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IMPACTS OF FOREST MANAGEMENT ON GENETIC DIVERSITY OF LODGEPOLE PINE: ASSESSMENT USING DNA-BASED MOLECULAR MARKERS

1998

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ABSTRACT

We examined the effects of different methods of forest regeneration on genetic diversity of lodgepole pine (*Pinus contorta* var. *latifolia*) using two different DNA-based molecular markers [randomly amplified polymorphic DNA (RAPDs), and simple sequence repeats (SSRs)]. Genetic diversity was estimated for 30 individuals per population for the following stand types: 1) mature lodgepole pine (> 100 yr); 2) 20-30 year old harvested stands left for natural regeneration; 3) 20-30 year old planted stands; and one group of 30 operationally-produced seedlings. There was no significant difference in the genetic diversity of young (20 to 30 years old) stands, established after harvesting by either planting or natural regeneration, and that of mature, unharvested stands. For both types of markers naturally regenerated stands had lower (not significant) expected heterozygosity than planted or unharvested stands. The seedling population (assessed by RAPDs only) had expected heterozygosity and allele frequencies similar to the unharvested stands. Allelic richness and diversity were much higher for SSRs than for RAPDs. Both markers showed that there was very little genetic differentiation among populations with over 94% of the total genetic variation accounted for by variation within populations. Genetic distance measures and ordination of the allele frequency data showed that there was little effect of geographic location or stand type on genetic relatedness of populations.

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INTRODUCTION

Genetic diversity provides the template for adaptation and evolution of populations and species and, 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 been long 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 to 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 seed lots from which planted stands are derived, with subsequent effects of selection during nursery production possibly further modifying genetic diversity.

In recent years there has been increasing interest in the use of DNA based molecular 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 in a wide variety of genetic studies of trees (Bucci and Menozzi 1995; Van den ven and McNicol 1995; Khasa and Dancik 1996; Bucci et al. 1997) and 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 (Smith and Devey 1994; Dow et al. 1995; Byrne et al. 1996; Echt et al. 1996).

As a dominant marker type, RAPDs are visualized as presence or absence of a band. Thus, it is impossible to verify whether any individual is a homozygote or heterozygote. We simply have to assume that absence of a band indicates the individuals homozygous for the recessive allele. Calculation of genetic diversity values, then, must assume the population is in Hardy-Weinberg equilibrium and the data do not allow for the determination of allelic richness, effective number of alleles, or for the calculation of fixation indices. SSRs, on the other hand, are co-dominant and tend to have multiple alleles per locus such that individuals can be identified as homozygotes or heterozygotes. The data can, therefore, be used to compare observed and expected heteroygosities, to calculate effective number of alleles and fixation indices, and to test whether the population is in Hardy-Weinberg equilibrium.

In this study we used RAPDs and SSRs to examine the impact of reforestation method on genetic diversity in stands of lodgepole pine located in the foothill regions 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.

METHODS AND MATERIALS

Material

Needle tissue was collected from lodgepole pine trees in twelve populations 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[1]	Stand type	Age[2]	Site prep[3]	RAPD	SSR
H-U-1	Hinton (T54 R25)	Unharvested	> 100 yrs	N/A	X	X
H-U-2	Hinton (T54 R25)	Unharvested	> 100 yrs	N/A	X	X
H-U-3	Hinton (T54 R25)	Unharvested	> 100 yrs	N/A	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 yrs	N/A		X
G-P-1	Grande Prairie (T65 R07)	Planted	19	Yes		X
G-NR-1	Grande Prairie (T65 R07)	Natural Regen	19	Yes		X

[1] *General location (township, range)*
[2] *Age since fire (unharvested stands) or harvest (planted, naturally regenerated stands)*
[3] *Mechanical site preparation*

