GENETIC EFFECTS OF DOMESTICATION IN WESTERN HEMLOCK, *TSUGA HETEROPHYLLA*

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ABSTRACT

Rates of genetic diversity were inferred from allelic variation in western hemlock (*Tsuga heterophylla* (Raf.) Sarg) using starch gel electrophoresis. Nine enzyme stains were employed to identify fourteen isozyme loci. The concluding allelic variation was then used to compare differing orchard conditions with natural stands of western hemlock. The main goal was to measure the effectiveness of seed orchard and seed collection protocol in maintaining natural genetic structure. A measure of gene diversity, H_t was fairly high for both the natural populations (0.146) and the seed orchard populations, within which it ranged from 0.141 for full-sib orchards (FS) to 0.164 for the offspring from supplemental mass pollination (SMP). Large amounts of genetic variation in western hemlock accords with the life history and patterns of morphological and physiological variability. This trend was also seen in observed heterozygosity H_o (0.154) for the SMP compared to the unimproved orchards (0.136). These results indicate that the maintenance of genetic diversity may be higher for the SMP treatment. This is expected as SMP have been shown to minimize self-fertilization, increase the genetic base through the introduction of desirable genotypes and improve parental balance. Another possible explanation is the crossing of distinct lines in the orchard therefore leading to more diverse populations. As a conclusion homoginzation of allelic diversity, as a result of genetic drift within the orchards has not been shown to lead to less genetic diversity.

Keywords: Western hemlock, *Tsuga heterophylla*, seed orchard, domestication, allozymes, genetic variation

INTRODUCTION

Western hemlock (*Tsuga heterophylla* (Raf.) Sarg) is distributed mainly in a narrow zone along the Pacific Coast from the Kenai Peninsula in Alaska to Northern California (Figure 1). Stands of western hemlock are among the most productive in the world (PACKEE 1990), and rank third in British Columbia for annual volume cut (OWENS & MOLDER 1984). This conifer has good to excellent pulping characteristics, and its fiber is a major source for groundwood, thermomechanical, kraft, and sulfite pulps (PACKEE 1990). In addition, its fine grain and resin free characteristics make it a suitable finishing lumber. This species also plays a major ecological role, being an important browse species for deer and elk as well as making up a significant portion of forest canopies in both western Canada and the northwestern United States.

In British Columbia, seed orchards are used to provide seedlings for reforestation. The growth cycle of vegetative buds and cones is well understood and this information has allowed the efficient production of nursery grown seedlings. Advancement in breeding practices has led to genetic improvement projects. Trees are selected for superior fitness and are incorporated into breeding plans. Such selection can lead to loss of genetic variation and an elevated percentage of inbreeding. Without genetic variance there can be no adaptive response by the individual (HEDRICK 1985). As a consequence, the higher inbreeding may lead to depression in fitness. Therefore it is important to understand the effects that breeding programs will have on genetic diversity.

In outcrossed species such as western hemlock, erosion of population genetic diversity can lead to a loss in viability and reproductive success (MOSSELER *et al.* 1994). This can therefore hinder the ability of the species to respond to selection pressures. As a consequence changes in our environment can conclude in selection for this reduced fitness. This can have profound consequences on ecosystem stability. Therefore, sustaining genetic diversity is an important factor in the maintenance of stability in managed areas. With the use of seed orchards to restock natural systems, genetic consequences must be realized.

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When a sub-sample of a population is used to represent a large area of a species range, genetic diversity can be compromised. With this, inbreeding and inbreeding depression arise as an issue. Inbreeding occurs when mates are more closely related than they would be if they had been chosen at random from the population (CROW & KIMURA 1970). Mating of closely related individuals may increase the probability of homozygosity in recessive lethal alleles. A special concern must be taken when dealing with small populations, such as seed orchards, as the opportunity of mating with relatives increases with decreasing population size (FRANKLIN 1980). To date little molecular work has looked at the above issues in western hemlock. The maintenance of genetic diversity in breeding and production populations of commercially valuable species is a priority in all breeding programs, but has seldom been verified by direct genetic assays of levels of variation. However, the problems with identifying rare alleles and the general limitations of isozymes for characterizing genetic variation will limit our inferences.

The goal of this study is to interpret levels of genetic differentiation under different seed orchard treatments. The orchard treatments tested in this study include full-sib, supplemental mass pollination and unimproved. Our purpose is then to (1) identify rates of genetic diversity under differing orchard conditions compared to natural stands of western hemlock and (2) identify the effectiveness of seed orchard and seed collection protocol in maintaining natural genetic structure.

