

SPATIAL GENETIC STRUCTURE OF ALLOZYME POLYMORPHISMS WITHIN POPULATIONS OF *PINUS STROBUS* (PINACEAE)¹

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The population structure of genetic variation for four allozyme loci was investigated for two populations (one old growth, OG, and the other logged, SS) of eastern white pine (*Pinus strobus*). Both seedlings and reproductive adults were studied for both study populations. Spatial autocorrelation statistics were used to examine the distribution of allozyme polymorphisms. The spatial genetic structure in adults of population OG indicated that individual genotypes were distributed in a structured, isolation-by-distance manner, consistent with observed levels of pollen and seed dispersal. In contrast, adult genotypes in population SS were nearly randomly distributed, probably as a result of logging. Nonetheless, spatial structuring of genotypes of seedlings occurred at both sites, indicating the power of limited seed flow, as well as temporal Wahlund effects, to create structure. None of four loci in both seedling populations showed a significant departure from Hardy-Weinberg proportions, whereas one and two significant deviations were found for loci in the two respective adult populations. These departures may be attributed to episodic reproductive events.

Key words: allozyme; eastern white pine; Pinaceae; *Pinus strobus*; spatial autocorrelation; spatial genetic structure.

Eastern white pine (*Pinus strobus* L.) is one of the most common and widespread conifers in the eastern United States. Yet little is known about the structure of genetic variation within populations and of the mating system of white pine, in contrast to important conifers in the western United States. Spatial structure of genetic variation and the outcrossing rate are the two primary determinants controlling the mating system, levels of inbreeding, and hence the dynamics of genetic variation within plant populations. They also interact with natural selection through inbreeding depression.

There is known to be a high level of genetic variation among populations of white pine. Several studies have demonstrated high levels of variation for various morphological traits among populations (Abubaker and Zsuffa, 1990; Beaulieu and Simon, 1994a, 1995b; review by Buchert, 1994), and these levels are typical of pines and other conifers (e.g., reviews by Brown, 1979, and Hamrick and Godt, 1990). Typical of conifers, a high level of variation for isozyme loci among populations of white pine has been demonstrated (Eckert, Joly, and Neale, 1981; Beaulieu and Simon, 1994b), and more recently the same has been found to be true for random amplified DNA (RAPD) (Echt and Nelson, 1997) and microsatellite genetic markers (Echt et al., 1996; Echt and May-Marquardt, 1997).

It has also been shown that white pine suffers severe inbreeding depression, like nearly all other conifers studied. For example, progeny resulting from self-fertilization suffer low rates of germination and high rates of early abortion, mortality, and abnormality, as well as reduced growth rates at the seedling and later stages (e.g., Fowler, 1965a; Blake and Yeatman, 1989; El-Kassaby, Meagher, and Davidson, 1993; review by

Mitton, 1992), and this is true of eastern white pine (Johnson, 1945; Fowler, 1965b).

Pinus strobus has high levels of dispersal of pollen by wind (e.g., Wright, 1953) and has winged seed that travel relatively long distances, easily 20 m or more (e.g., Critchfield, 1980). Unlike most other conifers, its mating system has been studied only once, in two stands in Quebec (Beaulieu and Simon, 1995a). The measured outcrossing rate was indistinguishable from 1.0, and this is atypical of conifers, which usually have rates in the range of 0.8–0.9. There are various reasons to expect that the rate in *P. strobus* could vary from the 0.80 to 1.0 among stands. Biparental inbreeding also strongly affects inbreeding coefficients. Moreover, variations among the relatively low selfing rates typical of conifers scarcely affects the spatial distribution of individuals. However, even moderate limits to dispersal can cause spatial structuring of genotypes, or genetic isolation by distance, within populations, and hence subsequent biparental inbreeding. By studying the spatial structure of genetic variation, we characterize the major determinant of biparental inbreeding.

In this article we quantify the spatial genetic structure within two populations of *P. strobus* in order to gain insights into the levels of inbreeding at the seedling and adult stages and to provide information for regeneration. Moreover, we combine study of a logged stand with one that is old growth, in order to obtain an initial assessment of the possible effects of logging on spatial structure and hence biparental inbreeding.