Most harvested sites were subjected to mechanical site preparation one or two years following harvesting to create favorable microsites for planting or natural regeneration (Table 1). In the Hinton area the planted stands were planted seven to nine years after harvesting while the Grande Prairie stand was planted three 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 populations from an adjacent township in harvest planning area Athabasca 27 (similar elevation, aspect, site types as in Athabasca 16). Stands in the Hinton area were all located in the Upper Foothills Natural Region while those in the Grande Prairie area were from the Lower Foothills Natural Region (Alberta Environmental Protection 1994). In addition we used RAPDs to analyze a single

population 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 50 m apart. The tissue was kept cool until storage at -70°C. RAPD and SSR loci, DNA extraction and 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 hrs 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 minutes, then photographed with a digital imager. Gel Pro Analyzer™ Ver. 2.0 software (Media Cybernetics) was used to detect alleles. Bands were grouped if their mobilities differed by less than 3%. A total of 10 RAPD loci, derived from four primers, were scored (see Hicks et al. 1998). A spreadsheet (EXCEL) 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 at 40 W for 2.5 hr on a 6% denaturing acrylamide gel containing 7M Urea using TBE. 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 and Boyle 1996) 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 be found in the population if all alleles were in the same 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, μ = the overall mean, M_i = the ith marker type, S_j = the jth stand type, MS_{ij} = the interaction of marker and stand type, $P(S)_{k(j)}$ = the kth population within stand type, and $e_{l(ijk)}$ = random error associated with population within stand type by marker. Differences were considered significant at α = 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 multi-locus genotypic structure, whereas univariate measures, such as genetic distance cannot (Yeh et al. 1985; Knowles 1985).

RESULTS AND DISCUSSION

Impact of regeneration method

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).

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

Source of Variation	df	*p*
Marker type	1	0.01
Stand type[1]	2	0.25
Marker by Stand type	2	0.74
Population within stand type	9	0.59

[1] *Including unharvested, naturally regenerated, and planted stands*

On average, however, naturally regenerated stands showed lower H_E than in unharvested stands (11% for RAPD loci; 4% SSR loci; Table 3, Fig. 1). 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. Knowles (1985) found 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 populations to have 'similar' diversity. To thoroughly examine the impacts of forest management on genetic diversity it is essential to have proper replication of stand types and to determine whether differences are statistically significant.

Table 3. Genetic variation for the 13 populations base on 10 RAPD loci or 5 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[1]	SSR	N	na[2]	ne[3]	H_O[4]	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	**GP-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	**GP-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	**GP-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[5]	F_{ST}[6]	**SSRs**	H_T	F_{ST}	F_{IS}[7]
		0.46	0.061		0.74	0.028	0.360

[1] *H_E = expected heterozygosity (Nei's unbiased estimate)*
[2] *na = Observed number of alleles*
[3] *ne = Effective number of alleles (Kimura and Crow 1964)*
[4] *H_O = observed heterozygosity*
[5] *H_T = expected heterozygosity for the species, based on these populations*
[6] *F_{ST}= Proportion of genetic variability that is due to population differentiation*
F_{IS} = Deficiencies of heterozygotes relative to Hardy-Weinberg expectations

The high levels of diversity in planted stands concur with the high level of diversity we found in the one population of operationally-produced seedlings, which had among the highest H_E values measured (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). Thus procedures for commercial seed collection and nursery seedling production do not appear to modify genetic diversity, relative to natural stands (Silen and Osterhaus 1979; Campbell and Sorensen 1984; Muona et al. 1988).