MATERIALS AND METHODS

The geographic origin of the natural and seed orchard source populations are given in Table 1 and Figure 1. Approximately 50 individuals were sampled from each of the 22 populations. The natural populations were collected throughout the 70's and 80's by the B.C. Ministry of Forests. Families used in orchards were selected from wild collections carried out by the Ministry of Forests. All seed orchard material used in this study was collected from F1 offspring. The unimproved seed orchards under went open-pollination. The full-sib orchards are controlled crosses. The pollen buds were removed from part of a branch, and female strobili were isolated with a paper/plastic bag; next pollen from only one other parent was applied. This eliminates the possibility of contamination. In general 20 isolation bags are used on one ramet of one clone and each approximately cover 20 plus cones. To meet B.C.'s diversity requirements for public lands a minimum of 5 crosses are used for each full-sib treatment. The SMP

Table 1. Population codes, year of collection and geographic origin of *T. heterophylla* seed.

Seedlot	Location	Year	Latitude	Longitude	Elevation (m)
2685	Holberg	1975	50° 38′	128° 05′	92
2753	Toba River	1975	50° 30′	124° 10′	366
3471	Ucona River	1978	49° 40′	126° 00′	475
4088	Fleet River	1979	48° 38′	124° 04′	370
4538	Sombrio Creek	1978	48° 32′	124° 18′	365
4692	Camper Creek	1982	48° 34′	124° 30′	300
4787	Sechelt	1976			
7832	Nanaimo River	1987	49° 00′	124° 10′	550
9789	UBC Res. Forest	1985	49° 17′	122° 33′	275
18784	Kaouk Jnc.	1982	50° 05′	126° 59′	60
46152	Unknown natural		48° 50′	123° 45′	600
60160	S. O.# 126 – unimproved	1993	50° 27′	127° 09′	188
60352	S. O.# 126 – SMP	1998	50° 30′	126° 53′	95
60351	S. O.# 126 – full-sibs	1998	50° 02′	125° 52′	121
6883	S. O.# 133 – unimproved	1990	50° 00′	124° 30′	140
61060	S. O.# 133 – SMP	1999	50° 12′	125° 08′	300
60624	S. O.# 133 – full-sib	1997	50° 00′	125° 00′	300
60183	S. O.# 136 – unimproved	1993	49° 38′	126° 08′	169
60224	S. O.# 156 – unimproved	1995	52° 43′	131° 40′	241
60319	S. O.# 130 – unimproved	1995	48° 47′	124° 18′	595
60374	S. O.# 143 – unimproved	1998	49° 54′	124° 29′	745
60067	S. O.# 127 – unimproved		50° 37′	127° 19′	616

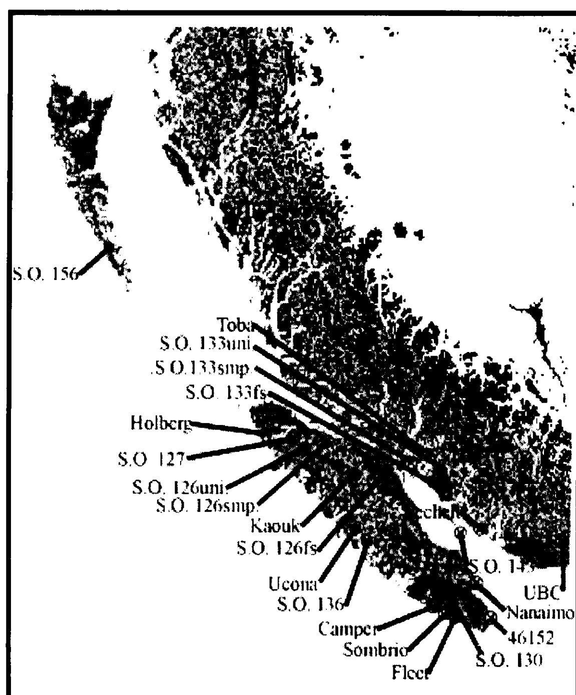

Figure 1. Locations of *Tsuga heterophylla* source populations in British Columbia.

(Supplementary Mass Pollinations) orchards had pollen from best trees dumped on receptive clones. Female strobili are receptive before any surrounding pollen are released. Therefore stored best pollen can be applied with contamination minimized. This is done by hand or with a blower. Breeding value for SMP treatments are based on applied pollen and concluding seed production given estimated contamination.

Seed was removed from the cone and was stored at 4 °C until germination. The germination process included a 72 hour soaking in distilled water at 4 °C followed by a 3 week period in which the seeds were left on wet filter paper at room temperature. Seedlings were removed once they had reached 2 cm in length and placed in cold storage (4 °C).

