Genetic structure, diversity, and inbreeding of eastern white pine under different management conditions

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Abstract: Resource sustainability requires a thorough understanding of the influence of forest management programs on the conservation of genetic diversity in tree populations. To observe how differences in forest structure affect the genetic structure of eastern white pine (*Pinus strobus* L.), we evaluated six eastern white pine sites across the 234 000 acre (1 acre = 0.4046856 ha) Menominee Indian Reservation in northeastern Wisconsin (45°00′N, 88°45′W). The six sites sampled for nuclear and chloroplast DNA microsatellite markers were of contrasting densities and managed by different management systems: shelterwood, pine release, plantation, and old growth. Three of the sites had natural regeneration, which was also sampled. Mean values of spatial genetic autocorrelation were positive in all mature populations and variable; the strongest spatial structuring of genes occurred in the least disturbed old-growth site ($I - E(I) = 0.031$). Genetic structuring at the historical old-growth site fit the isolation-by-distance model for a neighborhood size of 130 individuals. Significant inbreeding occurred in five populations, but the seedling or sapling populations as a group ($f = 0.088$) are significantly less inbred than the local mature populations ($f = 0.197$). The increase in heterozygosity between generations was attributed to harvesting having reduced the spatial genetic structure of the mature trees.

Résumé : L'utilisation durable des ressources requiert une connaissance approfondie des effets des programmes d'aménagement forestier sur la conservation de la diversité génétique des populations d'arbres. Afin d'étudier comment les différences de structure de peuplement affectent la structure génétique de pin blanc (*Pinus strobus* L.), les auteurs ont évalué six forêts de pin blanc réparties à travers la réserve indienne de Menominee, qui couvre 234 000 acres (1 acre = 0.4046856 ha) et qui est située dans le nord-est du Wisconsin (45°00′N, 88°45′O). Les six sites étudiés avaient des densités de peuplement contrastées et étaient aménagés selon différents régimes : coupe progressive, coupe de dégagement du pin, plantation et forêt ancienne. Trois des sites supportaient une régénération naturelle qui fut également échantillonnée. La diversité génétique a été caractérisée à l'aide de marqueurs microsatellites de l'ADN nucléaire et chloroplastique. Les valeurs moyennes d'autocorrélation spatiale génétique étaient positives pour toutes les populations d'arbres maturees et elles étaient variables : la plus forte structuration spatiale de la diversité génétique a été observée pour le site le moins perturbé qui supportait une forêt ancienne ($I - E(I) = 0{,}031$). La structure génétique de ce site s'ajustait au modèle d'isolement par la distance avec une taille de population de 130 individus. Une endogamie significative a été observée pour cinq populations, mais les populations de semis ou de jeunes arbres pris dans leur ensemble ($f = 0{,}088$) étaient significativement moins consanguines que les populations locales d'arbres matures ($f = 0{,}197$). L'augmentation en hétérozygotie d'une génération à l'autre pouvait s'expliquer par la récolte des arbres qui a réduit la structure génétique spatiale des cohortes d'arbres matures.

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Introduction

A fundamental requirement for improved forest management is an understanding of the dynamics of genetic variation within and among tree populations. The gene pools of natural populations are not static, but constantly changing through the interactions of site characteristics (e.g., density, age structure, and dispersal distance) with the forces of evolution (mutation, mating system, gene flow, genetic drift, and natural selection). Forest management practices can alter these interactions and hence alter the partitioning of genetic variation within populations. Therefore, characterization of the changes in genetic diversity that result from forest management is essential for sustaining our forest resources (sensu lato, Lindenmayer and Franklin 2003). While easily measured phenotypes such as stem form, crown mass, and diameter growth are often used to evaluate the successful management of the quality of crop trees, effects at the ge-

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Table 1. Site conditions for nine populations of eastern white pine on the Menominee Indian Reservation.

Site	Management system	UTM, 16T NAD27 (Northing, Easting)	Site area (ha)	Mature tree age (years)[a]	Seedling or sapling age (years)[a]	Adult tree and plantation sampling ratio[b]
Camp 1	Shelterwood	4978456 0357117	53	165	3	1:20
Minnow Creek	Pine release	4977170 0371648	33	130	40	1:2
Oconto Line	Shelterwood	4977568 0382314	39	165	9	1:20
Plantation	Plantation	4968153 0380512	24	—	12	1:20
Potato Patch	Shelterwood	4995071 0373749	18	160	—	1:20
School Pines	Old growth	4993514 0368254	24	240	—	1:1

[a]Age at sampling is the age of the even-aged pine population since its origination at the site and was determined from previously collected forest inventory data.

[b]Ratio of samples to census, the closest seedling or sapling from base of sampled mature tree was also sampled.

netic level have largely been ignored and are rarely incorporated into forest management programs (Ennos et al. 1998).

Generally, the partial removal of a coniferous forest is expected to decrease effective gene flow and increase inbreeding of naturally regenerating progeny (El-Kassaby et al. 2003). This is because, for wind-pollinated species, a low density population of mature trees is expected to increase the spatial genetic structure of the population by increasing the probability of pollination by spatially proximal relatives (Epperson 2003). Likewise, inbreeding should be limited in a high density forest because the pollen cloud for a tree in a dense population would contain a relatively small proportion of its own pollen; therefore, most seeds would be a product of outcrossing (Farris and Mitton 1984; Knowles et al. 1987; El-Kassaby et al. 2003). Hence, studying the effects of forest management on gene flow and spatial genetic structure can help us better understand how changes in breeding neighborhoods affect the inbreeding levels and genetic diversity of naturally regenerating progeny (Wright 1946). Pollen and seed dispersal are key processes for determining the spatial genetic structure of plant populations. Powerful inferences can be made about the dispersal processes that generate spatial genetic patterns through spatial autocorrelation analysis, a robust method for indirectly measuring dispersal, which is the total variance of dispersal distances (Epperson 2005). Dispersal provides an estimate of the neighborhood size, or the number of breeding individuals in a population (Wright 1946); thus, a good point estimate for Wright's neighborhood size (N_E) can be based on Moran's I coefficient (Moran 1950) for the first distance class (Epperson 2005).

