estimates of N_{ep} are themselves fine, but the differences between them might involve changes in the freedom of pollen flow (σ^2) or differences in density (d), and probably both. We inevitably obtain ($d\sigma^2$) as a product, and we cannot separately estimate the two parameters (without additional assumptions), but we can say that for real populations, the ecological impact of forest management has the potential to affect pollen-mediated gene flow and change effective neighborhood size.

Most available studies that examine the impact of landscape change on pollen movement are conducted in fragmented ecosystems and in tropical habitats (Smouse and Sork, 2004). As mentioned in the Introduction, several studies have shown an increase in gene flow (Nason and Hamrick, 1997), while a few others showed a decline in pollen-mediated gene flow (Rocha and Aguilar, 2001; Fuchs et al., 2003). The critical issue is whether the pollinator behavior or the pollinator species can adjust to greater inter-plant distances sufficiently or whether the degree of isolation is too great. Because forest management does not change the landscape as greatly as does fragmentation, it is unlikely to reduce gene flow per se, even when the canopy is opened or when clearings are created locally. In fact, as long as adult densities are sufficient, selective thinning and the creation of small clear-cut areas appear to promote pollen-mediated gene movement. Dyer (2002) has shown increased pollen flow in the treatment areas for wind-pollinated *P. echinata*, and we have now shown it for an insect-pollinated species as well. More studies of insect-pollinated species are needed before we can generalize these findings, but we can postulate that while forest management can influence pollen movement, the changes are more subtle than those produced by serious population decimation and fragmentation.

Comparative statistical analysis—Our new comparative for TWOGENER, the A&W test, can be used to test the hypothesis that pollen structure differs across management regimes. The approach can also be used to test hypotheses about the impact of different types of landscape change or about different ecological settings. As with all statistical procedures, the method has statistical power that is sensitive to sample sizes. The Ω coefficients, being differences of Φ_{ft} coefficients, have sample variances that are the sums of variances of Φ_{ft} coefficients, and the Φ_{ft} coefficients themselves can have large variances (Smouse et al., 2001). We have commented elsewhere (Smouse et al., 2001) that for a fixed total sample size, the best way to reduce this variance is to set the number of offspring per seed parent $K \sim \Phi_{ft}^{-1}$ and then maximize the number of seed parents, J, subject to the constraint that $JK = N$. Averaged over the six MOFEP sites, $K = 8$ offspring would have been optimal; 10 offspring per tree were more than adequate. Having set the number of offspring per seed parent at $K = 10$, the only way to reduce the variance of Φ_{ft} is to increase the total sample size (N) and hence the number of seed parents sampled (J). We sampled 252 adults, but reduced seed set and germination rates reduced the available number of seed-parents to 150. The available numbers of seed parents were obviously sufficient to establish that all three Φ coefficients were substantially greater than 0, but they were evidently not sufficient to reduce the variances enough to render some of the Ω tests significant.

Having said that, the question arises as to how many seed parents we would need. Under the null hypothesis that all three Φ_{ft} values are the same (i.e., $\Phi_U = \Phi_S = \Phi_C = \Phi$), a theoretical approximation of the variance of Φ_{ft} is provided by Falconer (1981):

$$\sigma^2_\Phi = \frac{1}{(J-1)} \frac{2[1 + (K-1)\Phi_{ft}]^2(1-\Phi_{ft})^2}{K(K-1)} = \frac{SS}{(J-1)}, \quad (6)$$

where J is the number of sampled seed parents and K is the numbers of offspring sampled per seed parent. For the study at hand, the pooled estimate of the average value is $\Phi = 0.128$.