Starch gel electrophoresis was chosen as the molecular genetic technique for this study. Fourteen loci were identified using nine isozyme stains. Two electrophoresis systems were used to optimize the resolution of each locus. These included one continuous system, Morpholine-citrate pH 8.0 (CLAYTON & TRETIAK 1972) and one discontinuous system, Sodium-borate pH 8.0 (POULIK 1957). The stains used with the morpholine system included: shikimate dehydrogenase (SKDH), 6-phosphogluconate dehydrogenase (PGD), malate dehydrogenase (MDH), fructose-1,6-diphosphatase (FDP) and isocitric dehydrogenase (IDH). The stains used with the Sodium-borate system included: Aspartate aminotransferase (AAT), Glutamate dehydrogenase (GDH), Phosphoglucoisomerase (PGI) and Phosphoglucomutase (PGM).

The gel was produced with a 12 % starch (Starch-art corp.) and 5 % sucrose (Sigma) concentration. This volume allowed 5 useable slices per gel for the same isozyme extract. Different populations were placed on the same gel to allow comparison between alleles of different populations. The running time for the Poulik running systems was 4 hours at 230 V and the running time for the Morpholine system was 4 hours at 160 V. The histochemical staining solutions were obtained from MURPHY *et al.* (1996) and ACQUAAH (1992). The banding pattern was recorded visually and each allele was denoted by an integer depending upon their mobility. Alleles were defined consecutively with 1 representing the fastest band. One tetramer, (MDH) was defined and it was scored as a monomer for two positions within the banding pattern, hence two loci for this stain.

Observed and expected mean heterozygosity, percentage of polymorphic loci, and mean number of alleles per locus were calculated from allelic frequencies using the BIOSYS-2 computer program (SWOFFORD & SELANDER 1981, BLACK IV 1997). The diversity index (H_t) was calculated using POPGENE, version 1.32 (YEH *et al.* 1997). The dendogram was produced using the computer program „gd" (http://genetics.forestry.ubc.ca/ritland/programs.html) whose algorithm is described in RITLAND (1989).

RESULTS

Mean heterozygosity ranged from 0.112 to 0.160 for the 11 natural populations collected throughout British Columbia, while the seed orchards ranged from 0.120 to 0.167 (Table 2). Mean number of alleles per locus ranged from 1.4 for the Camper population to 2.0 for the Holberg population. The percentage of polymorphic loci ranged from 28.6 for populations, Fleet, U.B.C., Camper and S.O 143un to 50.0 for populations Holerg, Toba and Ucona. Mean heterozygosity, mean number of alleles, H_t and the percentage of polymorphic loci have also been expressed as an average for each of the grouped seed orchard samples, SMP, FS, UN as well as the natural population (Table 3).

Both the unimproved and the SMP seed orchards show higher mean number of alleles per locus compared with the natural populations, but the full-sib orchards resulted in lower estimates. This can be contrasted to the percentage of polymorphic loci which showed a higher value in the natural populations. The SMP population showed higher heterozygosity (0.154)

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Table 2. Mean heterozygosity, (both expected Hardy-Weinberg (H_E) and Direct count (H_O), mean sample size per locus (*SS*), mean number of alleles per locus (*NA*) and percentage of loci that are polymorphic (*%P*) for 11 natural populations, 11 seed orchards of *T. heterophylla* (standard errors in parentheses).

