Theor Appl Genet (1999) 98: 793–801 © Springer-Verlag 1999

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# Effects of reforestation methods on genetic diversity of lodgepole pine: an assessment using microsatellite and randomly amplified polymorphic DNA markers

Received: 18 August 1998 / Accepted: 17 September 1998

**Abstract** We examined the effects of different methods of forest regeneration on the genetic diversity of lodgepole pine (*Pinus contorta* var 'latifolia') using two different DNA-based molecular markers [randomly amplified polymorphic DNA (RAPDs) and microsatellites or simple sequence repeats (SSRs)]. Genetic diversity was estimated for 30 individuals in each of four populations for the following three stand types: (1) mature lodgepole pine (>100 years); (2) 20- to 30-year-old harvested stands left for natural regeneration; (3) 20- to 30-year-old planted stands (4 stands of each type); and one group of 30 operationally produced seedlings. There was no significant effect of stand type on expected heterozygosity, although allelic richness and diversity were much higher for SSRs than for RAPDs. Expected heterozygosity ranged from 0.39 to 0.47 based on RAPDs and from 0.67 to 0.77 based on SSRs. The number of alleles per locus for SSRs ranged from 3 to 34 (mean 21.0), and there was a significant relationship between sequence repeat length and the number of alleles at a locus. Both marker types showed that over 94% of the variation was contained within the populations and that the naturally regenerated stands sampled had lower (not significant) expected heterozygosity than the planted or unharvested stands. The group of seedlings (assessed by RAPDs only) had expected heterozygosity and allele frequencies similar to those of the unharvested stands. Genetic distance measures were higher than obtained previously in the species using isozyme markers. There was no correlation between the two marker types for pair-wise genetic distances based on populations analyzed by both methods. Pair-wise genetic distance measures and an ordination of allele frequencies for both marker types showed little effect of geographic location or stand type on genetic similarity.

**Key words** *Pinus contorta* · Silviculture · Reforestation · Gene conservation · RAPD · SSR · DNA analyses

Communicated by P. M. A. Tigerstedt

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## Introduction

Genetic diversity provides the template for adaptation and evolution of populations and species. Therefore, maintenance of genetic variation is an important objective of biodiversity conservation. Commercial management and breeding of plant species often leads to changes in the pattern of, and usually reductions in, genetic diversity (Harlan 1975), and loss of genetic diversity in herbaceous crop species has long been recognized as a potentially serious problem (Frankel and Bennett 1970; Harlan 1975). Scientists and managers have also shown concern about the potential for loss of genetic diversity in commercially managed forest tree species (Libby et al. 1969; Jasso 1970; Richardson 1970; Ledig 1992; Rogers and Ledig 1996). Indeed, conservation of genetic diversity may be one of the most important issues influencing future forestry practices (Boyle 1992; Namkoong 1992).

Studies examining the impact of forest harvesting and regeneration on genetic diversity have produced mixed results. Knowles (1985) found no difference in genetic diversity between fire-origin and artificially regenerated stands of jack pine and black spruce. In

contrast, Gömöry (1992) reported that planted stands of Norway spruce had significantly less genetic diversity than unharvested or naturally regenerated stands. Even with wild seed collections, inadvertent loss of genetic diversity or shifts in allele frequencies may result from selection during seed collection, processing and seedling production (Silen and Osterhaus 1979; Campbell and Sorensen 1984; El-Kassaby and Thomson 1996; Stoehr and El-Kassaby 1997). On the other hand, selection during nursery production may be less intense than that experienced at the early establishment phase in natural stands (Muona et al. 1988), leading to higher levels of genetic diversity in artificially regenerated stands.

Lodgepole pine (*Pinus contorta* var 'latifolia') is an early successional conifer that has a continuous distribution in western North America, exhibits wide ecological amplitude and is an important commercial species in west-central Alberta, Canada and elsewhere (Ying et al. 1984). Wind-pollinated conifers typically show very high levels of within-population genetic variation and relatively less differentiation among populations (Guries and Ledig 1981; Yeh 1981; Hamrick and Godt 1989). This has been verified for lodgepole pine in western Canada, with 96–98% of variation in isozymes found within populations (Yeh and Layton 1979; Dancik and Yeh 1983; Yang et al. 1996). Still, isozyme and growth studies have provided evidence of population differentiation related to latitude and altitude (Illingworth 1976; Yeh et al. 1985).

Following forest harvesting, lodgepole pine may be regenerated artificially, by planting seedlings or naturally. On naturally regenerated sites cone-bearing branches are dispersed across the site by drag scarification, and subsequent regeneration depends upon natural seed cast from the serotinous cones which open in response to the warm air temperatures at the ground surface. For planted stands, cone-bearing branches are removed from the site during harvesting, and areas are subsequently planted with nursery-grown seedlings derived from local, wild-collected, bulked seed sources. Both methods have implications for genetic diversity in regenerated stands. Fewer serotinous cones may open in cutblocks than would occur naturally following fire, potentially leading to lower diversity in harvested, naturally regenerated stands. In contrast, the bulking of seed from many populations within the breeding region may result in higher genetic diversity in planted stands, but lower diversity among them. Subsequent effects of selection during nursery production would possibly further modify genetic diversity.