MATERIALS AND METHODS

Plant materials—From September to November 1998, a total of 452 individuals were mapped at two sites at Hartwick Pines State Park located near Grayling, Michigan, USA. Needles were collected from 120 adults, contiguous over an area of ~1 ha (density ≈ 120 adult trees/ha) of a virgin, old-growth stand (OG). These trees were all very large individuals (~35–55 m tall). In addition, if a seedling was present near each tree, it was sampled (90 seedlings total). There was a complete absence of individuals >2 yr old but <35 m tall. Other woody plants associated with white pine in population OG are red pine, hemlock, maple, and beech. In addition, 121 contiguous adults

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were sampled within an ~1-ha area of a second stand (hereafter referred to as SS), located a few kilometers from OG. The SS site had been logged near the turn of the century, but undisturbed since. At the SS white pine is the dominant species, naturally regenerated, and unevenly aged. Adult trees with diameters at breast height (dbh) >20 cm were sampled. Although this delimitation was somewhat arbitrary, it allowed the sample to be concentrated on individuals that are reproductive-aged (especially for female function) and that are most likely to have been produced soon after (or in some cases possibly before) logging. In addition, seedlings <30 cm in height were sampled such that one seedling nearest each sampled adult tree was sampled (121 seedlings). At the SS all size classes were well represented, including those >30 cm tall but having dbh <20 cm. However, by focusing on small seedlings the sample addresses structure in the most recent cohort of regeneration. At the SS other woody plants were more diverse, including in addition to those listed above for OG, balsam fir and black spruce. Collected needles were kept on ice, transported to the laboratory, and stored at 4°C until protein extraction.

Enzyme extraction and electrophoresis—Needles were cut finely and crushed with a mortar and pestle. A phosphate-polyvinylpyrrolidone extraction buffer (Mitton et al., 1979) was added to the leaf samples to facilitate crushing and to aid enzyme stabilization. The crushed extract was absorbed onto 4 × 6-mm wicks cut from Whatman 3MM chromatography paper, and the wicks were stored at -70°C until needed for allozyme analysis. Electrophoresis was performed using 10% starch gels. Out of several enzyme systems that were resolved using three gel/electrode buffer combinations, we concentrated on four loci that produced very clear banding patterns and that had sufficiently high levels of polymorphism. A Poulik buffer system, a modification (Haufler, 1985) of Soltis et al. (1983) system 6, was used to resolve triosephosphate isomerase (*Tpi-2*). A histidine citrate buffer system, a modification (Chung and Kang, 1994) of Soltis et al. (1983) system 11, was used to resolve phosphoglucosomerase (*Pgi-2*) and malate dehydrogenase (*Mdh-2*). Soltis et al. (1983) system 7 was used to resolve aspartate aminotransferase (*Aat-3*). Stain recipes followed Soltis et al. (1983). Putative loci were designated sequentially, with the most anodally migrating isozyme designated 1, the next 2, and so on. Similarly, alleles were designated sequentially with the most anodally migrating alleles designated a. The genetic bases of the loci examined here have been also well documented in other studies (e.g., Eckert, Joly, and Neale, 1981; Beaulieu and Simon, 1994a).

Data analyses—For all statistical analyses, seedlings and adults were analyzed separately. Observed heterozygosity was compared to Hardy-Weinberg (H-W) expected values using Wright's (1922) fixation indices (F). These indices were tested for deviations from zero by chi-square tests (Li and Horvitz, 1953). Nei's (1973, 1977) gene diversity formula for G_{ST} was used to evaluate the distribution of genetic diversity among populations. In addition, a chi-square statistic was used to detect significant differences in allele frequencies among samples for each locus (Workman and Niswander, 1970).

Spatial autocorrelation analysis was conducted separately for alleles of each polymorphic locus (Sokal and Oden, 1978). Alleles that were represented by less than five copies in a sample were excluded as being insufficiently informative for spatial analysis. For diallelic loci, only one allele was considered because the second allele would contribute identical information. The genotypic data were coded so that allele frequency values of 1.0, 0.5, or 0.0 were assigned to homozygotes for that allele, heterozygous for that allele, or genotypes with no copies of that allele, respectively. All pairs of individuals were considered as joins and assigned to one of the ten distance classes according to the Euclidean distance separating the two trees. The bounds for the distance classes were chosen such that there were sufficient numbers of pairs and so that mutually exclusive distance classes were formed. The first distance class is particularly critical and its upper bound was chosen to be approximately equal to 1.5 times the square root of the inverse of the sample density. Such a bound insured that most pairs of near-neighbor individuals are included in distance class one. Moran's I statistics (Sokal and Oden, 1978) were calculated for each of ten distance classes by

$$I = N \sum_i \sum_j (W_{ij} Z_i Z_j) \left(\sum_i \sum_j W_{ij} \sum_i Z_i^2 \right)^{-1}$$