Population	code	SS	NA	%P*	H_O	H_E**
HOLERG	2685	48.6 (0.8)	2.0 (0.1)	50.0	0.143 (0.071)	0.171 (0.051)
TOBA	2753	48.4 (0.8)	1.9 (0.2)	50.0	0.149 (0.072)	0.146 (0.045)
UCONA	3471	49.1 (0.5)	1.9 (0.2)	50.0	0.131 (0.069)	0.145 (0.045)
FLEET	4088	49.3 (0.5)	1.6 (0.1)	28.6	0.117 (0.071)	0.132 (0.054)
SOMBRIO	4538	49.6 (0.4)	1.6 (0.2)	42.9	0.143 (0.075)	0.147 (0.052)
CAMPER	4692	48.9 (1.1)	1.4 (0.1)	28.6	0.119 (0.075)	0.114 (0.051)
SECHELT	4787	50.9 (1.1)	1.6 (0.2)	42.9	0.16 (0.076)	0.148 (0.049)
NANAIMO	7832	50.0 (0.0)	1.9 (0.2)	35.7	0.14 (0.072)	0.131 (0.046)
UBC	9789	48.4 (1.0)	1.7 (0.2)	28.6	0.112 (0.073)	0.137 (0.054)
KAOUK	18784	46.4 (2.0)	1.6 (0.2)	42.9	0.149 (0.075)	0.147 (0.053)
UNKNOWN	46152	50.0 (0.0)	1.5 (0.1)	42.9	0.161 (0.079)	0.131 (0.048)
SO126UN	60160	47.6 (1.3)	1.8 (0.2)	42.9	0.146 (0.074)	0.154 (0.051)
SO126SMP	60352	50.0 (0.0)	1.8 (0.2)	42.9	0.167 (0.076)	0.158 (0.053)
SO126FS	60351	50.0 (0.0)	1.6 (0.2)	35.7	0.154 (0.079)	0.149 (0.057)
SO133UN	6883	48.8 (1.2)	1.9 (0.2)	35.7	0.143 (0.076)	0.135 (0.047)
SO133SMP	60106	50.0 (0.0)	1.8 (0.2)	35.7	0.141 (0.074)	0.158 (0.056)
SO133FS	60624	49.3 (0.7)	1.6 (0.2)	42.9	0.12 (0.071)	0.123 (0.042)
SO136UN	60183	48.0 (1.5)	1.8 (0.2)	42.9	0.145 (0.072)	0.147 (0.048)
SO156UN	60224	49.0 (1.5)	1.9 (0.1)	35.7	0.126 (0.07)	0.14 (0.049)
SO130UN	60319	50.4 (1.6)	1.7 (0.2)	35.7	0.136 (0.072)	0.145 (0.053)
SO143UN	60374	49.1 (0.6)	1.7 (0.2)	28.6	0.12 (0.073)	0.121 (0.049)
UNKNOWN	60067	47.8 (1.2)	1.8 (0.2)	35.7	0.142 (0.074)	0.15 (0.051)

* A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.
** Unbiased estimate for Hardy Weinberg expectation (Hdy Wby) (see NEI, 1978).

Table 3. Average mean heterozygosity (both expected Hardy-Weinberg (H_E) and Direct count (H_O)), average mean sample size per locus (*SS*), average mean number of alleles per locus (*NA*), average percentage of loci that are polymorphic (*%P*) and the genetic diversity index, H_t for 10 natural populations, 6 unimproved (UN) seed orchards, 2 supplemental mass pollinated (SMP) seed orchards and 2 full-sib (FS) orchards of *T. heterophylla*.

Population	*SS*	*NA*	*%P**	H_O	H_E**	H_t
Total mean	49.1	1.7	38.9	0.139	0.142	
Natural mean		1.7	40.3	0.136	0.142	0.146
S. O. UN mean		1.8	36.9	0.136	0.14	0.142
S. O. SMP		1.8	39.3	0.154	0.158	0.164
S. O. FS mean		1.6	39.3	0.137	0.136	0.141

* A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.
** Unbiased estimate for Hardy Weinberg expectation (Hdy Wby) (see NEI, 1978).

overall compared with all other groups. The lowest average heterozygosity was identified in the natural populations. The measure of gene diversity (H_t) portrayed a similar story with the SMP seed orchard showing the highest genetic diversity (0.164). But in contrast to the basic heterozygosity measures, both the FS and unimproved orchards fell lower than the natural population shown in Table 3.

A dendogram mapping allelic differences between each population is presented as Figure 2. Three distinct groups can be inferred. These are: (1) Sombrio, Holberg, Fleet, UBC and S.O. 133smp, (2) Toba, S.O. 133smp and S.O. 133fs and (3) the remaining populations. It can be noted that the standard error of branch length, depicted as the thicker shaded branches, is quite large, making most inferences fairly non-significant.

The lack of significant structure is not surprising given the low value of maximal genetic divergence (0.008).

Allele frequencies are presented in Appendix A. Some alleles are present in the natural populations that are not present in the seed orchards. These include population 1, *Pgd-1 A*, *Pgd-2 A*, population 2, *Mdh C*, *Idh-1 D*, population 3, *Idh-1 C*, population 8, *Idh-1 B* and population 9, *Pgm-1 D* and *Pgm-2 D*. Some alleles are also present in the seed orchards that are not present in the natural populations. These include population 12, *Pgm-1 C*, population 14, *Skdh-2 C*, population 17 and 18, *Pgm-2 B* and population 21, *Pgi-C*. All of the rare alleles presented have frequencies between .015 and .025.