In recent years there has been increasing interest in the use of DNA-based markers for a variety of applications in population genetics, conservation and tree improvement. Both RAPD (randomly amplified polymorphic DNA) and microsatellite (or simple sequence repeat, SSR) markers show much promise in this regard (Haymer 1994). RAPDs have been recently used to: identify hybrid spruce (Khasa and Dancik 1996); quantify genetic diversity in Norway spruce (Bucci and Menozzi 1995); identify clones and quantify genetic variation in Sitka spruce (Van den ven and McNicol 1995); assess genetic distance in *Pinus leucodermis* (Bucci et al. 1997) and quantify genetic variation in other native plants and crop species (Huff et al. 1993; Skroch and Nienhuis 1995). SSR markers have been used to quantify genetic diversity and examine population differentiation in agricultural crops (Morgante et al. 1994; Hamann et al. 1995; Maughan et al. 1995; Mörchen et al. 1996) and trees, including radiata pine (Smith and Devey 1994), bur oak (Dow et al. 1995), *Eucalyptus* sp. (Byrne et al. 1996) and eastern white pine (Echt et al. 1996).

Since RAPDs are a dominant marker type, the presence or absence of a band is defined as representing two alleles at a locus and, therefore, calculation of genetic diversity values must assume a Hardy-Weinberg equilibrium. Consequently, these data do not allow for the determination of allelic richness, effective number of alleles or for the calculation of fixation indices. Isabel et al. (1995) showed that expected heterozygosity is underestimated, while population differentiation estimates are inflated, when based upon RAPD banding phenotypes as opposed to the actual genotypes. SSRs, on the other hand, are co-dominant and tend to have multiple alleles per locus so that individuals can be identified as homozygotes or heterozygotes. The data can, therefore, be used to compare observed and expected heterozygosities and to calculate effective number of alleles and fixation indices.

In the study presented here we used RAPDs and SSRs to examine the impact of reforestation method on genetic diversity in stands of lodgepole pine located in the foothill region of west-central Alberta. Specifically, we quantified genetic variability for three stand types: unharvested mature lodgepole pine stands; harvested stands which were left for natural regeneration; harvested stands which were planted with nursery-grown seedlings; and in one group of seedlings produced (operationally) for reforestation. A secondary objective of the study was to compare RAPD and SSR markers with respect to the estimates of genetic variation they provide.

## Materials and methods

### Material

Needle tissue was collected from lodgepole pine trees in 12 stands within the Forest Management Agreement areas of Weldwood of Canada Ltd (Hinton Division) (9 populations) near Hinton, Alberta (53°N 117°W) and Weyerhaeuser Canada Ltd (Grande Prairie) (3 populations) near Grande Prairie, Alberta (55°N 118°W) (Table 1). Three different stand types were sampled: unharvested (fire-origin) stands ( > 100 years), harvested (clearcut) planted stands (19–33 years old) and harvested (clearcut) naturally regenerated stands (19–33 years old) (Table 1).

**Table 1** Populations used in the study: location, stand type, age, site preparation and molecular markers used for analysis

| | Population location[a] | Stand type | Age[b] (years) | Site preparation[c] | RAPD | SSR |
|---|---|---|---|---|---|---|
| H-U-1 | Hinton (T54 R25) | Unharvested | >100 | No | X | X |
| H-U-2 | Hinton (T54 R25) | Unharvested | >100 | No | X | X |
| H-U-3 | Hinton (T54 R25) | Unharvested | >100 | No | X | X |
| H-P-1 | Hinton (T54 R24) | Planted | 32 | Yes | X | X |
| H-P-2 | Hinton (T54 R24) | Planted | 28 | Yes | X | |
| H-P-3 | Hinton (T54 R24) | Planted | 33 | No | X | |
| H-NR-1 | Hinton (T54 R24) | Natural Regen | 32 | No | X | X |
| H-NR-2 | Hinton (T54 R24) | Natural Regen | 33 | Yes | X | |
| H-NR-3 | Hinton (T54 R24) | Natural Regen | 25 | Yes | X | |
| Seedlings | Hinton (T54 R24) | Seedlings | | | X | |
| G-U-1 | Grande Prairie (T65 R08) | Unharvested | >100 | No | | X |
| G-P-1 | Grande Prairie (T65 R07) | Planted | 19 | Yes | | X |
| G-NR-1 | Grande Prairie (T65 R07) | Natural Regen | 19 | Yes | | X |