TABLE 1. Fixation indices (F) for four polymorphic loci in two populations of *Pinus strobus*. OG = old growth site, SS = second site.

| Locus | OG | | SS | |
|--------------|-----------|----------|-----------|----------|
| | Seedlings | Adults | Seedlings | Adults |
| <i>Aat-3</i> | 0.116 | 0.424*** | 0.092 | 0.317*** |
| <i>Mdh-2</i> | 0.103 | -0.101 | -0.042 | 0.013 |
| <i>Pgi-2</i> | — | 0.015 | — | 0.253*** |
| <i>Tpi-2</i> | -0.017 | -0.025 | -0.026 | -0.017 |

*** $P < 0.001$.

where N is number of individuals; W_{ij} is a join in the weighting matrix, where W_{ij} is set equal to 1.0 if i th and j th individuals are in the distance class and zero otherwise; $Z_i = X_i - X$, $Z_j = X_j - X$; and the variables X_i and X_j are the genotypic scores for i th and j th individuals, respectively, and X is the mean score for all individuals. Each I value was used to test for significant deviations from the expected values, $E(I) = -1/(N-1)$ (Cliff and Ord, 1981), under the random distribution null hypothesis. A significant positive value of Moran's I indicates that pairs of individuals in the distance class considered have similar gene frequencies (genotypes), whereas a significant negative value indicates that they have dissimilar gene frequencies. Overall significance of individual correlograms was tested using Bonferroni's criteria (Sakai and Oden, 1983). Statistical analyses were performed using the SAAP program (version 4.3) written by D. Wartenberg.

RESULTS

Analysis of fixation indices showed that three (21%) F values out of 14 cases differed significantly from zero (Table 1). Two of these occurred in the SS adults and one in the OG adults. Denoting with the letters A or S for adults and seedlings, respectively, the mean values of G_{st} were 0.015, 0.017, 0.027, 0.021, 0.002, and 0.008, for OGA vs. OGS, OGA vs. SSS, SSA vs. SSS, SSA vs. OGS, SSS vs. OGS, and OGA vs. SSA, respectively. All differences are small, scarcely more than expected from sampling error. There is little difference between the two stands, whether at the adult stage or the seedling stage.

The spatial autocorrelation coefficients, Moran's I , calculated for seedlings and adults in each of the two populations, are shown in Tables 2 and 3. For seedlings and adults in population OG, Moran's I values were significantly different (at the 5% level or lower) from the expected value ($E[I] = -0.011$ and -0.008 , respectively) in 7 (14%) of 50 cases and in 13 (18.6%) of 70 cases, respectively, and the overall correlograms were significant for two (40%) of five alleles and for two (28.6%) of seven alleles, respectively (Table 2). For the first distance class (0 to <15 m), zero and three significant positive cases were observed in the seedling and adult populations, respectively, whereas no significant negative cases were detected in the first distance class. The average correlograms were similar for seedlings and adults for the OG site. They indicate that the highest relative levels of genetic similarity occur among individuals separated by <15 m. Spatial genetic structure among seedlings in population SS was very similar to that in population OG. However, a different result was observed among adults in population SS. For seedlings and adults in population SS, Moran's I values were significantly different from the expected value ($E[I] = -0.008$) in 10 (20%) of 50 cases and in 8 (10%) of 80 cases, respectively, and the overall correlogram was significant for one (20%) of five alleles in the seedling population (Table 3). There were no significant correlograms for the adults in population SS.

TABLE 2. Spatial autocorrelation coefficients (Moran's I) in the old growth population of *Pinus strobus* for ten distance classes. The upper table is for the seedlings ($N = 90$); the lower is for the adults ($N = 120$). * $P < 0.05$, ** $P < 0.01$.