The Menominee Nation (Keshena, Wisconsin) has harvested trees on their reservation for more than 140 years (Loew 2001). Forest-management goals balance growth with removals (from harvesting and natural losses), while maintaining tree vigor and the native species diversity (Landis 1992). The 234 000 acre (1 acre = 0.4046856 ha) reservation contains approximately 17 000 acres of eastern white pine (*Pinus strobus* L.). Resident populations consist predominantly of mature trees >50 cm diameter at breast height, most of which are managed for sustainable timber harvests. The current study was the first to compare the population genetic structures of managed and naturally regenerating populations of eastern white pine. We used f statistics as well as spatial autocorrelation analyses to examine the distribution of microsatellite markers in nine populations of eastern white pine and to estimate dispersal at the least disturbed site. Our goal was to determine whether forest management practices were maintaining the genetic diversity of eastern white pine on the Menominee Indian Reservation. We were particularly interested in the genetic differences between local mature populations and the naturally regenerating seedlings and saplings. Contrary to our hypothesis that inbreeding would be increased in the seedling and sapling populations of the harvested sites, we found significantly less inbreeding in the natural regeneration than the local mature populations, most likely from harvesting having reduced the spatial genetic structure of the mature populations.

Materials and methods

Species, study sites, and sample populations

Eastern white pine is a long-lived monecious conifer that is primarily outcrossed with wind-dispersed pollen and seed (Ledig 1998). The wind-dispersed pollen and seed of plant species are often deposited within a few metres of the source and are rarely transported beyond 1000 m for pollen and 200 m for seed (Levin 1981). Some of the longest dispersal distances reported for pine are 762 m for slash pine (*Pinus elliottii* Engelm.) pollen (Wang et al. 1960) from an isolated tree, up to 60 m for eastern white pine seed within a stand, and over 200 m for eastern white pine seed in open areas (Wendel and Smith 1990). Generally, conifers have a high genetic load (Ledig 1998) and eastern white pine has been shown to suffer from severe inbreeding depression (Johnson 1945). It is a widespread species across northeastern USA and Canada and is quite genetically diverse among individual trees (Echt et al.1996) and across its geographical range (Rajora et al. 2000; Marquardt and Epperson 2004; Nijensohn et al. 2005). Eastern white pine is an even-aged species that often coexists with a variety of other species. Six eastern white pine sites (Table 1), separated by 5–28 km and other forest cover types, were evaluated across

the Menominee Indian Reservation in northeastern Wisconsin (45°00′N, 88°45′W). Forest cover type is a classification system based on trees that predominate in an area. The age of the eastern white pine was based on previously collected data from forest inventory databases. Five of the six sites were managed for pine-timber harvesting: three were under shelterwood management, one was a pine-release site, and one was regenerating as an eastern white pine plantation. Shelterwood management (Nyland 1996) is a natural regeneration strategy in which new eastern white pine seedlings regenerate from seed dispersed from the overstory after 50% crown cover removal. In pine-release management (Nyland 1996), existing young eastern white pine trees are released from competition with an undesirable overstory by 100% crown removal. Three of the actively managed sites had mature and either seedling or sapling age classes (Table 1). The areas of study (six mature age classes and three juvenile) will be referred to as populations.

Relatively low densities of mature eastern white pines characterized two sites and both were undisturbed by live pine timber harvest. School Pines was a mesic site where succession was naturally moving the composition to shade-tolerant hardwoods. While eastern white pine made up a substantial portion of the overstory composition 50 years prior to sampling, mortality had greatly reduced the density of eastern white pine to approximately <15 trees/ha. The only forest management at School Pines had been salvage logging and thinning of hardwoods. The other low density site, Minnow Creek, was a brushy savanna-like system that was maintained by periodic surface fires prior to 1930. The mature eastern white pine trees were scattered across the site at approximately <15 trees/ha; there were no live eastern white pines harvested from Minnow Creek. With the advent of fire control in the 1930s, eastern white pine and other species developed beneath the fire survivors; thus, the younger eastern white pine regenerated from a rather restricted number of seed sources. Because the younger eastern white pine originated under a suppressing overstory of aspen (*Populus* spp.) and pin oak (*Quercus palustris* Muenchh.), they were smaller than open-grown pine would have been at that age, and were of sapling size. Camp 1, Oconto Line, and Potato Patch were dense sites composed of approximately >50–100 trees/ha of mature eastern white pine at the time of sampling. Over the previous 30 years, these sites had been thinned from below at least twice at 15 year intervals, selectively removing the least vigorous trees. (In contrast, sites thinned from above means the removal of the most vigorous trees). In the late 1980s, shelterwood treatments were applied to Camp 1 and Oconto Line, which had regenerated eastern white pine in the understory. In 1995, Potato Patch was cut to leave 50% crown closure and had no pine regeneration. Plantation comprised eastern white pine seedlings planted in 1990 from 2-year-old nursery stock originating from the Wisconsin State Nursery, at a density of approximately 1700 seedlings/ha. The site composition was originally a plantation of 70-year-old pole timber (i.e., oak and other hardwoods suitable for use in paper manufacturing) that was clear-cut in 1989. There were no pre-existing pines at Plantation. The area had been a savanna typically maintained by surface fires and only succeeded to trees with fire control in the early 1900s.

