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Levels of genetic diversity at different stages of the domestication cycle of interior spruce in British Columbia

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Abstract Concerns over the reductionist nature of the domestication of forest-tree species focus on the possibility of potential genetic erosion during this process. To address these concerns, genetic diversity assessments in a breeding zone the Province of British Columbia "interior" spruce (*Picea glauca* × *engelmanni*) program was conducted using allozyme markers. Genetic-variation comparisons were made between natural and production (seed orchard) populations as well as seed and seedling crops produced from the same breeding zone's seed orchard. The natural population sample consisted of a total of 360 trees representing three stands within each of three watersheds present in the Shuswap-Adams low-elevation zone of interior British Columbia. Small amounts of genetic differentiation were observed among the nine natural populations (4%) and this was attributable to extensive gene flow ($N_m = 7$). Consequently, the sum of these nine populations was considered as a baseline for the genetic variation present in the breeding zone. The comparisons between the seed orchard and the breeding zone produced a similar percentage of polymorphic loci ($\%P = 64.7\%$) while the expected heterozygosity (H_e) (0.207 vs 0.210) and the average number of alleles per locus (2.7 vs 2.4) were slightly lower in the seed orchard. A total of seven natural populations' rare alleles ($P < 0.007$) were not present in the orchard population, while one allele was unique to the orchard. The $\%P$ increased to 70.6% in the seedlot, but dropped to the natural populations level (64.7%) in the plantation. The observed increase in $\%P$ was a result of pollen contamination in the orchard. It is suspected that the reduction in the plantation was caused by an unintentional selection in the nursery. Simulated roguing in the orchard did not drastically reduce H_e even if up to 50% of the orchard's clones were rogued. However, roguing was associated with a reduction in the average number of alleles per locus (i.e., sampling effect).

Key words Genetic diversity · Breeding zone · Phenotypic selection · Seed orchard · Seed and seedling production · *Picea glauca* × *engelmanni*

Introduction

The maintenance of genetic diversity throughout the tree-breeding operation, and ultimately the domestication process, of forest trees is of vital importance. Crop-plant domestication programs are reductionist in nature; thus the importance of maintaining and conserving genetic variation increases with the expected progressive reduction of breeding individuals as the programs advance (Francis 1981). There are several steps in the forest-tree domestication process in which genetic variation could be reduced if sufficient safeguards are not considered. These are: phenotypic selection, breeding (tree improvement cycle), and seed and seedling production (El-Kassaby 1995; El-Klassaby and Namkoong 1995).

Studies assessing the impact of phenotypic selection on the genetic diversity of seed-orchard populations have indicated that seed orchards harbour levels of genetic variation that are similar to, or even greater than, that found in their natural population counterparts (Knowles 1985; Cheliak et al. 1988; Bergman and Ruetz 1991; Chaisurisri and El-Kassaby 1994; El-Kassaby et al. 1994; El-Kassaby and Ritland 1996; Williams et al. 1995). However, other less-obivious potential sources of genetic erosion could occur during the seed and seedling production phases.

Maximum genetic variation in seed-orchard crops can only be attained if all parents contribute equally to

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the gametic pool. However, this assumption is hardly fulfilled and it is commonly observed that only a small portion of any orchard's parents predominantly contribute to the orchard seed crop (see El-Kassaby et al. 1986 for a review). In fact the so-called "20/80" rule (i.e., 20% of the seed-orchard parents contribute 80% of the cone/seed crop) was coined to demonstrate the commonality of the reproductive output inequality in seed-production populations (Anonymous 1976). The impact of the reproductive output differential could be further compounded if reproductive phenology variation exists among seed-orchard parents (El-Kassaby and Askew 1991). Moreover, as seed orchards get older and their associated progeny test data become available, further parental reduction is expected through the genetic roguing of poor clones. The dynamics among the additional reduction of the parental population number, as well as its reproductive output and phenological differences, will further exacerbate the expected genetic variation levels of seed-orchard crops.