| Locus | Distance class ^a | | | | | | | | | | P^b | q^c |
|---------------|-----------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|---------------|-------|-------|
| | 1 (15 m) | 2 (25 m) | 3 (35 m) | 4 (45 m) | 5 (55 m) | 6 (65 m) | 7 (75 m) | 8 (85 m) | 9 (95 m) | 10 (105 m) | | |
| Seedlings | | | | | | | | | | | | |
| <i>Aat-3a</i> | 0.06 | 0.04 | 0.02 | -0.01 | -0.04 | -0.11* | -0.07 | -0.05 | 0.05 | 0.03 | 0.148 | 0.511 |
| <i>Mdh-2a</i> | 0.07 | 0.02 | -0.03 | 0.05* | 0.03 | -0.06 | -0.14** | -0.08 | -0.08 | 0.03 | 0.051 | 0.655 |
| <i>Mdh-2b</i> | 0.07 | -0.05 | -0.06 | 0.03 | 0.01 | -0.02 | -0.02 | -0.03 | -0.05 | -0.01 | 0.671 | 0.228 |
| <i>Mdh-2c</i> | 0.03 | 0.06 | 0.08** | 0.02 | -0.06 | -0.05 | -0.09* | -0.14* | -0.04 | -0.02 | 0.081 | 0.117 |
| <i>Tpi-2a</i> | -0.05 | 0.01 | -0.02 | -0.03 | -0.04 | 0.00 | 0.09* | 0.03 | -0.03 | -0.05 | 0.135 | 0.029 |
| Average | 0.04 | 0.02 | -0.00 | 0.01 | -0.02 | -0.05 | -0.05 | -0.05 | -0.03 | -0.00 | | |
| Adults | | | | | | | | | | | | |
| <i>Aat-3a</i> | 0.01 | -0.01 | 0.04 | -0.04 | -0.02 | 0.00 | -0.03 | 0.01 | -0.08 | 0.01 | 0.591 | 0.455 |
| <i>Mdh-2a</i> | 0.04 | -0.03 | -0.07* | -0.01 | 0.03 | 0.04 | -0.03 | -0.04 | -0.06 | 0.02 | 0.262 | 0.639 |
| <i>Mdh-2b</i> | 0.10* | -0.07* | -0.07* | -0.00 | 0.03 | 0.03 | -0.04 | -0.06 | -0.03 | 0.02 | 0.138 | 0.266 |
| <i>Mdh-2c</i> | -0.01 | -0.00 | 0.00 | -0.01 | 0.01 | -0.01 | -0.07* | 0.02 | 0.00 | -0.01 | 0.343 | 0.095 |
| <i>Pgi-2a</i> | 0.11** | -0.08* | -0.04 | -0.03 | 0.06* | 0.02 | -0.06 | -0.03 | 0.00 | -0.00 | 0.082 | 0.119 |
| <i>Pgi-2c</i> | 0.05 | -0.07* | -0.03 | -0.01 | 0.05* | -0.01 | -0.07 | 0.01 | 0.07 | -0.02 | 0.254 | 0.873 |
| <i>Tpi-2a</i> | 0.10** | 0.13** | -0.03 | -0.07* | -0.04 | -0.05 | -0.01 | 0.01 | -0.04 | 0.00 | 0.000 | 0.029 |
| Average | 0.06 | -0.02 | -0.03 | -0.02 | 0.02 | 0.00 | -0.04 | -0.01 | -0.02 | -0.00 | | |

^a Upper bound for distance class.

^b Overall correlogram significance (Bonferroni approximation).

^c Allele frequency.

Overall, the average correlograms for the adults and seedlings for OG and the seedlings for SS were similar and indicate short distance autocorrelations, whereas that for the SS adults indicates an essentially random distribution of genotypes over the sample area.

DISCUSSION

The amount of spatial structure in the adults of the undisturbed old-growth population (OG) appears to be small but consistent with studies of other species with similar levels of seed and pollen dispersal. The average value of Moran's I statistics for the shortest distances of separation was 0.05,

comparable to values of 0.07 for *Maclura pomifera* (Schnabel, Lauschman, and Hamrick, 1991), 0.08 for *Gleditsia triacanthos* (Schnabel and Hamrick, 1990), 0.08 for *Psychotria nervosa* (Dewey and Heywood, 1988), and ~ 0.05 for *Quercus laevis* (Berg and Hamrick, 1995).