Sampling and marker analysis

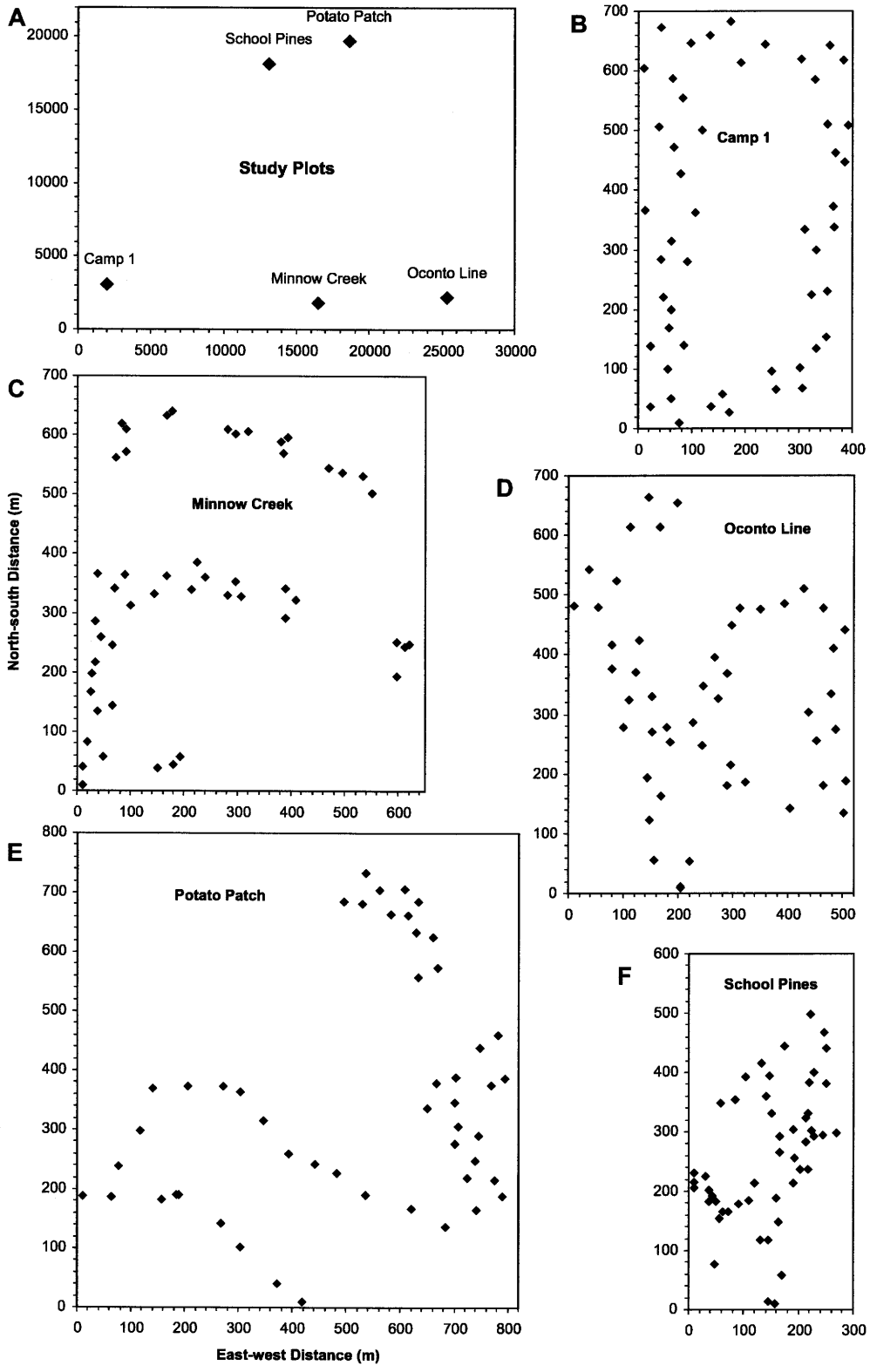
Needle and bud tissues were collected from 50 overstory eastern white pine trees at six sites using a 0.22–250-caliber target rifle during late summer and fall of 1996. Fifty regenerated seedlings or saplings were also sampled from each of three sites: Minnow Creek (saplings), Camp 1 (seedlings) and Oconto Line (seedlings). Sampling intensities of mature trees are listed in Table 1. Seedling and sapling samples were taken at the base of a sampled mature tree. All sample locations were mapped using Universal Transverse-Mercator and North American Datum of 1927 (UTM-NAD27) coordinates obtained through global positioning system (GPS) data gathered at the field plots. Single-tree coordinates were obtained® community base station (Trimble Navigation Ltd., Sunnyvale, California) was used to differentially correct the GPS data for accuracy to 5 m. Geographic distributions of mature trees sampled at the five sites used for spatial analyses are shown in Fig. 1. Fresh tissue was transported to the Rhinelander Forestry Sciences Laboratory (Rhinelander, Wisconsin) and stored at –20 °C until DNA was extracted.