DISCUSSION

Our finding of large amounts of genetic variation in western hemlock, and little genetic differentiation of populations, accords with the life history and patterns of morphological and physiological variability exhibited by this species. Western hemlock has a large, continuous, geographic distribution, and is wind pollinated; therefore it should show little population differentiation (MOSSELER *et al.* 1994), with perhaps less than 10 % of the variation occurring among population, as gauged by studies with similar species (HAMRICK & GODT 1990). MALAVASI & PERRY (1993) found, in a shade tolerance study, physiological variability within populations. A common garden study of 21 western hemlock provenances from California to Alaska showed latitudinal differentiation for cold hardiness, survival and seedling growth factors (KUSER & CHING 1980, 1981). However, in the range of 46° to 51° no trend was found. Foster and LESTER (1983) found similar results for height, with no differentiation in the 3° latitude distribution of hemlock in Washington State. Interestingly, KING (1991) did find significant differentiation between trees below vs. above 600 m elevation.

Seed orchards vs. natural populations

Tsuga heterophylla, an outcrossing wind pollinated species, showed a relatively high gene diversity (H_t) in its natural populations, 0.146 (Table 3). This finding is similar to other outcrossing wind pollinated species (HAMRICK *et al.* 1992). *T. heterophylla* had a lower H_t value than that determined as an average value in gymnosperms (0.281) but was similar to an average value determined for long-lived perennials, 0.148 (HAMRICK *et al.* 1992). The unimproved and full-sib seed orchards showed a slight decrease in H_t (0.142) but this was not significant (Table 3). This trend was also seen in mean observed heterozygosity (H_o). The natural populations H_o (0.136) did not differ significantly from the unimproved and full-sib seed orchards, 0.136 and 0.137 respectively (Table 3). It is important to note that the observed heterozygosities are not significantly different than the expected heterozygosities (H_e), indicating that the different populations are in Hardy-Weinberg equilibrium. These results indicate that gene diversity was retained in the unimproved and full-sib seed orchards. This result was also seen in the percentage of polymorphic loci which showed a slight decrease for orchard populations compared with natural populations (Table 3).

In contrast the SMP seed orchards showed different results. The SMP orchards had a higher H_t (0.164) than the unimproved orchards (0.142) (Table 3). This trend was also seen in H_o (0.154) for the SMP compared to the unimproved orchards (0.136) (Table 3). These results indicate that gene diversity may be higher for this treatment. This is expected as SMP has been shown to: 1) minimize self-fertilization (EL-KASSABY & RITLAND 1986) 2) increase the genetic base through the introduction of desirable genotypes (HADDERS 1984) and 3) improve parental balance (REYNOLDS & EL-KASSABY 1990). All of these factors would result in higher levels of genetic diversity. The percentage of polymorphic loci worked in parallel with the H_t results. The SMP seed orchards were measured at $P = 39.3$ % where as the unimproved seed orchards were measured at $P = 36.9$ % (Table 3). Although slight, there is a decrease in the polymorphic estimate when comparing the SMP treatment to the natural populations (40.3 %) (Table 3). This is most likely the result of a limited genetic base in the orchard population. It should also be noted that the mean number of alleles did not differ significantly between the natural and seed orchard populations.

The dendogram showed limited pattern (Figure 2), but does indicate three groups. These groups show little correlation between the seed orchards and the source areas of the orchards that the natural populations are located in. This result suggests that gene flow in the orchards has crossed distinct lines therefore leading to more diverse populations. Genetic drift within the orchards has not seemed to homogenize the populations therefore leading to less genetic diversity. This was shown earlier in the mean heterozygosity (H_o) and genetic diversity (H_t) levels. One issue that may arise from high rates of gene flow is the loss of rare alleles as rare alleles may harbor the genetic differentiation that allow a population to adapt to a changing ecosystem.

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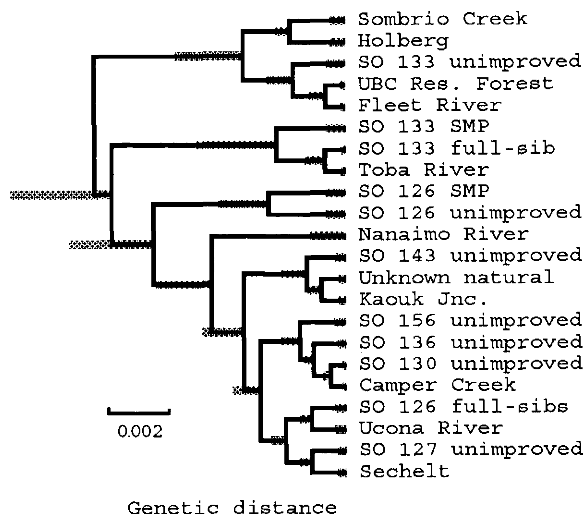

Figure 2. Dendrogram of 22 seed orchard and natural *T. heterophylla* populations.