As in seed production, seedling-production practices could affect the genetic variation. Seedling production represents a unique case where biological (seed dormancy and germination rate and speed) and management (thinning and culling) factors play a significant role in affecting the level of genetic variation of seedling crops. El-Kassaby and Thomson (1996) presented a case where the relationship between seed biology and nursery management practices introduced an unintentional directional selection, thus reducing the expected genetic variation of seedling crops.

Clearly, there are several steps in the forest-tree domestication process where the genetic diversity could be affected. In the present study, the cumulative effects of phenotypic selection, breeding, and seed and seedling production are evaluated using allozyme markers. The genetic diversity levels of a breeding zone of the interior spruce complex (*Picea glauca* × *engelmanni*) was determined from nine natural populations. This level of genetic variation was assessed in a seed orchard representing the same breeding zone, in a bulk seedlot collected from the same seed orchard, and in a 2-year-old plantation established from the same seedlot.

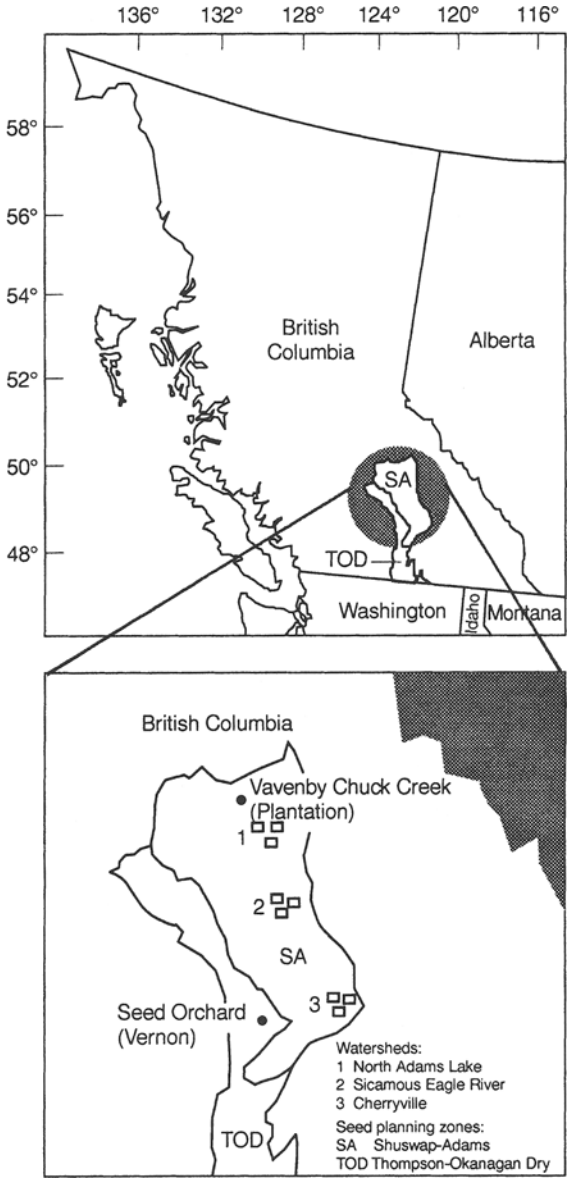

Fig. 1 Location of the nine "interior spruce" natural populations sampled from the Shuswap-Adams breeding zone in the southern interior of British Columbia, as well as of the seed orchard and plantation studied

Materials and methods

Sample collection

The materials used in this study were sampled from the "interior spruce" complex Shuswap-Adams low-elevation zone (SAL) which is located in the southern interior of British Columbia (Fig. 1). The "interior spruce" complex consists of pure white spruce [*Picea glauca* (Moench) Voss], Engleman spruce [*P. engelmanni* (Parry)], and their hybrids. Due to the lack of reproductive barriers between the two species and their fertile hybrids, as well as their similar growing habits, they are collectively called "interior spruce" (El-Kassaby et al. 1988). Three watersheds were sampled in the SAL breeding zone along an elevation band between 500 and 1450 m. Every watershed was represented by three natural stands (see Table 1, for sample size). The level of genetic variation observed in these nine populations was considered as a reference "bench mark" for comprison with the level of genetic variation that is present in the seed orchard, seedlot, and plantation. Dominant vegetative buds were collected from a total of 360 dominant, mature trees using a helicopter during the late winter breeding of 1994 (Table 1, Fig. 1).