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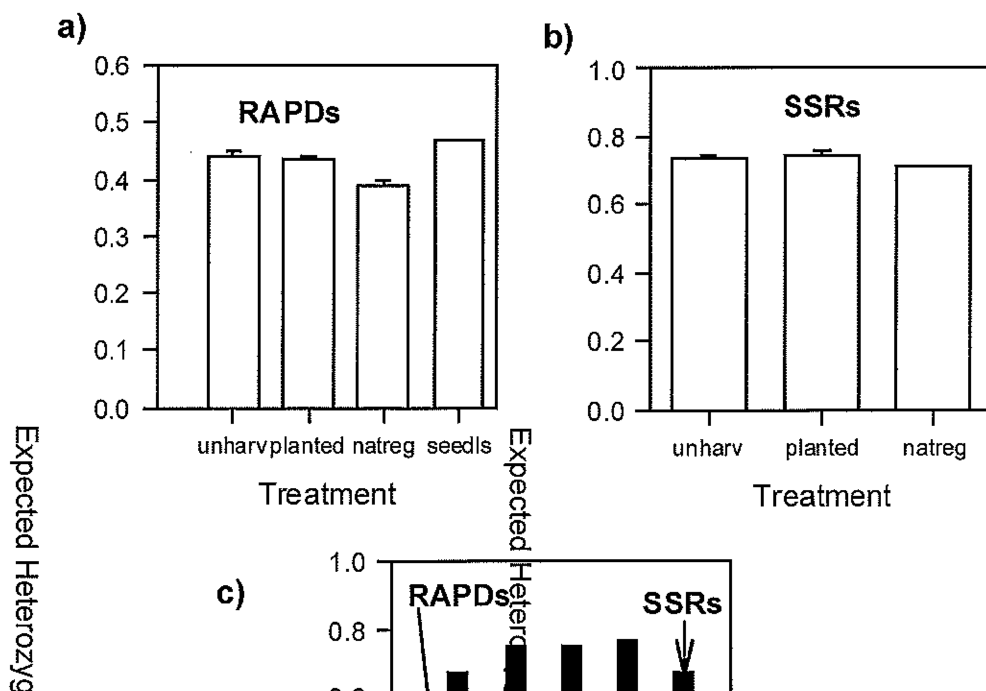

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literature. Using 20 RAPD loci in Norway spruce (*Picea abies*), Bucci and Menozzi (1995) measured an average H_E = 0.334. Average H_E for SSR loci in both radiata pine (*Pinus radiata;* two loci, 96 individuals, Smith and Devey 1994) and bur oak (*Quercus macrocarpa;* three loci, one population, Dow et al. 1995) was approximately 0.70. In a study of eastern white pine (*Pinus strobus*), Echt et al. (1996) found an average observed heterozygosity (H_O) of 0.52 with an average 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 to 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. For the five 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. 1), although this is not surprising given that these populations showed little variation in H_E.

Table 4. Mean genetic variation for the 10 RAPD loci and five SSR loci across N populations.

RAPD	N	H_E[1]	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								

[1] *H_E, na, ne, H_O, F_{IS}, F_{ST} as in Table 2*

RAPD loci showed relatively little variation in H_E (0.34 to 0.49) as compared to SSR loci (0.51 to 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). There was more variation in F_{ST} among loci for RAPDs (0.011 to 0.126) than for SSRs (0.02 to 0.044).

Table 5 Mean length of repeat sequence at the five SSR loci and total number of alleles detected at each. There was a significant correlation between the two (r^2 = 0.606)

SSR	length base pair	Total # alleles detected
APC3	102.0	13
APC9	131.9	34

APC11	161.6	27
APC13	136.7	28
APC15	100.8	3

Population differentiation
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 [*Pinus rigida* (Guries and Ledig 1981), *Pinus banksiana* and *Pinus contorta* (Dancik and Yeh 1983), *Picea mariana* (Knowles 1991; Wang and Macdonald 1992; Sproule and Dancik 1996); and *Larix laricina* (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 micro-scale 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 release of seeds from serotinous cones. Thus family structure could easily develop in populations and lead to some degree of inbreeding, simply reflecting breeding among related individuals which are spatially grouped (Linhart et al. 1981; Dancik and Yeh 1983; Yeh et al. 1985; Sproule and Dancik 1996).

Although we can reasonably expect some level of apparent inbreeding (positive F_{IS} values) in lodgepole pine 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 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).

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 vs 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).