The allele frequencies are listed for all loci in the 22 populations (Appendix A). Eight alleles were recorded in the natural populations that were not present in any of the seed orchard populations. All alleles were very rare being found in only one of the natural populations studied. Within the populations their allele frequencies ranged from 0.020 to 0.025 (Appendix A). In comparison, 4 alleles were present in the seed orchards that were not present in the natural populations. These alleles were also only found in one seed orchard for each. Their frequencies were also very low, ranging from 0.010 to 0.020. CHAISURISRI & EL-KASSABY (1994) noted in a similar study on Sitka spruce (*Picea sitchensis* (Bong.) Carr.) that sampling breadth may be the reason for such findings. The source ranges differed between the seed orchards and natural populations. All of the orchard cones were collected throughout the range of western hemlock, where as the natural populations were centered around Vancouver Island and the Lower Mainland.

Comparison to other hemlock species

Very little molecular work has been performed on hemlock. ZABINSKI (1992) looked at isozyme variation in eastern hemlock (*Tsuga canadensis* (L.) Carr.) and found very low rates of H_t, (0.04). The proportion of polymorphic loci was also very low for this species, 0.10. This is unexpected as much higher values have been observed for outcrossing, wind pollinated, long lived conifers with a very wide range. The hypothesis presented as an explanation are that of FOWLER & MORRIS (1977). They point to a population bottleneck or a series of bottlenecks during the Pleistocene.

ALLY *et al.* (2000) also found lower than expected levels of H_t in Mountain hemlock (*Tsuga mertensiana*). They found an H_t of 0.093 and a percentage of polymorphic alleles of 0.33. They point to two reasons for this relatively low estimate. First being the bottleneck hypothesis, presented earlier, and second, the genetic depauperacy of southern refugial populations hypothesized by CWNYAR & MACDONALD (1987). They also note that flight of mountain hemlock seed is short and much more likely to promote family structure and the accumulation of local genetic differences. This is in contrast to western hemlock, which has been recorded, under windy conditions, with 1.6 km of seed travel (ISAAC 1930).

Comparison to similar studies with other conifers

CHAISURISRI & EL-KASSABY (1994) performed a similar study with Sitka spruce (*Picea sitchensis* (Bong.) Carr.). In this study 10 range-wide natural populations were compared to 1 seed orchard population. Major finding include a non-significant higher value for mean heterozygosity in the seed orchard population when compared to the natural populations. It is also noted, by looking at genetic distance, that the seed orchard is similar to three of the natural populations located within the area where the parent seed orchard seed was collected. Increased levels of heterozygosity in the seed orchard are hypothesized as being the result of sampling breadth in parent tree sampling (CHAISURISRI & EL-KASSABY 1994). As a conclusion CHAISURISRI & EL-KASSABY (1994) note that the seed orchard, composed of a production population of 139 clones, was sufficient to prevent loss of genetic variability.

EL-KASSABY & RITLAND (1996) performed a seed orchard study to identify the impact of selection and breeding on genetic diversity in Douglas-fir (*Pseudotsuga menziesii*). In this study two generations of seed orchards were compared against their 49 wild progenitor populations. Measures of heterozygosity, polymorphic loci and divergence were found to be similar or higher in the domesticated populations. EL-KASSABY & RITLAND (1996) therefore concluded that selection and breeding had not lead to a significant decrease in genetic variation. This was thought to be linked to the seed source used to stock the orchards as it was pooled from a widely distributed natural population. They also noted that although the first generation seed orchard was not significantly different from the natural populations, the second generation was. This is thought to be the result of interbreeding that formed the advanced

seed orchard generation.

The dynamics of rare alleles in these domesticated populations needs study, using different types of genetic markers. Isozymes are well known to encompass a very restricted portion of the total genome of an organism. In a Douglas-fir study isozymes were shown to not fully measure the losses of variation that occur in the initial stages of domestication (EL-KASSABY & RITLAND 1996). The ideal markers would lie adjacent to loci controlling physiologically and ecologically important characters, and lacking knowledge of the locations of these genes, would at least be numerous and highly polymorphic. Further analysis should therefore be initiated.

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Appendix. Allozyme frequencies for the 22 seed orchard and natural populations of Western hemlock collected in British Columbia.