The observed values of Moran's I statistics for distance class 1 (average value 0.06) for the OG adults can be used to estimate total dispersal under the standard isolation by distance model (Table 4 of Epperson, Huang, and Li, 1999), and thus the observed value corresponds approximately to a Wright's neighborhood size of 100 individuals. This number of individuals is contained in an area approximately equal to ($\sim 84\%$) the total area of the sample. Using the relationship $N = 4\pi\sigma^2$,

TABLE 3. Spatial autocorrelation coefficients (Moran's I) in the second site population of *Pinus strobus* for ten distance classes. The upper table is for the seedlings ($N = 121$); the lower is for the adults ($N = 121$). * $P < 0.05$, ** $P < 0.01$.

| Locus | Distance class ^a | | | | | | | | | | P^b | q^c |
|---------------|-----------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|---------------|-------|-------|
| | 1 (15 m) | 2 (25 m) | 3 (35 m) | 4 (45 m) | 5 (55 m) | 6 (65 m) | 7 (75 m) | 8 (85 m) | 9 (95 m) | 10 (105 m) | | |
| Seedlings | | | | | | | | | | | | |
| <i>Aat-3a</i> | 0.08* | 0.02 | -0.06* | -0.04 | 0.02 | -0.00 | -0.01 | -0.04 | -0.03 | 0.02 | 0.240 | 0.475 |
| <i>Mdh-2a</i> | 0.07* | -0.03 | -0.06* | 0.02 | 0.06* | -0.03 | -0.06 | -0.05 | -0.02 | 0.05 | 0.161 | 0.591 |
| <i>Mdh-2b</i> | 0.02 | -0.01 | -0.03 | -0.03 | -0.03 | 0.02 | -0.00 | -0.05 | 0.09* | -0.01 | 0.121 | 0.260 |
| <i>Mdh-2c</i> | -0.02 | 0.00 | -0.00 | 0.02 | -0.03 | 0.06* | -0.07* | -0.04 | -0.04 | 0.01 | 0.154 | 0.149 |
| <i>Tpi-2a</i> | 0.05 | 0.04 | 0.02 | 0.03 | 0.02 | -0.09** | -0.02 | -0.06 | 0.01 | -0.14** | 0.001 | 0.029 |
| Average | 0.04 | 0.00 | -0.03 | 0.00 | -0.01 | -0.01 | -0.03 | -0.05 | 0.00 | -0.01 | | |
| Adults | | | | | | | | | | | | |
| <i>Aat-3a</i> | -0.04 | -0.02 | -0.01 | -0.03 | 0.03 | -0.01 | 0.03 | -0.05 | -0.01 | 0.02 | 1.000 | 0.595 |
| <i>Mdh-2a</i> | 0.00 | -0.03 | 0.03 | -0.01 | 0.02 | -0.07* | 0.02 | -0.05 | -0.01 | 0.00 | 0.388 | 0.665 |
| <i>Mdh-2b</i> | -0.00 | -0.04 | 0.01 | -0.01 | -0.02 | -0.07* | 0.05* | 0.00 | -0.04 | 0.04 | 0.233 | 0.236 |
| <i>Mdh-2c</i> | -0.04 | 0.01 | 0.03 | -0.01 | 0.01 | -0.01 | -0.04 | -0.01 | -0.03 | -0.02 | 0.850 | 0.099 |
| <i>Pgi-2a</i> | -0.10* | 0.01 | 0.02 | -0.03 | 0.03 | -0.02 | -0.00 | 0.03 | 0.00 | -0.08* | 0.116 | 0.145 |
| <i>Pgi-2b</i> | 0.01 | 0.01 | -0.06* | 0.03 | -0.01 | -0.01 | -0.04 | -0.03 | -0.05 | 0.08* | 0.145 | 0.058 |
| <i>Pgi-2c</i> | -0.04 | 0.04 | -0.06* | -0.00 | 0.01 | 0.02 | -0.02 | -0.01 | -0.06 | 0.03 | 0.460 | 0.789 |
| <i>Tpi-2a</i> | 0.02 | -0.04 | -0.00 | 0.03 | -0.00 | -0.03 | 0.02 | -0.03 | -0.06 | -0.01 | 0.687 | 0.022 |
| Average | -0.02 | -0.01 | -0.01 | -0.00 | -0.01 | -0.03 | 0.00 | -0.02 | -0.03 | 0.01 | | |

^a Upper bound for distance class.

^b Overall correlogram significance (Bonferroni approximation).