The studied seed orchard is located close to the SAL breeding zone (Table 1, Fig. 1). The seed orchard was established in 1981 as a grafted orchard containing 103 clones with a total of 990 ramets on 2.25 ha. Three out of the one-hundred-and-three clones flower very sporadically, and thus were not included in the study. As in the natural stand sample collection, dormant vegetative buds were sampled from the 100 clones included in the study.

To monitor the cumulative effects of the various factors affecting seed production (i.e., seed-orchard dynamics) which might affect the

Table 1 Location, sample size, and population code of the "interior spruce" populations included in the study

Population	Sample size	Latitude	Longitude	Population code
North Adams Lake 1	40	51°40′	119°10′	NAL1
North Adams Lake 2	41	51°30′	119°20′	NAL2
North Adams Lake 3	38	51°25′	119°20′	NAL3
Sicamous Eagle River 1	61	50°50′	118°50′	SER1
Sicamous Eagle River 2	30	50°55′	118°40′	SER2
Sicamous Eagle River 3	30	50°55′	118°55′	SER3
Cherryville 1	40	50°20′	118°30′	C1
Cherryville 2	40	50°25′	118°20′	C2
Cherryville 3	40	50°20′	118°20′	C3
Breeding zone[a]	360			
Seed orchard	100	50°20′	119°10′	
Seedlot	120			
Plantation	120	51°40′	119°45′	

[a] The breeding zone consists of the sum of the nine natural populations

genetic variation, a bulk seedlot sample from the 1990 seed crop (seedlot # 06914 of British Columbia's Provincial seed inventory) was obtained for evaluation. The seedlot was also used for production to establish a plantation with 1-year-old seedlings in the northern part of the breeding zone in 1993 (Table 1, Fig. 1). From this seedlot, a total of 120 embryos were genotyped to determine the level of genetic diversity in the seed crop. In addition, dormant buds were collected from 120 plantation seedlings. It is expected that the level of genetic variation present in the seedlot and its plantation will reflect the biological processes, as well as the management practices of both the seed orchard and the nursery. At the time of sampling, there was no obvious mortality in the plantation as indicated by a lack of empty plantable spots; thus the plantation is a good representative of nursery production.

All dormant bud samples were stored on ice after collection and transported in coolers to the laboratory where they were kept at −20°C. Seeds were kept dry at 4°C until analysis.

Electrophoretic analysis

Analysis of isozyme variation was carried out only with loci that could be scored in all tissue types and genetic entries (i.e., vegetative buds and embryos). A total of 11 enzyme systems encoded by 17 loci were scored. A copy of the original data set is available upon request. For further details of electrophoresis see El-Kassaby and Ritland (1995) and references herein. The Menedelian inheritence for the isozyme systems studied has been confirmed for seed tissue (King and Dancik 1983; Stewart and Schoen 1986) and for bud tissue (Cheliak and Pitel 1984).

Genetic variation analysis

Allozyme variation was analyzed using BIOSYS-1 (Swofford and Selander 1981) and GENESTAT (Lewis and Whitkus 1989). Allele frequencies, the number of alleles per locus, the percent polymorphic loci, and the average observed and expected hetrozygosities were derived for each population , the seed orchard, the bulk seedlot, and the seedling populations. In addition, Nei's (1973) G_{ST} and Wright's (1965) F_{IT} and F_{IS} statistics were calculated for the natural populations to obtain estimates of the distribution of genetic variation and to reveal levels of inbreeding. Genetic-distance dendrograms were constructed using Nei's (1973) and the UPGMA (Sneath and Sokal 1973) algorithm to illustrate the genetic relationship among: (1) natural stands, and (2) among the breeding zone (the sum of the nine popoulations), the seed orchard, its seedlot and the resulting seedling crop (i.e., plantation).