Locus	Allele	Population										
		1	2	3	4	5	6	7	8	9	10	11
Aat-1	(N)	50	50	50	50	50	50	50	50	50	50	50
	A	0.99	0.99	0.98	0.98	1	1	0.981	0.94	1	0.98	0.98
	B	0.01	0.01	0.02	0.02	0	0	0.019	0.06	0	0.02	0.02
Aat-2	(N)	50	50	50	50	50	50	53	50	50	50	50
	A	0.9	0.93	0.91	0.97	0.87	0.98	0.837	0.98	0.96	0.97	0.98
	B	0.1	0.07	0.09	0.03	0.13	0.02	0.163	0.02	0.04	0.03	0.02
Gdh	(N)	40	40	49	50	50	50	52	50	48	50	50
	A	0.6	0.8	0.837	0.6	0.7	0.8	0.808	0.88	0.583	0.82	0.86
	B	0.4	0.2	0.163	0.4	0.3	0.2	0.192	0.12	0.417	0.18	0.14
Pgi	(N)	50	50	50	50	50	50	50	52	50	50	50
	A	0.01	0.01	0.03	0.01	0	0	0	0.03	0	0	0.01
	B	0.99	0.99	0.97	0.99	1	1	1	0.97	1	1	0.99
	C	0	0	0	0	0	0	0	0	0	0	0
Pgm-1	(N)	45	45	45	45	50	50	52	50	40	30	38
	A	0.956	0.944	0.978	1	0.98	1	1	1	0.975	0.95	0.934
	B	0.044	0.056	0.022	0	0.02	0	0	0	0	0.05	0.066
	C	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0.025	0	0
Pgm-2	(N)	45	45	45	45	50	50	52	50	40	30	38
	A	1	1	1	1	1	1	1	1	0.975	1	1
	B	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0.025	0	0
Skdh-1	(N)	50	49	49	50	50	50	52	50	50	50	50
	A	0.07	0.173	0.102	0	0.02	0	0.019	0.02	0.05	0.13	0.18
	B	0.88	0.786	0.878	0.9	0.9	0.9	0.846	0.86	0.91	0.72	0.74
	C	0.05	0.041	0.02	0.1	0.08	0.1	0.135	0.12	0.04	0.15	0.08
Skdh-2	(N)	50	49	49	50	45	35	37	50	49	40	40
	A	0.49	0.214	0.378	0.4	0.556	0.443	0.351	0.58	0.439	0.463	0.475
	B	0.51	0.786	0.622	0.6	0.444	0.557	0.649	0.42	0.561	0.538	0.525
	C	0	0	0	0	0	0	0	0	0	0	0
Pgd-1	(N)	50	50	50	50	50	50	52	50	50	50	50
	A	0.02	0	0	0	0	0	0	0	0	0	0
	B	0.98	1	1	1	1	1	1	1	1	1	1
Pgd-2	(N)	50	50	50	50	50	50	52	50	50	50	50
	A	0.02	0	0	0	0	0	0	0	0	0	0
	B	0.98	1	1	1	1	1	1	1	1	1	1

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Appendix. Allozyme frequencies for the 22 seed orchard and natural populations of Western hemlock collected in British Columbia.