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 to 0.007, Dancik and Yeh 1983). This is, no doubt, partly due to the fact that we used only polymorphic loci in this study whereas isozyme studies include both polymorphic and

monomorphic loci. It may also reflect the greater power of DNA-based markers for detecting differences among populations or the more rapid evolution of non-coding parts of the genome. Bucci et al. (1997) found much higher (five times) genetic distance values for RAPDs vs 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 pairwise comparisons for the five 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 genetic relatedness of populations (Fig. 2). Using genetic distance calculations and ordination of isozyme data Gömöry (1992) showed divergence in allele frequencies for planted compared to naturally regenerated stands of Norway spruce. Ordination of our RAPD allele frequency data, verified that the seedlings had similar allele frequencies to the three 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

Table 6. Genetic distance among populations based on a) RAPDs and b) SSRs (Nei's unbiased measure, Nei 1978). Lower numbers indicate populations which are more similar in terms of their allele frequencies.

a) RAPDs

Popn	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

a) SSRs

Popn	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

traits in lodgepole pine was more closely associated with steep elevational gradients but was affected little by geographical 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 lac ... ence as measured by these non-c ... vould be preferable to base geneti ... have so many alleles.

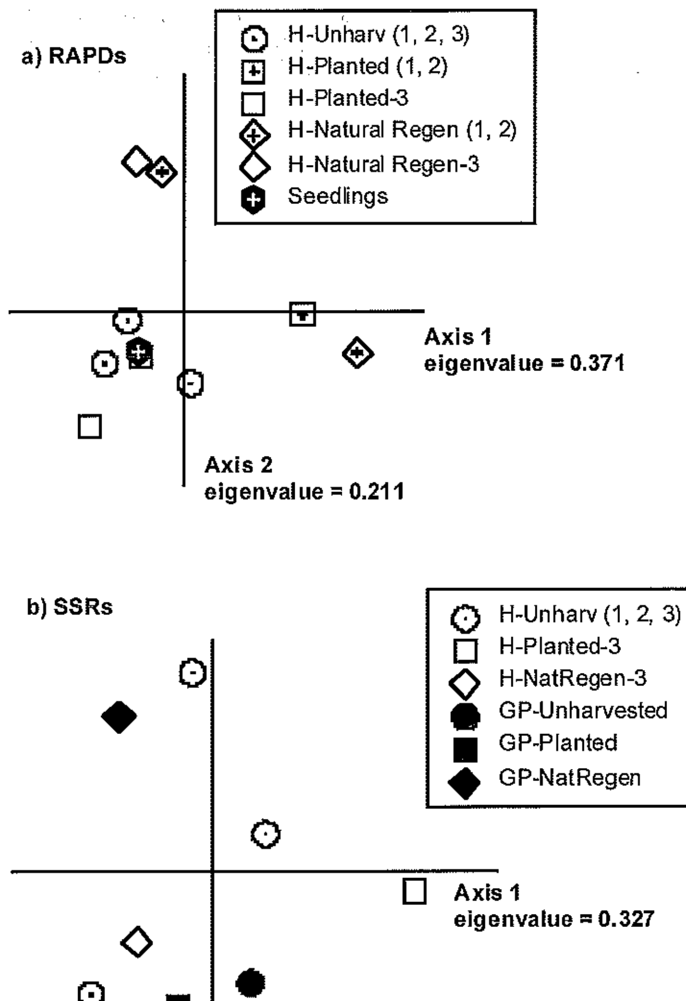

Figure 2 R ... 0 populations); and b) SSRs (8 po ... ir genetic relatedness. The eigenva ... y the set of variables associated ... e analysis (Tabachnick and Fidell 19

Future con

In th ... ark for comparison of genetic dive ... s changes in genetic diversity witl ... rees could experience changes in ... ade intolerant there is typically ve ... nt phase. Stands do, however, e ... rough the self-thinning phase. This ... e to bottleneck effects, or possibly ... d thus greater mortality during the ... id of *Pinus sylvestris* Tigerstedt e ... es up to 100 years old but the olde ... m. We are currently in the process ... n patterns of genetic diversity of ... stand age, elevation, silvicultural t

CONCLUSIONS

Our results suggest that regeneration of lodgepole pine after harvesting, by both planting and natural regeneration, results in young populations (20 to 30 years old) with similar levels of genetic diversity as mature (> 100 year), unharvested stands. Within the geographic range examined, there was no relationship between stand type or geographic location and genetic relatedness among populations. Both RAPD and SSR markers proved to be powerful tools for quantifying genetic diversity and examining genetic relationships among populations.

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