Results and discussions

Population diversity (baseline information)

The 17 loci studied showed detectable polymorphism in at least one population (data not shown). The number of monomorphic loci per population varied between two and four. The NAL3 was the only population with monomorphic LAP-2 and polymorphic MDH-1, while the opposite trend was observed in the remaining eight populations (data not shown). Five private alleles were observed and they were scattered over five different populations. The number of alleles per locus varied between two (seven loci) and four (two loci), while the remaining eight loci expressed three alleles (data not shown).

The total number of alleles per population ranged between 33 (NAL3) and 40 (NAL1), with an overall average of 36 alleles (Table 2). The average number of alleles per locus was 2.16 across populations, varying from 1.9 for NAL3 (the population with the lowest number of alleles) to 2.4 for NAL1 (the population with the highest number of alleles) (Table 2). It is noteworthy that population NAL3 harbors one private allele in spite of its low allelic number (Table 2). With the exception of the SKD-1 locus for NAL-3, the same allele was most common in all populations. An average of 62.7% of the loci were polymorphic within populations, with individual populations ranging from 52.9% (NAL3) to 70.6% (SER1 and SER2). The average expected diversity (genetic diversity) within a population was 0.203. The SER3 population has the highest (0.226), while NAL3 had the lowest (0.154), diversity (Table 2). For all populations, differences between observed and expected hetrozygosities were not significant indicating that the populations are in Hardy-Weinberg equilibrium (differences were based on comparing bounds of confidence intervals at the 95% significance level) (Table 2).

Total genetic diversity values varied between 0.006 and 0.439, producing an average over all loci of 0.210

Table 2 Estimates of heterozygosity parameters for the nine "interior spruce" populations, breeding zone, seed orchard, seedlot, and plantation included in the study (standard errors in parentheses)[a]

Population	A_T	$\bar{A}$	$\%P^b$	H_o	H_e
NAL1	40	2.4	58.8	0.157(0.039)	0.188(0.040)
NAL2	37	2.2	58.8	0.154(0.033)	0.174(0.037)
NAL3	33	1.9	52.9	0.150(0.038)	0.154(0.038)
SER1	38	2.3	70.6	0.193(0.038)	0.220(0.041)
SER2	35	2.1	70.6	0.184(0.038)	0.221(0.042)
SER3	35	2.1	58.8	0.214(0.051)	0.226(0.049)
C1	36	2.1	64.7	0.226(0.045)	0.215(0.041)
C2	35	2.1	64.7	0.209(0.042)	0.204(0.040)
C3	38	2.2	64.7	0.212(0.045)	0.223(0.046)
Mean	36.33	2.16	62.73	0.1888	0.2028
Breeding zone	46	2.7	64.7	0.189(0.036)	0.210(0.039)
Seed orchard	40	2.4	64.7	0.194(0.040)	0.207(0.043)
Seedlot	38	2.2	70.6	0.209(0.040)	0.219(0.043)
Plantation	39	2.3	64.7	0.194(0.042)	0.215(0.044)

[a] See Table 1 for population code; A_T = total number of alleles per population; $\bar{A}$ = average number of alleles per locus; $\%P$ = percentage of polymorphic loci; H_o = observed heterozygosity; H_e = expected heterozygosity
[b] 95% criterion

(Table 3). The inter-locus variation in within-population genetic diversity is large: values ranged from 0.006 to 0.421 with an average over all loci of 0.203 (Table 3). Among population-differentiation values varied between zero and 0.097, and the proportion of total genetic diversity among populations was 0.035, indicating that about 4% of the detected genic variation is due to inter-populational gene differences (Table 3). Thus, the majority of genic variation (96%) resided within populations (Table 3). This level of population differentiation is typical of most conifers (El-Kassaby 1991; Ledig 1986; Hamrick and Godt 1989) and similar to that reported for white spruce by Cheliak et al. (1985, 1988) and Alden and Loopstra (1987). However, greater population differentiation was reported when mariginal populations were included in the analysis (Tremblay and Simon 1989).