Our empirically determined variance of Φ_{ft} is a bit smaller than the theoretical value in Eq. 6, but that too is inversely proportional to the numbers of seed parents, so it is convenient to write the variance of a pairwise comparison of two Φ_{ft} estimates, say $\Omega_{12} = (\Phi_1 - \Phi_2)$, as $\hat{\sigma}^2_\Omega = [SS_1/(J_1 - 1) + SS_2/(J_2 - 1)]$. The criterion $(\hat{\Omega}_{12}/\hat{\sigma}_\Omega)$ is a nonparametric analogue of the two-sample t test, whose nominal $\alpha = 0.05$ threshold value is 1.645 (for a one-tailed test). Our empiric null distributions, obtained by permutational shuffling of the A_{ij} and W_{ij} values, were very similar to those of the two-sample t test, with the empiric $P = 0.05$ threshold criteria for all three tests in the 1.60–1.66 range. Using 1.70 as a conservative target for declaring ''significance'' in the one-tailed nonparametric case, if we were to set $J_1 = J_2$ (balanced sampling for the two treatments), then to meet the $\alpha = 0.05$ per-comparison rate threshold for differences of the size we actually encountered, we would have needed $J_S = J_C$ to be approximately 110 observations for the comparison of selective-cut and clear-cut treatments, substantially larger than the numbers of seed parents actually sampled ($J_S = 74$ and $J_C = 43$). For a comparison of selective cut and uncut control, we would have needed $J_S = J_U$ to be about 60, collectively a bit larger (and better balanced) than the actual numbers of seed parents ($J_S = 74$ and $J_U = 33$) used. For a comparison of the clear-cut and uncut control, which differ more substantially, we would have needed only $J_U = J_C$ to be about 25 observations, less than the numbers of seed parents we actually used ($J_C = 43$ and $J_U = 33$). Two caveats are in order. (1) While we can determine how large a difference we want to detect, in routine practice, we will have little (if any) a priori information on the sizes of the Ω criteria to be expected, so it is probably a good idea to

opt for larger (rather than smaller) numbers of seed trees for each of the scheduled treatments. (2) If we insist on small experiment-wise error rates, rather than small per comparison error rates, we will need to increase the numbers of seed parents accordingly.

Adult spatial structure—We attempted to correct for the influence of an underlying genetic gradient in the adult population on our estimates of pollen pool structure. However, the StAMOVA analysis (Dyer et al., 2004) did not detect any significant impact of spatial structure on the pollen donor population. That result does not mean that the *Cornus* population spread over the overall MOFEP region does not exhibit any genetic gradient. Indeed, preliminary analyses had detected a small gradient for *Cornus florida* across the entire MOFEP region (Sork, unpublished results), but that gradient is evidently too subtle to have any meaningful influence on the pollen structure estimates used here to gauge pollen movement, which evidently occurs over much shorter distances.

Adult inbreeding—For the most part, the inbreeding coefficients of the adult population were not significant. In one case, the adults exhibited a negative *F*, or excess of heterozygotes, which led to a slight decrease in the estimate of effective pollen donor number when the estimate was adjusted. However, this correction did not change the overall conclusions. This empirical finding raises questions about the extent to which inbreeding among adults causes bias in the pollen pool structure. Austerlitz and Smouse (2001b) show that the inflation in Φ_{ft} is comparable to the size of the inbreeding coefficient. It is clear from Eq. 5 that if $F > 0$, we are overestimating Φ_{ft}, but if $F < 0$, we are underestimating. The inflation/deflation is the same amount as the inbreeding, because the relationship is approximately given by $\hat{\Phi}_{ft} = \tilde{\Phi}_{ft}(1 + F)$. A value of $F = 0.02$ means we have 2% inflation of Φ_{ft}; if $F = 0.10$, then we have a 10% inflation. For most tree species, inbreeding is unlikely to be that high, but there are species for which *F* can be quite large.

Conclusions—Our findings indicate that small changes in landscape context can influence pollen movement. In this case, the creation of clearings and the thinning of the forest promoted greater pollen movement, especially for those trees located near the area of treatment. Thus, a generic concern that silvicultural treatment per se may negatively affect future genetic diversity or increase reproductive isolation is contraindicated. We hasten to add that we conducted these studies across a landscape with extensive forest cover and a focal tree species that occurred in high density. We cannot conclude what would happen when the pairwise distance between reproductive adults is significantly greater, which might occur for species that either occur in low abundance or that have had adult densities reduced through several cycles of silvicultural treatment. In our temperate forest setting, insect pollinators appear able to adapt to small changes in forest structure. In fact, if one is concerned about neighborhood size, genetic bottlenecks, or future genetic diversity, the evidence reported here indicates that, as long as a sufficiently large number of seed parents remain to provide adequate reproduction and to avoid a genetic bottleneck in the effective number of mothers, silvicultural management may not negatively affect the effective number of pollen parents, and hence subsequent genetic diversity in *Cornus florida*.

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