[a] General location (township, range)
[b] Age since fire (unharvested stands) or harvest (planted, naturally regenerated stands)
[c] Mechanical site preparation

Most harvested sites were subjected to mechanical site preparation 1 or 2 years following harvesting to create favorable microsites for planting or natural regeneration (Table 1). In the Hinton area the planted stands were planted 7–9 years after harvesting, while the Grande Prairie stand was planted 3 years after harvesting. In the Grande Prairie area the unharvested, planted and naturally regenerated populations represented neighboring stands (< 2 km apart). In the Hinton area, the naturally regenerated and planted stands were randomly selected from one township within harvest planning area Athabasca 16. All mature stands in Athabasca 16 had been harvested, so we randomly collected unharvested stands from an adjacent township in harvest planning area Athabasca 27 (similar elevation, aspect and site types as in Athabasca 16). Stands in the Hinton area were all located in the Upper Foothills Natural Subregion, while those in the Grande Prairie area were from the Lower Foothills Natural Subregion (Alberta Environmental Protection 1994). In addition we used RAPDs to analyze a single group of 30 nursery-grown seedlings which had been pre-screened to meet the minimum size and quality criteria and were ready for planting in the Hinton area.

Current-year needle tissue was collected for 40 trees per population from individuals that were a minimum of 30 m apart. The tissue was kept cool until storage at $-70^{\circ}$C. RAPD and SSR loci, DNA extraction and polymerase chain reaction (PCR) protocols are as described elsewhere (Hicks et al. 1998). Ten RAPD loci and five SSR loci were used to analyze the populations as summarized in Table 1. Five populations were analyzed by both methods. Bootstrapping (calculation of expected heterozygosity based on random sub-samples of between 10 and 40 individuals per population) demonstrated that analysis of 30 individuals was sufficient to accurately quantify genetic variation in these populations (Hicks 1997). Thus, all subsequent analyses were based on 30 individuals per population.

## Analysis

RAPD-PCR products were visualized on vertical agarose gels subjected to electrophoresis for 6 h at 3 V/cm or overnight at 1.25 V/cm in TBE (0.1 *M* TRIS-HCl, 0.1 *M* borate, 0.01 *M* EDTA, pH 8.0). Gels were stained with ethidium bromide (0.5 μg/ml) for 30 min, then photographed with a digital imager. GEL PRO ANALYZER™ Ver. 2.0 software (Media Cybernetics) was used to detect alleles. Bands were grouped together if their mobilities differed by less than 3%. A total of ten RAPD loci, derived from four primers, were scored (see Hicks et al. 1998). A spreadsheet was used to calculate: expected heterozygosity ($H_E$; Nei's unbiased estimate for small population size, Nei 1978) for each locus and each population; species-level expected heterozygosity based on these populations ($H_T$); the proportion of variation that is due to population differentiation ($F_{ST}$) for each locus and over all populations (Hartl and Clark 1989); and genetic distance (Nei 1978).

SSR-PCR products were subjected to electrophoresis in TBE at 40 W for 2.5 h on a 6% denaturing acrylamide gel containing 7 *M* Urea. X-ray images of dried gels were used to score alleles by hand. Alleles were sized using a molecular-weight ladder generated from a sequencing reaction of known DNA sequence. Five SSR loci were scored [APC 3, 9, 11, 13, 15 (APC = Alberta *Pinus contorta*); Hicks et al. 1998]. Alleles at each locus were assigned letter codes, and POPGENE (Yeh et al. 1997) was used to calculate allele frequencies and estimates of genetic variation as follows: expected heterozygosity (Nei's unbiased estimate, Nei 1978) for each locus, population and the species (based on these populations); observed heterozygosity; average number of alleles; effective number of alleles (number of alleles that would result in that level of heterozygosity if all alleles were in equal frequency; Hartl and Clark 1989); F-statistics [the proportion of variation due to population differentiation ($F_{ST}$), deficiency of heterozygotes relative to Hardy-Weinberg expectation ($F_{IS}$) (Hartl and Clark 1989; Weir 1990)], and genetic distance (Nei 1978). Paetkau et al. (1997) found Nei's unbiased measure of genetic distance to be effective for fine-scale examination of population variation using SSR markers.

Analysis of variance (ANOVA) was used to examine differences in expected heterozygosity as a function of marker type (RAPD or SSR), stand type (unharvested, naturally regenerated, planted) and population using the following model:

$$Y_{ijkl} = \mu + M_i + S_j + MS_{ij} + P(S)_{k(j)} + e_{l(ijk)}$$

where $Y_{ijkl}$ = the observation, $\mu$ = the overall mean, $M_i$ = the $i$th marker type, $S_j$ = the $j$th stand type, $MS_{ij}$ = the interaction of marker and stand type, $P(S)_{k(j)}$ = the $k$th population within the $j$th stand type, and $e_{l(ijk)}$ = random error associated with population within stand type by marker. Differences were considered significant at $\alpha = 0.05$. Principal Components Analysis (PCA) ordination (using CANOCO, Ter Braak 1988) was used to examine relationships among populations based on multilocus allele frequencies for both RAPD and SSR data. This type of multivariate analysis has proven useful in understanding patterns of population differentiation because it can reveal multilocus genotypic structure, whereas univariate measures, such as genetic distance, cannot (Yeh et al. 1985; Knowles 1985).