Total DNA was purified from either bud or needle tissue using the method of Guillemaut and Maréchal-Drouard (1992). Stock DNA solutions were diluted to 5.0 ng/μl in $T_{10}E_1$ (10 mmol/L Tris-Cl pH 8.0, 1 mmol/L EDTA) for amplification by polymerase chain reaction (PCR). We surveyed allele-length variation in 3 chloroplast microsatellite markers (*Pt15169*, *Pt30204*, *Pt63718*; Vendramin et al. 1996) and 10 nuclear microsatellite markers, but a detectable frequency of null alleles at three nuclear loci (data not shown) resulted in only seven loci being reported here (*Rps1b*, *Rps2*, *Rps6*, *Rps39*, *Rps50*, *Rps84*, and *Rps127*; Echt et al. 1996). Chloroplast and nuclear markers were used in the population genetic analyses. Only nuclear markers were used in the spatial genetic analyses. PCR was conducted separately for each primer pair. Reaction mixtures contained 1 ng/μL DNA template in 10 μL of reaction buffer. The reaction buffer consisted of 50 mmol/L Tris-Cl pH 9.0, 20 mmol/L $(NH_4)_2SO_4$, 3.5 mmol/L $MgCl_2$, 12% sucrose, 0.2 mmol/L cresol red (Routman and Cheverud 1994), 100 μg/mL gelatin, 200 μmol/L each dNTP, 200–800 nmol/L each primer (forward primer was fluorescent-dye labeled), and 0.04 U/μL AmpliTaq® DNA polymerase (PE Biosystems, Foster City, California). A touchdown-amplification protocol (Echt et al. 1999) with a modified target-annealing temperature of 55 °C was performed using PTC-100 or PTC-200 thermocyclers (MJ Research Inc., Watertown, Massachusetts). PCR products were diluted in deionized water and two to three loci were pooled in one lane for electrophoresis with CXR fluorescent ladder (Promega Corp., Madison, Wisconsin) as the internal size standard. Fragment separation and analysis were done with an ABI Prism® 373 DNA analyzer and GeneScan® analysis software (PE Applied Biosystems, Foster City, California). PCR and gel electrophoresis were conducted at the Rhinelander Forestry Sciences Laboratory.

Statistical analyses

Each sample population was considered separately for most of the statistical analyses. For the population genetics analyses, nuclear and chloroplast loci were analyzed sepa-

Fig. 1. Distance plots of individual study sites used in the spatial analyses (A) and of individual mature trees sampled at the five sites (B–F). Plots B–F are drawn to the same scale.



rately for nine populations. Observed number of alleles and expected heterozygosity (H_E; Nei 1973) and allele frequency (x_i; Nei 1973) were used to measure genetic diversity using Genetic data analysis (GDA) (Lewis and Zaykin 2001) and Popgene (Yeh et al. 1997), respectively. Deficits in heterozygosity were measured for each population using Weir's fixation index (f; Weir and Cockerham 1984) using Fstat (Goudet 1995, 2002). Significant reductions in heterozygosity from Hardy–Weinberg equilibrium were tested at the 1% level using permutations (Manly 1997) to generate P

values for f (1260 randomizations). Permutation tests (10 000 randomizations) were also used at the 5% level to test significant differences in f values between mature trees and naturally regenerated seedlings or saplings. Relative divergence of the nine populations combined (using Fstat) was estimated by Weir's θ (Weir and Cockerham 1984), which is equivalent to F_{ST} (Wright 1943), which measures the correlation between random genes drawn from the subpopulation relative to the total population. Significant differences in nuclear allele frequencies among populations were tested by bootstrapping over loci to provide 99% confidence intervals for θ (Weir 1996). Bootstrapping could not be performed for samples with only one locus (i.e., chloroplast haplotypes). To measure significant differences in chloroplast allele frequencies between pairs of populations (using Fstat), a log-likelihood test statistic (G statistic; Goudet et al. 1996) was formed by permuting multilocus genotypes (α = 0.01; 36 000 randomizations) among samples to generate P values, as described in Petit et al. (2001), where α measures the difference between the desired confidence level and certainty.

For the spatial analyses, five mature populations were evaluated; each allele at each nuclear locus was analyzed separately. For a given allele, the genotype for each tree was converted to an X value of 0, 0.5, or 1.0, according to the number (none, one, or two) of alleles carried in the genotype (Sokal and Oden 1978). For a locus that had more than two alleles, all alleles were used for spatial analyses. Only one allele was considered at diallelic loci, because in this case the second allele contributes identical information. Spatial analyses were not carried out for alleles that were represented in only one individual in a sample, because such cases are considered noninformative for spatial analysis (Epperson 2005). Distance classes were based on the Euclidean distances between pairs of trees. In all cases, five distance classes were used. Because the sample sizes were relatively small, nearly all of the spatial autocorrelation was contained in the first distance class. As the first distance class usually obtains disproportionate statistical power when measuring spatial genetic structure caused by isolation-by-distance (Oden 1984) and avoids several unresolved statistical problems (Epperson 2005), we present these values in detail. The upper bounds were chosen to include at least 200 pairs of trees and to provide sufficient statistical power for detecting spatial autocorrelation within each population (Epperson et al. 1999; Epperson 2005); therefore, the first distance class had a lower bound of zero and upper bounds of 100 m for School Pines and 150 m for the other four populations.

For each analysis, Moran's I (Sokal and Oden 1978) was calculated for each distance class using Saap (Wartenberg 1989). Each I value was tested for significant deviations from the expected value, $E(I) = -1 / (n - 1)$, under the null hypothesis of a spatially random distribution (Cliff and Ord 1981). In all cases, the sample size was near 50; hence, the expected I value is approximately –0.02. A significant positive I value indicates that pairs of individuals in a distance class have similar or correlated values of the trait, whereas a significant negative value indicates that they have dissimilar values. Each set of I values for mutually exclusive distance classes, which is known as an I correlogram, was tested for statistical significance using Bonferroni's criteria (Sakai and Oden 1983). The mean I correlograms were calculated for all alleles at each locus separately, and further averaged over all loci. Epperson (2004) states such mean values are similar, but not necessarily identical, to the multivariate approach used by Smouse and Peakall (1999). To adjust for small sample sizes, we subtracted the means of the observed I values from the expected I values ($I - E(I)$); Walter and Epperson 2004) for the first distance class. To correct for sampling by taking porosity into account (i.e., the proportion of individuals sampled; Epperson et al. 1999), we adjusted the predicted I values by interpolating and extrapolating from the data presented by Epperson et al. (1999; see Table 4) and comparing these values to the $I - E(I)$ values in this experiment. Additionally, we calculated join-count statistics for the total number of unlike genotypes (Sokal and Oden 1978) for the same array of distance classes using Jcsp (Epperson 1993). A test statistic of standard normal deviates (SND) was formed, which has an asymptotically standard normal distribution under the null hypothesis of a random spatial distribution. A SND value <–1.96 indicates a statistically significant excess of pairs of identical single-locus genotypes (i.e., a deficit of pairs with non-identical single genotypes), in which pairs are separated by a given range of Euclidean distances (Epperson 1993).