Table 3 Estimates of genetic diversity (Nei's gene diversity statistics and Wright's fixation index) for each locus over the nine "interior spruce" populations sampled from the Shuswap-Adams low-elevation breeding zone of interior British Columbia[a]

Locus	H_T	H_S	G_{ST}	F_{IT}	F_{IS}
FEST	0.044	0.043	0.025	0.273	0.259
IDH-2	0.076	0.075	0.015	0.057	0.038
PGM-1	0.422	0.421	0.003	−0.078	−0.094
PGI-1	0.014	0.014	0.000	0.400	0.384
PGI-2	0.330	0.328	0.007	−0.052	−0.069
6PGI-2	0.163	0.160	0.021	−0.008	−0.044
GDH	0.372	0.364	0.021	0.109	0.080
SKD-1	0.387	0.363	0.061	0.357	0.312
SKD-2	0.223	0.214	0.040	0.183	0.142
LAP-1	0.013	0.013	0.000	−0.006	−0.023
LAP-2	0.439	0.396	0.097	0.132	0.038
MDH-1	0.006	0.006	0.000	−0.003	−0.027
MDH-2	0.192	0.188	0.023	0.034	0.004
MDH-3	0.304	0.303	0.004	−0.002	−0.017
ACO-1	0.408	0.379	0.072	0.240	0.178
AAT-2	0.163	0.163	0.000	−0.009	−0.020
AAT-3	0.018	0.018	0.000	−0.008	−0.014
Mean	0.210	0.203	0.035	0.097	0.057

[a] H_T = total genetic diversity; H_S = within-population genetic diversity; G_{ST} = proportion of total diversity partitioned among populations; F_{IT} and F_{IS} = deviations of genotype frequencies from Hardy-Weinberg expectations over all populations and within individual populations, respectively

The level of gene flow present in the populations studied was determined using the relationship $[N_m = (1 - G_{ST})/4G_{ST}]$ (Slatkin and Barton 1989) and was very high (6.89). This gene flow estimate is considered to be among the highest reported for conifers (see Ellstrand 1992) and supports the results obtained on white and Englemann spruce mating systems (King et al. 1984; Cheliac et al. 1985; Shea 1987; Innes and Ringuis 1990) as well as inter-populational differentiation (see above). Estimating the expected outcrossing rate (t) practiced in these natural populations using the relationship $[t = (1 - F_{IS})/(1 + F_{IS})]$ (Nei and Syakudo 1958) produced an outcrossing rate of 89.2%. This value is high and similar to those reported for the species concerned (King et al. 1984; Cheliak et al. 1985; Shea 1987; Innes and Ringius 1990). The species large population size, its open-pollinated mating system, and its reproductive biology attributes (Owens and Molder 1979) are all factors contributing to the observed gene flow (see Hamrick and Godt 1989 for a review).

Estimates of genetic distances among the nine populations were determined (Table 4) and the genetic relationships among them are depicted in Fig. 2. The dendrogram did not produce any clusters of populations that could be attributed to geography (Fig. 2). It is interesting to note that population NAL3 formed an independent branch. The separation of NAL3 from the remaining populations is not surprising considering all the unique features of this population (see above). The fact that the NAL3 population is located in a high-elevation site might have contributed to the lack of gene exchange with the other populations. A unidirectional gene exchange from high- to low-elevation sites might have been the predominant mode (this was demonstrated by the presence of more monomorphic loci in the population). The overall genetic distance average value is very small (0.009) and this value was further reduced

Table 4 Genetic distance among the nine "interior spruce" populations sampled from the Shuswap-Adams low-elevation breeding zone of interior British Columbia

Population[a]	NAL1	NAL2	NAL3	SER1	SER2	SER3	C1	C2
NAL2	0.003							
NAL3	0.016	0.010						
SER1	0.009	0.009	0.037					
SER2	0.001	0.003	0.023	0.002				
SER3	0.004	0.012	0.032	0.004	0.001			
C1	0.004	0.008	0.025	0.008	0.001	0.004		
C2	0.004	0.004	0.025	0.007	0.000	0.004	0.001	
C3	0.008	0.011	0.033	0.002	0.002	0.002	0.007	0.004