**Table 2** Results of analysis of variance on expected heterozygosity ($H_E$)

| Source of variation | *df* | *P* |
|---|---|---|
| Marker type | 1 | 0.01 |
| Stand type[a] | 2 | 0.25 |
| Marker-by-stand type | 2 | 0.74 |
| Population within stand type | 9 | 0.59 |

[a] Including unharvested, naturally regenerated and planted stands

## Results and discussion

There was a significant difference in expected heterozygosity ($H_E$) among marker types but no effect of stand type, population, or marker-by-stand type interaction (Table 2). On average, however, the naturally regenerated stands showed lower $H_E$ than the unharvested stands (11% for RAPD loci; 4% SSR loci; Table 3, Fig. 1a, b). Gömöry (1992) found a 13% reduction in expected heterozygosity for planted (vs unharvested) stands and an 8% increase in diversity for naturally regenerated stands of Norway spruce (*Picea abies*), and reported this as a significant impact of artificial regeneration on genetic diversity, although no significance testing was done. In contrast, Knowles (1985) found that one planted and one naturally regenerated stand of jack pine (*Pinus banksiana*) had higher observed heterozygosity (13% and 16%, respectively) than an old, unharvested stand, but she considered all stands to have 'similar' diversity. To thoroughly examine the impacts of forest management on genetic diversity it is essential to have a proper replication of stand types and to test for statistical significance.

The high levels of diversity in the planted stands concur with the high level of diversity we found in the group of operationally produced seedlings, ($H_E = 0.47$ for RAPDs). These seedlings also appeared to be a good reflection of allele frequencies found in unharvested stands (see Table 6, Fig. 2 and discussion below; cf. El-Kassaby and Thomson 1996; Stoehr and El-Kassaby 1997). In contrast to previous suggestions (Silen and Osterhaus 1979; Campbell and Sorensen 1984; Muona et al. 1988), procedures for commercial seed collection and nursery seedling production do not appear to modify genetic diversity relative to natural stands.

Although it has been suggested that allelic richness measures may be more informative than measures of heterozygosity when examining changes in gene pools due to disturbance (Marshall and Brown 1975; Buchert et al. 1997), only co-dominant markers are useful for this purpose. We saw no significant difference in total or mean alleles per SSR locus among the three stand types (not shown) despite their different disturbance histories.

Expected heterozygosity as measured by SSRs was 1.7 times that obtained using RAPD markers [mean $H_E$ of 0.73 (range: 0.69–0.78, SSR) vs 0.43 (range: 0.34–0.48, RAPD); $H_T$: 0.74 vs 0.46, respectively]. In general, our values are comparable to the few published in the literature. Using 20 RAPD loci in Norway spruce (*Picea abies*), Bucci and Menozzi (1995) measured an average $H_E = 0.334$. Average $H_E$ for SSR loci in both radiata pine (*Pinus radiata*; 2 loci, 96 individuals, Smith and Devey 1994) and bur oak (*Quercus macrocarpa*; 3 loci, one population, Dow et al. 1995) was approximately 0.70. Pfeiffer et al. (1997) obtained an $H_E$ of 0.43–0.94 (7 SSR loci, Norway spruce) with 6–22, alleles

**Table 3** Genetic variation for the 13 populations based on ten RAPD loci or five SSR loci for *n* individuals per population, means across all populations for each marker type, and species-level estimates (based on these populations)

| RAPD | *n* | $H_E$[a] | SSR | *n* | na[b] | ne[c] | $H_O$[d] | $H_E$ |
|---|---|---|---|---|---|---|---|---|
| H-U-1 | 30 | 0.44 | H-U-1 | 28 | 10.8 | 7.07 | 0.46 | 0.67 |
| H-U-2 | 30 | 0.48 | H-U-2 | 29 | 13.0 | 7.95 | 0.44 | 0.77 |
| H-U-3 | 30 | 0.40 | H-U-3 | 29 | 12.8 | 7.44 | 0.48 | 0.76 |
| H-NR-1 | 30 | 0.42 | G-U-1 | 30 | 12.2 | 7.10 | 0.47 | 0.74 |
| H-NR-2 | 30 | 0.34 | H-NR-3 | 28 | 11.2 | 6.39 | 0.43 | 0.69 |
| H-NR-3 | 30 | 0.42 | G-NR-1 | 28 | 11.0 | 6.65 | 0.49 | 0.73 |
| H-P-1 | 30 | 0.47 | H-P-3 | 29 | 12.6 | 7.42 | 0.46 | 0.78 |
| H-P-2 | 30 | 0.38 | G-P-1 | 27 | 10.4 | 6.03 | 0.46 | 0.71 |
| H-P-3 | 30 | 0.45 | | | | | | |
| Seedlings | 30 | 0.47 | | | | | | |
| Mean | | 0.43 | Mean | | 11.8 | 7.01 | 0.46 | 0.73 |
| All populations | | RAPDs | $H_T$[e] | $F_{ST}$[f] | SSRs | $H_T$ | $F_{ST}$ | $F_{IS}$[g] |
| | | | 0.46 | 0.061 | | 0.74 | 0.028 | 0.360 |