Results

Genetic diversity

The eastern white pine forest of the Menominee Reservation is genetically diverse. A total of 65 nuclear alleles at seven variable microsatellite loci with a mean of nine alleles per locus were detected in the nine populations combined. A mean of 51% of the nuclear alleles are rare ($x_i \leq 0.050$) and 25% are very rare ($x_i \leq 0.015$). Allele frequencies were estimated separately for each population, and then mean percentages of rare and very rare alleles were calculated over populations. For the combined sampled populations, four alleles were observed at each of the three variable chloroplast microsatellite loci for a total of 12 alleles. Nineteen chloroplast haplotypes (multilocus haploid genotypes) were found for the three chloroplast loci, and when treated as single-locus alleles (i.e., chloroplast genome), the mean H_E is very high (Table 2). H_E was greatest for the chloroplast haplotypes, followed by the mean values for nuclear loci, and then by chloroplast loci. Nuclear H_E is high in all populations, ranging from 46% in the Plantation to 51% in Camp 1 and School Pines. The mean H_E ± standard error for the mature populations of Camp 1, Minnow Creek, and Oconto Line combined (0.49 ± 0.01) was identical to the mean for the associated seedling and sapling populations (0.49 ± 0.00).

Population genetic structure

Five populations have highly significant f values, ranging from 16% in the old-growth population (School Pines) to 21% in the mature pine-release population (Minnow Creek; Table 2). The Plantation population and the mature trees in the Potato Patch population were the only managed populations with low f values, 8% and 4%, respectively. Also, significantly less inbreeding (P = 0.030; one-tailed test) occurs

Table 2. Genetic diversity as measured by expected heterozygosity (H_E).

Sample population	$H_{E\ (NUC)}$	f	P value for f	$H_{E\ (CP)}$	$H_{E\ (HAP)}$
Camp 1	0.51	0.198	0.001	0.42	0.79
Camp 1/s	0.49	0.179	0.001	0.42	0.78
Minnow Creek	0.48	0.207	0.001	0.44	0.82
Minnow Creek/s	0.48	0.058	0.075	0.42	0.78
Oconto Line	0.49	0.188	0.001	0.50	0.83
Oconto Line/s	0.49	0.027	0.254	0.46	0.74
Plantation	0.46	0.075	0.034	0.44	0.80
Potato Patch	0.50	0.037	0.178	0.46	0.82
School Pines	0.51	0.157	0.002	0.46	0.76
Mean	0.49	0.125		0.45	0.79

Note: Inbreeding was measured by Weir's fixation index (f). Nine populations of eastern white pine were analyzed; seedling and sapling populations are designated with /s. Nuclear analyses were based on seven microsatellite loci, chloroplast analyses on three microsatellite loci, and the chloroplast haplotype analyses used three microsatellite loci. NUC, nuclear; CP, chloroplast; HAP, haplotype.

Table 3. Mean Moran's I statistics for the first distance class (100 m for School Pines and 150 m for all other populations), calculated over alleles within loci.

	Rps1b	*Rps2*	*Rps6*	*Rps39*	*Rps50*	*Rps84*	*Rps127*	Mean	I – $E(I)^a$
Camp 1	–0.013	–0.043	–0.003	–0.083	–0.021	0.023	0.120	–0.003	0.017
Minnow Creek	0.055	–0.075	–0.025	0.000	–0.043	–0.003	0.010	–0.012	0.008
Oconto Line	0.003	–0.005	–0.047	–0.023	0.015	–0.015	–0.040	–0.016	0.004
Potato Patch	–0.007	–0.034	0.056	–0.078	–0.003	0.008	0.050	–0.001	0.019
School Pines	0.047	0.008	–0.014	–0.030	–0.027	0.063	0.030	0.011	0.031

Note: Seven nuclear microsatellite markers were sampled in five mature eastern white pine populations.

[a]Mean observed I value minus expected I value of –0.02.

on average in the seedling and sapling age classes for the Camp 1, Minnow Creek, and Oconto Line populations (f = 0.088) compared with the mature trees in these populations (f = 0.197). The seedling population at Camp 1 was the only juvenile population with significant inbreeding (f = 0.179). Differentiation at nuclear loci for all populations combined was minimal and is not significant at the 1% level (mean θ = 0.001; confidence interval 0.000, 0.003) and was similar to the chloroplast haplotype analysis (θ = –0.001). Differentiation evaluated with pair-wise comparisons of the chloroplast haplotypes is significant at the 1% level for 2 of 36 comparisons (less than one significant value is expected by chance). There are no significant differences in allele frequencies between individual seedling or sapling populations and their corresponding mature populations.