[a] See Table 1 for population code

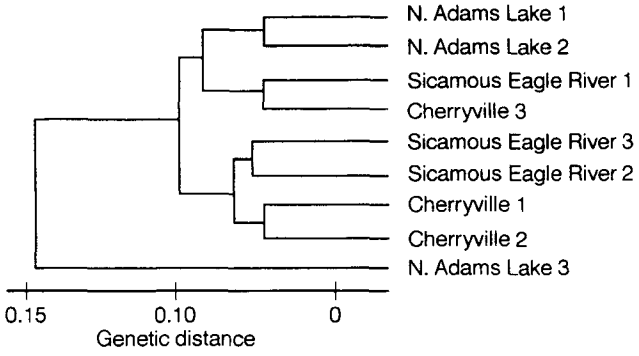

Fig. 2 Dendrogram depicting the genetic relationship among the nine "interior spruce" natural populations sampled from the Shuswap-Adams breeding zone in the southern interior of British Columbia

to 0.004 when population NAL3 was excluded, indicating a greater similarity among the remaining populations.

The results obtained from the gene-diversity analysis and the genetic distance all show the existence of extensive gene flow within the breeding zone. This high gene flow acted as a force that countered population differentiation and supported the use of the data obtained from the nine populations as "bench mark" or base-line for comparison with the remaining test populations (i.e., seed orchard, seedlot, and plantation).

Evaluation of the domestication process

The various analyses conducted on the nine populations were repeated for the four test populations (i.e., breeding zone, seed orchard, seedlot, and plantation) after grouping the nine natural populations into one breeding zone. The genetic diversity parameters for the four test populations are shown in Table 2.

As expected, the 17 loci studied were polymorphic in the breeding zone; however, this number was reduced to 14 loci in the three test populations studied (data not shown). The three loci (PGI-1, LAP-1, and MDH-1) that lost their heterozygosity in the remaining three test populations were characterized by the presence of rare alleles in the breeding zone (frequency ranged between 0.003 and 0.007) (data not shown). The frequencies of these alleles within any of the natural populations were low (<0.04). In addition, a total of another three alleles (FEST-4, AAT-2-5, and AAT-3-5) that were present in three polymorphic loci in the breeding zone were lost from the remaining test populations. Once again, these three alleles were rare (frequency <0.02) and private in some natural populations. In addition, a case of loss and gain was observed for the ACO-1-2 allele where the locus was lost in the seed orchard but appeared in the plantation, probably through pollen contamination in the seed orchard. It appears that the 100 clones sampled from the breeding zone did not successfully capture any of these seven alleles, thus this could be considered as an allelic loss. A third type of allelic loss was also observed when allelic frequencies across the test populations were compared. These include those alleles that are present in both the breeding zone and the seed orchard (i.e., which phenotypic selection was successful in capturing) but were lost due to the dynamics within seed and seedling production phases. These alleles were missing from: (1) the seedlot and plantation (PGI-2-5), (2) the seedlot alone (PGM-1-5), and (3) the plantation alone (AAT-3-3) (data not shown). On the other hand, allelic gain was observed for IDH-2-3. This allele is present in the seed orchard but was not present in the breeding zone, indicating that phenotypic selection was successful in capturing this allele while the intensive selection of nine populations failed to do so. Allelic gain was also observed for FEST-3, this allele is not present in either the breeding zone or the seed orchard but was observed in the seedlot and persisted in the plantation (data not shown). Pollen contamination represents the most probable source of this allele.