[a] $H_E$, Expected heterozygosity (Nei's unbiased estimate)
[b] na, Observed number of alleles
[c] ne, Effective number of alleles (Hartl and Clark 1989)
[d] $H_O$, Observed heterozygosity
[e] $H_T$, Expected heterozygosity for the species, based on these populations
[f] $F_{ST}$, Proportion of genetic variability that is due to population differentiation
[g] $F_{IS}$, Deficiencies of heterozygotes relative to Hardy–Weinberg expectations

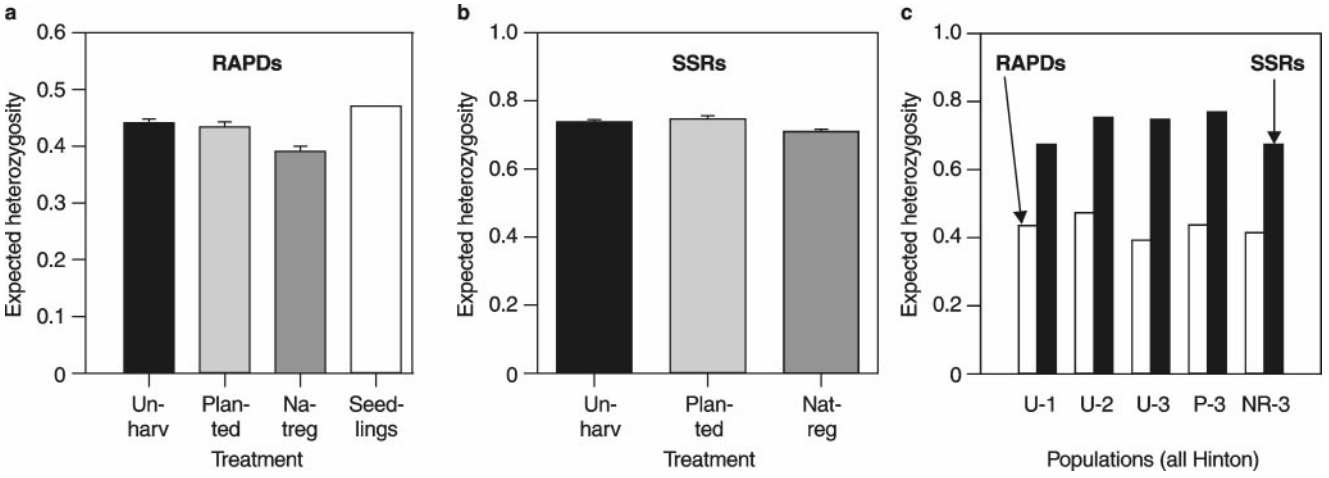


**Fig. 1a–c** Mean expected heterozygosity ($H_E$) (standard error) for the three stand types based on **a** RAPDs, **b** SSRs and **c** comparison of $H_E$ by both marker types for 5 populations. *Unharv* unharvested, *natreg* naturally regenerated

per locus. In a study of eastern white pine (*Pinus strobus*), Echt et al. (1996) found an average observed heterozygosity ($H_O$) of 0.52 with a mean of 5.4 alleles per locus (16 SSR loci, 16 trees). Our average $H_O$ was 0.46 with 11.8 alleles per SSR locus (Table 3).

Expected heterozygosities for both marker types are much higher than for isozyme studies of lodgepole pine (0.16–0.19), which typically include both polymorphic and monomorphic loci (Yeh and Layton 1979; Dancik and Yeh 1983; Yang and Yeh 1995). Isabel et al. (1995) found $H_E$ for black spruce based on isozymes (13 loci) was very similar to that based on RAPDs when the latter loci were selected randomly without regard to their monomorphic or polymorphic nature. Isozymes may also have lower diversity because some mutations will not produce detectable differences in isozyme mobility and others may be selected against if they result in a non-functioning enzyme. For the 5 populations we assessed by both RAPD and SSR markers there were differences in the rank order of populations (for $H_E$) depending on the marker type used (Table 3, Fig. 1c). This is not surprising given the slight differences in $H_E$ among these populations. RAPD loci showed relatively little variation in $H_E$ (0.34–0.49) as compared to SSR loci (0.51–0.95) (Table 4). This reflected the variation in number of alleles per SSR locus which, in turn, was correlated with SSR repeat length (Table 5). Pfeiffer et al. (1997) also found a correlation between SSR repeat length and alleles per locus. There was more variation in $F_{ST}$ among loci for RAPDs (0.011–0.126) than for SSRs (0.02–0.044).