Locus	Allele	Population										
		12	13	14	15	16	17	18	19	20	21	22
Aat-1	(N)	50	50	50	50	50	50	51	52	50	50	50
	A	0.97	1	0.99	0.99	1	0.98	0.931	0.981	0.98	1	1
	B	0.03	0	0.01	0.01	0	0.02	0.069	0.019	0.02	0	0
Aat-2	(N)	50	50	50	50	50	50	51	52	50	50	50
	A	0.94	0.96	0.96	0.94	0.91	0.92	0.961	0.962	0.96	0.97	0.88
	B	0.06	0.04	0.04	0.06	0.09	0.08	0.039	0.038	0.04	0.03	0.12
Gdh	(N)	50	50	50	50	40	50	51	52	50	50	50
	A	0.88	0.8	0.82	0.54	0.825	0.76	0.784	0.75	0.82	0.84	0.88
	B	0.12	0.2	0.18	0.46	0.175	0.24	0.216	0.25	0.18	0.16	0.12
Pgi	(N)	50	50	50	50	50	50	51	52	50	50	50
	A	0.01	0	0.02	0.01	0	0.02	0.01	0.01	0.01	0.02	0
	B	0.99	1	0.98	0.99	1	0.98	0.99	0.99	0.99	0.96	1
	C	0	0	0	0	0	0	0	0	0	0.02	0
Pgm-1	(N)	50	50	50	50	50	50	51	52	44	40	50
	A	0.97	0.99	0.99	0.98	1	1	0.961	0.981	1	0.988	1
	B	0.01	0.01	0.01	0.02	0	0	0.039	0.019	0	0.013	0
	C	0.02	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0	0
Pgm-2	(N)	50	50	50	50	50	50	51	52	44	40	50
	A	1	1	1	1	1	0.99	0.98	1	1	1	1
	B	0	0	0	0	0	0.01	0.02	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0	0
Skdh-1	(N)	50	50	50	50	50	49	50	52	50	50	50
	A	0.04	0.06	0.02	0.07	0.02	0.143	0.1	0.096	0.03	0.18	0
	B	0.69	0.63	0.87	0.77	0.87	0.776	0.88	0.788	0.92	0.74	0.91
	C	0.27	0.31	0.11	0.16	0.11	0.082	0.02	0.115	0.05	0.08	0.09
Skdh-2	(N)	50	50	33	50	50	29	30	30	50	39	50
	A	0.56	0.49	0.333	0.41	0.18	0.241	0.45	0.467	0.48	0.385	0.4
	B	0.44	0.51	0.652	0.59	0.82	0.759	0.55	0.533	0.52	0.615	0.6
	C	0	0	0.015	0	0	0	0	0	0	0	0
Pgd-1	(N)	50	50	50	50	50	49	50	52	50	50	50
	A	0	0	0	0	0	0	0	0	0	0	0
	B	1	1	1	1	1	1	1	1	1	1	1
Pgd-2	(N)	50	50	50	50	50	49	50	52	50	50	50
	A	0	0	0	0	0	0	0	0	0	0	0
	B	1	1	1	1	1	1	1	1	1	1	1

Appendix. Allozyme frequencies for the 22 seed orchard and natural populations of Western hemlock collected in British Columbia.

Locus	Allele	Population										
		1	2	3	4	5	6	7	8	9	10	11
Mdh	(N)	50	50	50	50	50	50	52	50	50	50	50
	A	0.9	0.91	0.95	1	0.95	0.98	0.904	0.96	0.98	0.94	0.9
	B	0.1	0.07	0.05	0	0.05	0.02	0.096	0.04	0.02	0.06	0.1
	C	0	0.02	0	0	0	0	0	0	0	0	0
Fdp	(N)	50	50	50	50	50	50	52	50	50	50	50
	A	0.5	0.5	0.49	0.48	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	B	0.5	0.5	0.51	0.52	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Idh-1	(N)	50	50	50	50	50	50	53	50	50	50	50
	A	1	0.98	0.98	1	1	1	1	0.98	1	1	1
	B	0	0	0	0	0	0	0	0.02	0	0	0
	C	0	0	0.02	0	0	0	0	0	0	0	0
	D	0	0.02	0	0	0	0	0	0	0	0	0
Idh-2	(N)	50	50	50	50	50	50	52	50	50	50	50
	A	0.02	0	0.01	0	0	0	0	0.01	0	0	0
	B	0.04	0.02	0.05	0.04	0.04	0	0.019	0.01	0.02	0	0.4
	C	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0	0
	E	0.94	0.98	0.94	0.96	0.96	1	0.981	0.98	0.98	1	0.96

Appendix. Allozyme frequencies for the 22 seed orchard and natural populations of Western hemlock collected in British Columbia.

Locus	Allele	Population										
		12	13	14	15	16	17	18	19	20	21	22
Mdh	(N)	50	50	50	50	50	49	50	52	50	50	50
	A	0.83	0.94	0.96	0.97	0.96	0.918	0.99	0.942	0.99	0.9	0.88
	B	0.17	0.06	0.04	0.03	0.04	0.082	0.01	0.058	0.01	0.1	0.12
	C	0	0	0	0	0	0	0	0	0	0	0
Fdp	(N)	50	50	50	50	50	49	50	52	50	50	50
	A	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	B	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Idh-1	(N)	50	50	50	50	50	49	50	52	50	50	50
	A	1	1	1	1	1	1	1	1	1	1	1
	B	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0	0
Idh-2	(N)	50	50	50	50	50	49	50	52	50	50	50
	A	0	0	0.01	0	0.02	0	0	0	0	0	0
	B	0	0.02	0.07	0.04	0.06	0.2	0.02	0	0.04	0.04	0.02
	C	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0	0
	E	1	0.98	0.92	0.96	0.92	0.98	0.98	1	0.96	0.96	0.98