The total number of alleles per test population ranged between 38 (seedlot) and 46 (breeding zone) (Table 2). The total number of alleles for all the test populations exceeded most individual natural populations and is higher than their average (Table 2). The average number of alleles per locus per test population varied from 2.2 for seedlot (the test population with the lowest number of alleles) and 2.7 for the breeding zone (the test population with the highest number of alleles) and in general is higher than that of most individual natural populations in spite of the observed progressive decline over test plantations (Table 2). The percentage of polymorphic loci within test populations ranged from

64.7% (breeding zone, seed orchard, and plantation) to 70.6% (seedlot) and once again is higher than the average over natural populations (Table 2). It should be emphasized that the inclusion of 100 clones collected throughout the breeding zone produced higher heterozygosity parameters in the seed-orchard population. This high heterozygosity was transmitted to the seed and seedlings produced from that seed orchard. Additionally, pollen contamination was proven to be a factor for the inclusion of some alien alleles, thus further increasing the level of heterozygosity. This influx of foreign pollen was detected by the presence of alleles not previously found in the orchard genotypes. It should be stated that the adaptive significance of these foreign alleles is not known at present. The observed lower number of alleles per locus in the test populations (seedlot and plantation) should be considered as a function of the loss of rare alleles due to sampling. In summary, it should be emphasized that four of the seven rare alleles lost were private and found in only one genotype in the breeding zone, while two lost rare alleles were confined to a single watershed.

Observed and expected hetrozygosities did not vary substantially among the four test populations. The expected diversity (genetic diversity) within test populations ranged between 0.207 and 0.219 and was higher than the average over natural populations (Table 2). Observed heterozygosities represent a direct count of alleles as an indication of genetic variation. Observed heterozygosity was lowest for the breeding zone, intermediate for the orchard and plantation, and highest for the seedlot (Table 2). This suggests that the seed orchard is a good representative sample of the breeding zone and that pollen contamination has contributed to the increased heterozygosity of the seedlot and plantation (Table 2).

The dendrogram, based on genetic distances among the test populations, was revealing and reflected the expected dynamics among these populations (Fig. 3). The seed orchard and the breeding zone were on the same branch confirming their genetic similarity. These results are similar to those reported for white spruce and other species where the level of genetic variation in natural populations was compared to that of phenotypic selection (Knowles 1985; Cheliak et al. 1988; Bergmann and Ruetz 1991; Chaisurisri and El-Kassaby 1994; El-Kassaby et al. 1994; El-Kassaby and Ritland 1996; Williams et al. 1995). Interestingly, the seedlot occupied an intermediate position in the dendrogram between that of breeding zone/seed orchard and the plantation branches (Fig. 3). Although the seedlot joined both the breeding zone/seed orchard major branch, it was in a separate branch by itself indicating that some genetic changes have taken place during the course of seed production in the seed orchard. These results are not surprising and in fact reflect the expected violations to the seed orchards' assumptions reported for white spruce (Schoen and Stewart 1986, 1987; Schoen et al. 1986; Denti and Schoen 1988) as well as many other species (see El-Kassaby 1989, 1992, 1995 for reviews). Finally, the detached position of the plantation in the dendrogram from the other three test populations further supports the contention that some form of genetic selection has taken place during the seed and/or seedling production. El-Kassaby and Thomson (1996) reported the existence of three cases of unintentional directional selection during nursery seedling production. The position of the four test populations on the dendrogram clearly demonstrates the dynamics of these populations. Seed-orchard populations in most cases are a good representative sample of the genetic variation that is present in natural populations (see references above). As the populations move from the seed orchard to seed and seedling production, genetic differences become apparent.

The effects of genetic roguing (i.e., truncation selection) on the resulting genetic variation of the seed orchard were investigated using the breeding values of the orchard clones (breeding values were based on 5-year progeny test data). Based on this simulated roguing, it was observed that the expected hetrozygosities remained high even if up to 50% of the orchard clones were removed (Table 5). These results support the notion of Savolainen and Yazdani (1991) and Savolainen and Karkkainen (1992) indicating that the effect of random genetic drift on depleting the hetero-

Fig. 3 Dendrogram depicting the genetic relationship among the four "interior spruce" test populations (breeding zone, seed orchard, seedlot, and plantation) investigated in the present study