SSR locus APC 15 had an observed heterozygosity similar to that expected under Hardy-Weinberg ($F_{IS} = -0.064$). All other loci showed a deficiency of heterozygotes ($F_{IS}$ ranged from 0.177 to 0.648) with an overall $F_{IS} = 0.360$ (Tables 3, 4). Heterozygote deficiencies have also been found in other wind-pollinated and dispersed conifers (Guries and Ledig 1981; Dancik and Yeh 1983; Knowles 1991; Wang and Macdonald 1992; Sproule and Dancik 1996; Liu and Knowles 1991). Heterozygote deficiencies can be caused by inbreeding (assortative mating), selection against heterozygotes, the Wahlund effect (population subdivision into separate breeding units) or selection-induced microscale differentiation (Brown 1978; Epperson 1990; Sproule and Dancik 1996; Knowles 1991; Bush and Smouse 1992). Lodgepole pine is an early successional species that regenerates quickly after fire by the release of seeds from serotinous cones. Thus, family structure could easily develop in populations and lead to some degree of inbreeding, simply reflecting outcrossing among related individuals which are spatially grouped (Linhart et al. 1981; Sproule and Dancik 1996).

Both marker types showed that the vast majority (94%, RAPDs; 97%, SSRs; based on $F_{ST}$) of genetic diversity was contained within populations and that there was relatively little differentiation among populations (Table 3). This is similar to what has been shown using isozyme markers (Dancik and Yeh 1983; Yeh and Layton 1979; Yeh et al. 1985; Yang et al. 1996) and has been attributed to extensive gene flow and large population size (Epperson and Allard 1989). In contrast, Bucci et al. (1997) found much higher $F_{ST}$ values in *Pinus leucodermis* for RAPDs than for isozymes. In our study, it does not appear that estimates of population differentiation are being inflated by the assumption of Hardy-Weinberg when using RAPD markers (Isabel et al. 1995).

Our values for $F_{IS}$ are considerably higher than published values based on isozyme markers for lodgepole pine (0.03, Dancik and Yeh 1983; 0.06, Yeh et al. 1985) or other conifers (Guries and Ledig 1981; El-Kassaby and Ritland 1996; Stoehr and El-Kassaby 1997). We found only one published study for which $F_{IS}$ values could be determined for SSR data. In 20 unrelated individuals of *Eucalyptus nitens* $F_{IS}$ was 0.306 with

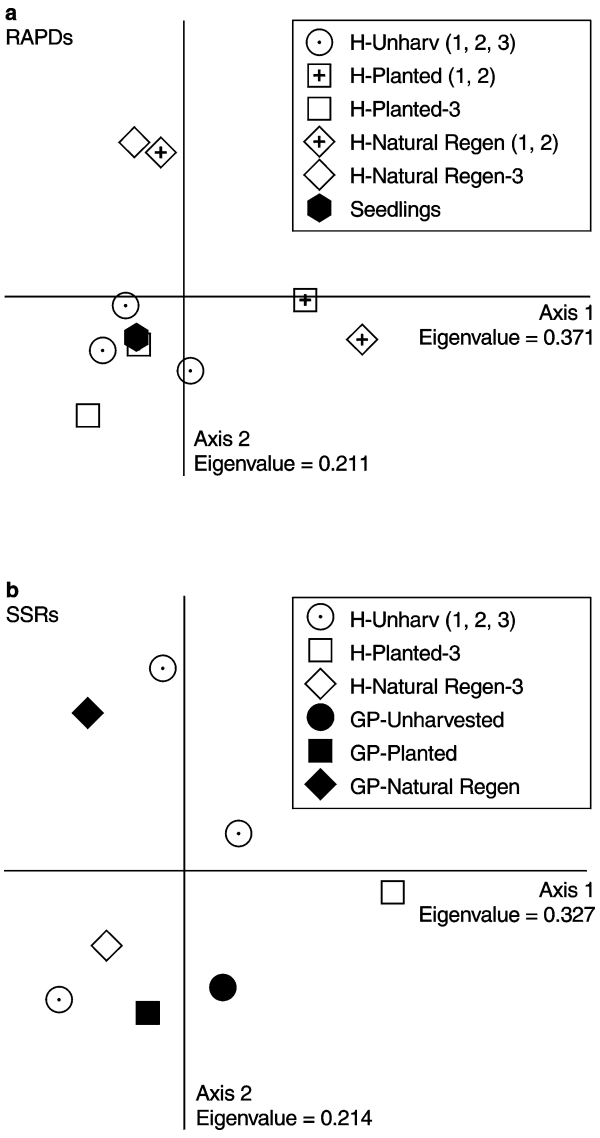


**Fig. 2a, b** Scores for the first two PCA axes based on **a** RAPD data (10 populations) and **b** SSR data (8 populations). The eigenvalues for axes 1 and 2 explain the variance accounted for by the set of variables associated with that axis, but there is no 'significance' level per se for the analysis (Tabachnick and Fidell 1989)