Spatial genetic structure

Spatial analyses were conducted on the five mature populations using seven nuclear microsatellite markers (Tables 3 and 4). We found that nearly all significant statistics were in the first distance class; hence, we focused on this class. This was not surprising because isolation-by-distance or any reasonable type of positive global autocorrelation tends to have positive values for the first distance class (Epperson 1993). Moreover, the statistics for the first distance class are highly predictive of the amount of global autocorrelation under genetic isolation-by-distance processes (Epperson et al. 1999). The mean values of I (averaged over alleles within each locus and then over all loci) are presented in Table 3. Based on these results, we determined that the degree of autocorrelation is generally small. The strongest autocorrelation occurred at the least disturbed site of the School Pines population, followed by the Potato Patch, Camp 1, Minnow Creek, and Oconto Line populations. Proper statistical tests of significance for such mean values (i.e., averaged over alleles within a locus) have been developed recently (Epperson 2004). However, those methods assume normality and are not appropriate for the present data set, owing to the small sample size and large numbers of rare alleles. Moreover, the same trends were observed for SND values of the total number of joins between unlike genotypes (Table 4), to which significance tests can be applied (Cliff and Ord 1981). There was compelling evidence of the autocorrelation of genotypes for *Rps2* and *Rps50* in the Oconto Line population; *Rps6* in the Potato Patch population; and *Rps1b*, *Rps84*, and *Rps127* for the School Pines population, with autocorrelation meaning the correlation among genotypes (of individuals) within the sampled population. All statistically significant SND values are negative, indicating positive autocorrelations. Except for the Oconto Line population, the inverse rank order of the mean SND among populations reflected those based on I.

Discussion

Inbreeding

H_E values were lower than expected (i.e., inbreeding) in five of the nine sampled populations. Eastern white pine, like other conifers, can suffer from severe inbreeding depression, resulting in reduced yields, lower seed germination rates, lower survival rates, and slower seedling growth (Ledig 1998). Inbreeding is typically low in naturally regen-

Table 4. Standard normal deviates (SND) values for the total number of joins between unlike genotypes for the first distance class (100 m for School Pines, 150 m for all other populations).

	Rps1b	*Rps2*	*Rps6*	*Rps39*	*Rps50*	*Rps84*	*Rps127*	Mean
Camp 1	–0.72	1.07	–0.89	0.83	0.58	–0.36	–1.06	–0.08
Minnow Creek	–1.37	1.31	0.07	0.24	0.51	0.70	–0.05	0.20
Oconto Line	–0.22	–2.42**	0.13	–1.92*	–3.62***	0.66	0.42	–1.00
Potato Patch	1.45	0.57	–2.10**	–1.12	–1.43	–1.82	–0.38	–0.69
School Pines	–2.27**	–1.20	–0.86	0.78	–0.23	–2.18**	–2.25**	–1.17
Mean	–0.62	–0.13	–0.73	–0.24	–0.82	–0.60	–0.66	–0.54

Note: Seven nuclear microsatellite markers were sampled in five mature eastern white pine populations. *, significant at the 0.10 level, two-tailed test; **, significant at the 0.05 level, two-tailed test; ***, significant at the 0.01 level, two-tailed test.

erating populations if they are not isolated or have large breeding populations, such as in *P. strobus* (Beaulieu and Simon 1994; Marquardt and Epperson 2004; Jones et al. 2006), and other pine species such as *Pinus brutia* Tenore (Panetsos et al. 1998), *Pinus contorta* Dougl. ex Loud. (Epperson and Allard 1984; Knowles 1984), and *Pinus ponderosa* Dougl. ex Laws. (Farris and Mitton 1984). In comparison, and similar to the results of this study, high inbreeding is reported in several conifer species, such as *Abies amabilis* Dougl. ex J. Forbes and *Tsuga heterophylla* (Raf.) Sarg. (El-Kassaby et al. 2003) and *Pinus halepensis* P. Mill. (Troupin et al. 2006).

Populations in which Hardy–Weinberg expectations of random mating were observed were the Minnow Creek sapling and Oconto Line seedling populations, the Plantation population (which is a synthetic population originating from multiple stands), and the Potato Patch population. Possible explanations for the losses in heterozygosity observed in the remaining populations were the presence of null alleles, the Wahlund effect resulting from population admixture, spatial genetic structure, or a self-fertilization or biparental inbreeding mating system. The presence of null alleles is unlikely, because there is no evidence of their presence in a previous study of eastern white pine in Michigan that was evaluated with an identical set of nuclear markers (Marquardt and Epperson 2004). A spatial Wahlund effect is expected if divergent subpopulations are pooled into a population sample. This type of spatial substructure is unlikely because all populations had the same gene frequencies; θ measured little variance among them. A temporal Wahlund effect from pooling diverging generations within populations is also unlikely, because pairwise comparisons of chloroplast haplotypes indicated that a lack of significant differentiation had occurred between the seedling and sapling populations and their corresponding mature populations. Therefore, spatial genetic structure and mating system are the most plausible explanations for significant inbreeding within populations.

Old-growth (sensu lato; Hunter and White 1997) populations of eastern white pine may or may not have deficits in heterozygosity. For example, we found a decrease in heterozygosity from Hardy–Weinberg expectations for the School Pines population ($f = 0.16$), which was similar to the mean inbreeding coefficient estimated for two virgin old-growth populations of eastern white pine in Ontario studied by Rajora et al. (2000). Using the reported mean values of observed heterozygosity (H_O) and H_E (Rajora et al. 2000), we calculated the mean f value for the two old-growth populations of eastern white pine as $f = 1 - (H_O / H_E) = 0.14$ (Nei 1977). In comparison, minimal inbreeding (mean $f = 0.01$) is estimated in an old-growth eastern white pine population in Michigan (Marquardt and Epperson 2004), which is 16 times lower than the value reported for the School Pines population in this study and 14 times lower than the mean value estimated in the Ontario study. Such comparisons among studies are possible because all three studies used largely similar sets of microsatellite markers developed for eastern white pine (Echt et al. 1996). The markers used in this study and in the Michigan study were identical, while the eastern white pine populations in Ontario were evaluated at 13 *Rps* loci, including five of the seven loci assayed in this study. Clearly, inbreeding can occur naturally in eastern white pine for long periods of time.