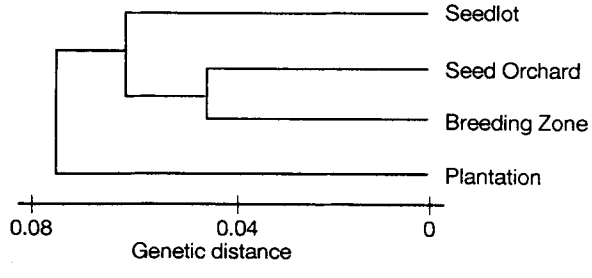

Table 5 Effects of genetic selection on expected heterozygosities simulated and under drift theory and alleles per locus in the Shuswap-Adams low-elevation "interior spruce" seed orchard containing 100 clones

No. clones rogued	Expected heterozygosity		No. alleles/locus
	Simulated	Drift theory	
0	0.207	0.207	2.35
10	0.209	0.206	2.35
20	0.208	0.205	2.29
30	0.209	0.205	2.18
40	0.212	0.205	2.18
50	0.205	0.205	2.12
60	0.198	0.204	2.12
70	0.196	0.203	2.00
80	0.198	0.202	1.94
90	0.191	0.186	1.76

zygosity in seed orchards is slow. The simulated expected heterozygosities actually droppped below those expected under the drift theory (He_t) only when more than 50 clones were removed (Table 5). This was demonstrated using the relationship $He_t = [1 - 1/(2N_e)](He_{t-1})$, where N_e = the variance-effective population size and t = the generation = 1 (Nei et al. 1975). This indicates that the depletion of heterozygosity caused by drift alone will only be high if severe roguing was practiced (Table 5). It is of interest to note that the resulting heterozygosity initially increased; however, this trend did not continue (Table 5). The connection between the individual clone breeding value and its hetrozygosity level is tenuous and cannot be clarified by the present study. Additionally, the number of alleles per locus dropped much more rapidly as a result of removing clones from the orchard (Table 5).

Conclusions

The "interior spruce" in the Shuswap-Adams low-elevation breeding zone has been found to be highly variable. Many conifer species maintain high levels of genetic diversity through extensive gene flow. This high amount of gene flow has been confirmed for the present study and is manifested in the lack of differentiation among the nine natural populations investigated. In the present study, only 3.5% of the total variation is attributable to variation among populations. Thus, by selecting many individuals from many populations, the largest amount of the total variation within the overall breeding zone is expected to be captured. This was confirmed in our study, as the 100 clones selected from many different locations within the SAL breeding zone captured most of the variation found in the natural populations.

The loss of rare alleles in the seed orchard is chiefly a function of sampling and was restricted to rare and private alleles. It is concluded that this may not represent a problem for gene conservation, as the rare alleles contribute little to fitness, and usually are the result of deleterious mutations or may be evolutionary relics (Lindgren and Gregorius 1976).

The effects of genetic selection based on the breeding value of the orchard clones (roguing) does not seem to affect expected heterozygosities, as long as not more than 50% of the clones are removed. At this level of genetic selection, heterozygosity would only drop from 0.207 to 0.205, while the number of alleles per locus would be reduced from 2.35 to 2.12.

One important aspect of this study is the demonstration of how the genetic variation present in seed orchards is being delivered to the plantation. Our results indicate that the seedling nursery practices have reduced two of the three genetic diversity parameters compared to the seedlot [percent polymorphic loci reduced from 70.6 to 64.7% (9% reduction) and observed heterozygosity reduced from 0.209 to 0.194 (7% reduction)]. One explanation for these reductions could be early mortality in the field. However, signs of early mortality were not observed in the plantation. Thus, the reduction in the observed genetic variation could be attributed to nursery practices.

In summary, the present data demonstrate that phenotypic selection within the studied breeding successfully captured the genetic variation present in the natural populations. The expected level of hetrozygosity in the resulting seedlot is identical to that predicted after a bottle-neck event $\{He_t = [1-(1/2*100)]*0.210 = 0.209\}$ (Nei et al. 1975). Seed-orchard biology and genetics, as well as nursery management practices, are worthy of genetic monitoring to avoid potential allelic losses.

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