observed and expected heterozygosities of 0.58 and 0.83, respectively (4 SSR loci, Byrne et al. 1996). A possible explanation for these higher inbreeding coefficients is that SSR loci are non-coding, and thus homozygous genotypes may be retained in the population longer than for allozymes which represent a more conservative portion of the genome and may be under selective pressure (Mitton 1994).

Genetic distance values (D) (Table 6) were fairly similar for both marker types (RAPD mean = 0.056; SSR mean = 0.063) but higher than for isozyme studies of lodgepole pine (D = 0.003–0.007, Dancik and Yeh 1983). This may reflect a greater power of these DNA-based markers for detecting differences among populations or the more rapid evolution of non-coding parts of the genome. It could also be due to the fact that both polymorphic and monomorphic loci are used for the calculation of genetic distances based on isozyme data, whereas only polymorphic loci are used for RAPDs and SSRs.

Bucci et al. (1997) found much higher (five times) genetic distance values for RAPDs than for isozymes but a strong correlation between pair-wise population distances by both marker types. In our study there was no correlation between distance values for the two marker types, based on pair-wise comparisons for the 5 populations analyzed by both methods (not shown). Based on the SSR data there was no greater differentiation between the Hinton and Grande Prairie regions than within either (average D within both regions = 0.069; between regions = 0.058). The seedling population was very similar to all other stand types in the Hinton region (D based on RAPDs = 0.036). Otherwise, there was no obvious pattern of similarity based on stand type or geographic location.

The ordination of allele frequencies also indicated that geographic location or stand type had little influence on similarity in allele frequencies (Fig. 2). Using genetic distance calculations and ordination of isozyme data, Gömöry (1992) showed divergence in allele frequencies for planted compared to naturally regenerated

**Table 4** Genetic variation for the ten RAPD loci and five SSR loci. Averages across *n* populations

| RAPD | *n* | $H_E$[a] | $F_{ST}$ | SSR | *n* | $F_{ST}$ | $F_{IS}$ | na | ne | $H_O$ | $H_E$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 10 | 0.485 | 0.011 | APC3 | 8 | 0.044 | 0.648 | 13 | 2.43 | 0.197 | 0.588 |
| 2 | 10 | 0.451 | 0.034 | APC9 | 8 | 0.020 | 0.390 | 34 | 19.48 | 0.565 | 0.949 |
| 3 | 10 | 0.466 | 0.037 | APC11 | 8 | 0.036 | 0.631 | 27 | 3.56 | 0.255 | 0.719 |
| 4 | 10 | 0.434 | 0.037 | APC13 | 8 | 0.020 | 0.177 | 28 | 16.22 | 0.757 | 0.938 |
| 5 | 10 | 0.480 | 0.056 | APC15 | 8 | 0.031 | − 0.064 | 3 | 2.05 | 0.527 | 0.512 |
| 6 | 10 | 0.377 | 0.116 | | | | | | | | |
| 7 | 10 | 0.341 | 0.126 | | | | | | | | |
| 8 | 10 | 0.362 | 0.117 | | | | | | | | |
| 9 | 10 | 0.482 | 0.048 | | | | | | | | |
| 10 | 10 | 0.390 | 0.057 | | | | | | | | |

[a] $H_E$, na, ne, $H_O$, $F_{IS}$ and $F_{ST}$ are as defined in Table 3

**Table 5** Mean length of repeat sequence at the five SSR loci and total number of alleles detected at each SSR locus. There was a significant correlation between the two ($r^2 = 0.61$)

| SSR | Length of base-pair | Total number of alleles detected |
|---|---|---|
| APC3 | 102.0 | 13 |
| APC9 | 131.9 | 34 |
| APC11 | 161.6 | 27 |
| APC13 | 136.7 | 28 |
| APC15 | 100.8 | 3 |

stands of Norway spruce. Ordination of our RAPD allele frequency data verified that the seedlings had similar allele frequencies to those of the 3 Hinton unharvested populations. The three Hinton naturally regenerated stands and one of the Hinton planted stands showed divergence from this core group.