^c Allele frequency.

we estimate the average (for both female and male parents) parent-offspring distance variance of ~ 796 m², i.e., an average standard deviation of ~ 28 m. It is likely that seed dispersal, $\sigma^2(s)$, is considerably shorter than pollen dispersal, $\sigma^2(p)$. The total (male plus female parent-offspring) variance is equal to $1/2\sigma^2(p) + \sigma^2(s)$ (Crawford, 1984). Hence, the estimated value would fit, for example, a standard deviation of distance of effective (i.e., fertilizing) pollen movement of 30 m and a standard deviation of seed dispersal distance of ~ 18.6 m, though there are many other combinations that would fit the equation. Typical direct measures of standard deviations for conifers are in the range of ~ 17 – 65 m for pollen (e.g., Wright, 1953) and ~ 17 – 35 m for seed (e.g., Epperson and Allard, 1989), though both may be considerably smaller in a closed-canopy setting. The seedlings at the OG site have spatial structure consistent with that of the adults under the standard genetic isolation by distance process.

The spatial distribution of genetic variation among adults at the second population (SS) is somewhat different, indeed it is essentially indistinguishable from a random distribution. Logging may have caused this difference in two ways. First, considerable removal of structure would be caused by intense but not “clear-cut” logging. The small-scale structuring (observed at the OG) would be reduced if not completely erased among those trees left uncut. Second, those trees initially regenerated after cutting would have structure produced only from a single generation of limited seed flow from the uncut trees. However, the fact that the seedlings at the SS have nearly as much structure as the seedlings and adults at the OG site indicates that substantial structure can be created within a single entire generation of regeneration and caused solely by limited seed flow. The data do not allow us to more precisely determine the manner of effect of logging on the structure.

It is of interest to note that regeneration may have been limited and episodic at the SS and OG sites, limited by seed sources at least during some periods. In 1998, some 20 man-hours were spent in searching for cones on trees at the OG site, and only 13 trees were observed to have mature cones, and three or less cones were observed on any one tree. Less time was spent searching the SS site, but it appeared that some trees had larger numbers of cones. In 1999, ~ 1 wk was spent at each site, searching and harvesting cones. At both sites fewer than one-fifth of trees were observed to have cones, and usually less than three cones were observed on a tree. At both sites there were one or two trees that had considerably larger numbers of cones. Undoubtedly, there were more cones than observed, but we are confident that at both sites many trees did not have any cones.

Beaulieu and Simon (1995a) found that four loci (*Aco* [aconitase], *Aat-3*, *Mdh-1*, and *Pgm-2*) in two adult populations of white pine from Quebec were in H-W equilibrium and their multilocus estimates of outcrossing rates were essentially 1.0. In the seed populations of their study, the *Aco* locus showed a significant departure from H-W equilibrium. However, our study populations revealed a reverse result. Fixation indices of the seedling populations (none of four loci showed a significant departure from panmixia when tested by chi-square statistics) were different from those from the adult populations (one and two significant loci). In addition, both the seedling populations were monomorphic at *Pgi-2* (*Pgi-2^c*). The result that the four loci examined in both seedling populations were in H-W equilibrium may indicate support for an outcrossing rate near 1.0. In addition, the highly positive *F* values

for *Aat-3* (OG) and *Aat-3* and *Pgi-2* (SS) in the two adult populations were significantly different from zero, indicating a significant excess of homozygotes over those expected under panmixia. In contrast, slight excesses of heterozygotes in adult populations and absence of such excesses or even presence of heterozygote deficits appear to be frequent in seed and seedling populations in other conifers (e.g., El-Kassaby et al., 1987; Knowles et al., 1987; Beaulieu and Simon, 1995a). Because inbreeding affects all genes, the observed departures from H-W equilibrium for both adult populations (as well as the relatively large values of G_{st} between adults and seedlings) in our study may be attributable to episodic reproductive events (leading to a temporal “Wahlund” effect) over generations. Such an effect could not be observed in a single cohort of seedlings. Although the white pine at the OG site are “unmanaged,” fire has been excluded at the site, leading to a high density of shade-tolerant species such as maple and hemlock, which in turn are probably why no seedlings survive more than two years. This disallows observation of the temporal Wahlund effect among young age classes at the OG.

In summary, spatial structure of genetic variation among the OG adults fits that expected for an equilibrium genetic isolation by distance model, and so does that of the seedlings. It appears that limited pollen flow and spatial structure combine to cause some biparental inbreeding in the natural state of white pine forest. However, it appears that some additional kinship within cohorts may be caused by a temporal Wahlund effect and episodic reproduction, but this part has been excluded in recent decades at the OG site, because of fire prevention. Logging apparently randomized the spatial structure of genetic variation among adults at the SS, but substantial structure occurs among regeneration seedlings, apparently caused by limited seed flow.

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