Spatial genetic structure

The spatial autocorrelation of nuclear DNA markers among the trees sampled within each mature population was weak. It was also variable, because the sites have been disturbed in various ways and were sampled at different intensities. It should be noted that "weak" spatial autocorrelation does not mean "unimportant". A small (i.e., weak) increase in the observed I value above expectations can impart substantial spatial genetic structure. Because our sampling rates varied among populations, it was appropriate to correct for sampling by taking sample porosity into account (Epperson et al. 1999). Epperson (2005) reviews sample design in detail and the indirect effects of sample porosity on the predicted amount of spatial autocorrelation.

The School Pines population site had been disturbed the least, and it exhibited the largest mean measures of spatial autocorrelation. All trees within the study area were sampled at this site. Most of the trees were approximately 240 years old, but the population had been rapidly declining in recent decades. Since 1950, approximately 80% of the eastern white pines had died with no regeneration; thus, given the current density of 15 trees/ha, the density circa 1950 would have been 75 trees/ha. The survivors, if spatially random with respect to genetic structure, can be compared with sampling only one in five of the historical (pre-1950) population, which was equivalent to sampling on a larger spatial scale (Epperson 2005). Hence, to infer the spatial genetic structure of old-growth populations on the Menominee Reservation, we may consider that we in effect "sampled" the pre-1950 School Pines population (prior to losses from old age) on a porosity of 5.0. Because our sample size was

small, we compared the mean of the observed I value minus the expected I value ($I - E(I)$; Walter and Epperson 2004). Indeed, the observed autocorrelation (adjusted for small sample size) falls in the range of values (I = 0.03–0.05) interpolated from Table 4 of Epperson et al. (1999), for a porosity of 5.0. Therefore, correcting for sampling by taking into account sample porosity, we inferred a mean spatial autocorrelation of approximately 0.04–0.05 for contiguous trees prior to 1950 (see porosity = 1.0 from Table 4; Epperson et al. (1999)).

The Minnow Creek population was the second least disturbed by human activities. The mature population had a density similar to that of the School Pines population, but it was sampled at a rate of one in two mature trees rather than contiguously. Note that for a wind-dispersed species, the increase in scale from sampling on a porosity of 2.0 rather than contiguously is not great enough to reduce the amount of autocorrelation expected for the first distance class for the former (interpolating from Table 4; Epperson et al. (1999)). Because both sites had been undisturbed by live-pine harvests and the Minnow Creek population had not experienced a notable decline of mature trees, one could have expected about the same level of autocorrelation for the mature population as that inferred for the historical (pre-1950) School Pines population, a mean I value of approximately 0.04–0.05. In fact, the spatial distribution of genetic variation of the mature population was nearly random. One explanation for this observed randomness is that the spatial genetic structure among established trees reflects the immigration of seeds from offsite seed donors. It seems unlikely that the mature trees at the Minnow Creek site had been progeny of a relict forest population, because the study population consisted of mature pines that had survived low-intensity fires in the past and were scattered across the site, with very few old eastern white pine stumps present at the time of sampling.

The other three sites had been highly disturbed by harvesting activities. Prior to being sampled for study, the Camp 1 and Oconto Line populations were reduced to 70% crown closure in 1989. The Potato Patch population was repeatedly thinned up until 1995, when the site was cut to 50% crown closure. All three sites were sampled on a scale of 1 in 20 of the mature-sized trees, which corresponds to a porosity of at least 40 ($0.5^{-1} \times 20$) with respect to the pre-thinned populations. Even if the spatial autocorrelations in the mature populations before thinning and sampling had values similar to the inferred historical value for the School Pines population (0.04–0.05), the expected I value for the first distance class would be <0.01–0.02 when correcting for porosity (extrapolating from Table 4; Epperson et al. 1999). Indeed, on average the observed I value minus the expected value for these three populations combined (I = 0.01) was <0.02 and roughly consistent with expectations (of the removal of spatial structure) when logging is modeled as spatially random sampling.

In summary, analyses of spatial genetic autocorrelation (for the mature populations) suggested weak positive structuring at the shortest distances; the strongest autocorrelation of genotypes occurred in the old-growth School Pines population, the least disturbed site. Spatial genetic structure can be influenced greatly by seed dispersal and mating system and is expected to be weak in natural tree populations that typically have continuous distributions, like eastern white pine and other conifers (Wright 1943, 1946), whose pollen and seed are widely dispersed by wind. The resulting high gene flow can disrupt the building of spatial genetic structure, as shown by near randomly distributed genotypes of *P. strobus* (second-growth population; Marquardt and Epperson 2004), *P. contorta* (Epperson and Allard 1989) and *Picea mariana* P. Mill. (BSP) (Knowles 1991). Nonetheless, the structuring of genotypes and inbreeding can still occur over time, owing to limited seed dispersal and mating between relatives, as reported for *P. strobus* (old-growth population; Marquardt and Epperson 2004; Jones et al. 2006) and for *P. halepensis* (Troupin et al. 2006), which is similar to the results reported for the old-growth population in this study.

Effects of harvesting on spatial genetic structure and inbreeding

The strongest spatial structuring of genes occurs in School Pines (the least disturbed site), followed by the mature Camp 1, Minnow Creek, Oconto Line, and Potato Patch populations, all of which are managed for pine-timber harvest. Mean $I - E(I)$ statistics were largely in agreement with the inverse rank order of the mean SND values, for which significant autocorrelation exists within the Oconto Line, Potato Patch, and School Pines populations. In studies that evaluate the effects of logging on the spatial genetic structure of forest trees, Knowles (1991) found random distributions of genotypes for both clear-cut and undisturbed *P. mariana* stands. Takahashi et al. (2000) reported greater spatial autocorrelation for a cutover site than a control site for *Fagus crenata* Blume, attributing the increased spatial autocorrelation to founder effects caused by forest cutting. In contrast with Takahashi et al. (2000) and similar to the results of this study, Marquardt and Epperson (2004) reported less spatial autocorrelation for a second-growth site of *P. strobus* than for an old-growth site, suggesting that logging decreased the spatial genetic structure. Therefore, based on these results, we suggest that harvesting decreased the spatial genetic structure in the three populations under shelterwood management: Camp 1, Oconto Line, and Potato Patch.