The lack of a correlation between genetic and geographic distance is consistent with previous isozyme studies that showed significant geographic variation only over a much wider range of the species distribution (Yeh and Layton 1979; Yeh et al. 1985). Yeh et al. (1985) suggested that altitudinal variation may be important at a smaller scale but our sampling was not intensive enough to test this. Rehfeldt (1988) also found that clinal variation of ecological traits in lodgepole pine was more closely associated with steep elevational gradients but was affected little by geographic differences. Also noteworthy in our data is the lack of concordance in population divergence as measured by the two marker types. This could simply reflect a lack of genetic drift among these populations such that divergence as measured by these non-coding regions of the genome is essentially random. Further, it would be preferable to base genetic distance measures on larger numbers of SSR loci, since they have so many alleles.

In this study we used old, unharvested stands as the benchmark for comparison of genetic diversity in harvested stands. We did not specifically address changes in genetic diversity with age although stands of forest trees could be reasonably expected to display changes in genetic diversity over time. Since lodgepole pine is very shade intolerant there is typically very little ingress into stands following the initial establishment phase. Stands do, however, experience considerable mortality and a reduction in density through the self-thinning phase. This mortality could lead to either a decline in genetic diversity, due to bottleneck effects, or possibly an increase if homozygotes demonstrate slower growth and thus greater mortality during the self-thinning phase (Mitton et al. 1981). In a very old stand of *Pinus sylvestris* Tigerstedt et al. (1982) demonstrated a deficiency of heterozygotes in trees up to 100 years old but the oldest trees (300–400 years) were in Hardy-Weinberg equilibrium. We are currently in the process of using isozymes to develop a comprehensive baseline on patterns of genetic diversity of lodgepole pine in northern Alberta, including relationship to stand age, elevation, silvicultural treatment and stand initiation history.

**Table 6** Genetic distance among populations based on RAPDs and SSRs (Nei's unbiased measure, Nei 1978)

RAPDs

| Population | H-U-1 | H-U-2 | H-U-3 | H-P-1 | H-P-2 | H-P-3 | H-NR-1 | H-NR-2 | H-NR-3 |
|---|---|---|---|---|---|---|---|---|---|
| H-U-1 | ****** | | | | | | | | |
| H-U-2 | 0.0469 | ****** | | | | | | | |
| H-U-3 | 0.0556 | 0.0456 | ****** | | | | | | |
| H-P-1 | 0.0376 | 0.0130 | 0.0320 | ****** | | | | | |
| H-P-2 | 0.0760 | 0.0644 | 0.0787 | 0.0528 | ****** | | | | |
| H-P-3 | 0.0356 | 0.0496 | 0.0523 | 0.0238 | 0.0772 | ****** | | | |
| H-NR-1 | 0.0568 | 0.0344 | 0.0425 | 0.0381 | 0.0691 | 0.0800 | ****** | | |
| H-NR-2 | 0.0939 | 0.0814 | 0.0460 | 0.0622 | 0.0243 | 0.1039 | 0.0737 | ****** | |
| H-NR-3 | 0.0570 | 0.0562 | 0.0736 | 0.0462 | 0.0658 | 0.0599 | 0.0231 | 0.0927 | ****** |
| SEEDL | 0.0335 | 0.0190 | 0.0279 | 0.0172 | 0.0529 | 0.0269 | 0.0400 | 0.0650 | 0.0402 |

SSRs

| Population | H-U-1 | H-U-2 | H-U-3 | H-P-3 | H-NR-3 | G-U-1 | G-P-1 |
|---|---|---|---|---|---|---|---|
| H-U-1 | ****** | | | | | | |
| H-U-2 | 0.050 | ****** | | | | | |
| H-U-3 | 0.065 | 0.051 | ****** | | | | |
| H-P-3 | 0.129 | 0.074 | 0.118 | ****** | | | |
| H-NR-3 | 0.018 | 0.039 | 0.045 | 0.099 | ****** | | |
| G-U-1 | 0.041 | 0.046 | 0.068 | 0.065 | 0.025 | ****** | |
| G-P-1 | 0.029 | 0.071 | 0.069 | 0.086 | 0.046 | 0.059 | ****** |
| G-NR-1 | 0.041 | 0.076 | 0.036 | 0.133 | 0.042 | 0.084 | 0.067 |

**Acknowledgments** We thank Lee Martens and Aubrey Webb for help with collections, Sandra O'Keefe for assistance in the laboratory, and Sally John for careful reading of this manuscript. We gratefully acknowledge funding from: the Alberta Environmental Protection and Enhancement Fund, Natural Sciences and Engineering Research Council (Canada), Forestry Canada, Foothills Model Forest, Weyerhaeuser Canada Ltd (Grande Prairie Operations), Weldwood of Canada Ltd (Hinton Division), Alberta Newsprint Company (Timber), Millar Western Forest Products Ltd, Canadian Forest Products Ltd, Manning Diversified Forest Products Trust Fund, and the University of Alberta Challenge Grants in Biodiversity. We declare that the tissue sampling undertaken for this project complied with the current laws in Canada at the time of this study.

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