Significantly less inbreeding was found in two of the three juvenile populations than in their corresponding mature populations. The effects of forest management on genetic diversity have been studied more extensively and often the results contradict the results of this study. For example, enhanced inbreeding following harvest has been reported in *Tsuga Canadensis* L. (Carr.) (Hawley et al. 2005), *Scaphium macropodum* (Miq.) Beumee ex K. Heyne (Lee et al. 2002), *Shorea megistophylla* Dipterocarpaceae (Murawski et al. 1994), and *Pinus sylvestris* L. (Yazdani et al. 1985). In comparison, Thomas et al. (1999) found that the breeding system of *P. contorta* was not affected by harvesting. More similar to the results for this study, Glaubitz et al. (2003) observed no significant increases in inbreeding for *Eucalyptus consideniana* following cutting, with f significantly reduced for one of four harvested sites.

The forest management practices studied in the present article are complementary to those in a previous paper (Marquardt and Epperson 2004) that indicated spatial genetic

structure is weak but positive in old-growth populations of eastern white pine, unless removed by logging. In concurrence (as discussed above), the removal of spatial genetic structure by harvesting is a reasonable explanation for the observed reduction in positive spatial autocorrelation of the mature nuclear genotypes. Spatially caused biparental inbreeding would then be reduced for seedlings established postdisturbance. Therefore, these results suggest that Menominee Reservation forest management practices up to the time of sampling reduced inbreeding, that is, increased heterozygosity, in naturally regenerating seedlings.

Estimating dispersal

Wright's (1946) N_E, or the number of mating individuals drawn at random from within a circle of area $4\pi\sigma^2$ and radius 2σ, can be used to estimate total parent-offspring dispersal (σ^2). To help ensure that genetic diversity is maintained in managed forests, a useful guide for the cutting of seed trees would be to anticipate how the influences of dispersal standardized by density (i.e., changes in breeding neighborhood size, NE) affect spatial genetic structure. For example, when seed dispersal is limited relative to the average spacing between maternal trees (i.e., low seed tree density), increased inbreeding in the regenerating seedlings (and increased autocorrelation) would be expected. However, the results of this study indicate that when seed dispersal is not limited relative to density and harvesting removes the spatial genetic structure of the reproducing mature trees, then inbreeding is reduced in the regenerating seedlings.

To estimate dispersal for the historical School Pines population, a neighborhood size of 130 trees was approximated from an inferred I value of 0.05 (see Discussion above) using Epperson's (2005) estimator, $N_E = \exp[(0.544 - I) / 0.102]$. When standardized for density, the total parent–offspring dispersal (σ^2), i.e., variance, and the combined seed and pollen dispersal distance (σ), i.e., standard deviation, can be estimated using the neighborhood formula for a monecious population, $N_E = 4\pi\sigma^2 d$ (eq. 6; Wright 1946), where d is the estimated pre-1950 population density. Thus, using a d value of 75×10^{-4} trees/m^2 (i.e., 75 trees/ha) led to a predicted value of approximately 1393 m^2 for σ^2 and 37 m for σ. Dispersal for seed ($\sigma^2 s$) can be considerably shorter than dispersal for pollen ($\sigma^2 p$), with $\sigma^2 = 1/2(\sigma^2 p) + \sigma^2 s$ (Crawford 1984). Given that σ^2 is equal to 1393 m^2, our results would correspond (for example) to a mean dispersal distance (σ) of 43 and 21.6 m for pollen and seed, respectively. These σ values were just one of many possible combinations and were in agreement with reported ranges of typical empirical measures: 17 (Wright 1952) to 69 m (Wang et al. 1960) for pine pollen, and 15 to 30 m for pine seed (Epperson and Allard 1984). In comparison, dispersal for the historical School Pines population (σ^2 = 1393 m^2) was approximately 1.7 times larger than the dispersal reported for one old-growth population of white pine in Michigan (σ^2 = 795 m^2; Marquardt and Epperson 2004) that had an identical autocorrelation value ($I - E(I)$ = 0.05). Although both eastern white pine populations are minimally disturbed old-growth of similar ages, the density of the Michigan population is 25% greater than the density estimated for the historical School Pines population, resulting in an increase in predicted dispersal in the latter.

Conclusions

Genetic diversity was high for all eastern white pine populations and evenly distributed among them. The spatial genetic structure of nuclear genes was variable in the managed mature populations and strongest at the least-disturbed old-growth site. Spatial autocorrelation at the historical old-growth site fit predictions for eastern white pine, based on pollen and seed dispersal and population density. H_E values were lower than expected within five populations and this was attributed to the combined effects of spatial genetic structure and inbreeding. Contrary to our hypothesis that inbreeding would be increased in the naturally regenerating populations at the harvested sites, we found significantly less inbreeding on average in the young trees than in the local mature trees. The observed reduction in inbreeding among juveniles is most likely because of disturbance such as harvesting, which decreases the spatial genetic structure of the mature trees. Only with an increased understanding of the changes that occur as a result of forest management (e.g., changes in breeding neighborhood sizes as a result of the cutting of seed trees) will it be possible to conserve genetic diversity in tree populations — a crucial step for sustainable resource management.

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