GENETIC DIVERSITY, DIFFERENTIATION AND MATING SYSTEM IN MOUNTAIN HEMLOCK (*TSUGA MERTENSIANA*) ACROSS BRITISH COLUMBIA

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ABSTRACT

Genetic diversity, mating system and the evolutionary history of 19 populations of mountain hemlock (*Tsuga mertensiana*) within British Columbia were inferred from genetic variation at 19 allozyme loci. Within populations, 32 % of the loci were polymorphic and expected heterozygosity was 0.087, which is ca. half the heterozygosity found in other conifers. Outcrossing rates did not significantly differ from 100 %. Populations showed moderate differentiation (G_{st} = 0.077), island populations showed considerably more differentation (G_{st} = 0.095) than mainland populations (G_{st} = 0.058), and an isolation-by-distance analysis suggests restricted gene flow. For the populations in southwestern British Columbia, there was a significant positive correlation between average expected heterozygosity and elevation, while expected heterozygosity was negatively correlated with latitude. This suggests that during a northward post-glacial range expansion, more northerly mountain hemlock populations suffered a loss in genetic variation due to this migration.

Keywords: *Tsuga mertensiana*, hemlock, western North America, allozymes, genetic variation, gene flow, bottleneck

INTRODUCTION

Most species of conifers are long-lived, outcrossing, wind-pollinated, wind-dispersed, and have large geographic ranges. Levels of genetic variation are high in conifers and populations show little genetic differentiation (HAMRICK *et al.* 1992, HAMRICK & GODT 1996) conforming to expectations under models of mutation, genetic drift and migration. However, historical events may also significantly affect present-day genetic structures. For example, lodgepole pine shows reduced allelic diversity at its northern periphery, possibly a result of repeated long distance founding events during post-glacial expansion (CWYNAR & MACDONALD 1987). Thus, against a background of life-history and mating system attributes of a species, population history can significantly contribute to the interpretation of genetic structure of in a species.

Mountain hemlock (*Tsuga mertensiana* Bong.) grows in the subalpine coastal and interior forests of British Columbia and Alaska. Found within a region between 300 to 1000m (1,000 to 3, 300ft) in elevation, mountain hemlock is a late-successional species. It is commonly found in pure stands or mixed with subalpine fir (*Abies lasiocarpa*), amabilis fir (*Abies amabilis*), Englelmann spruce (*Picea engelmanni*), subalpine larch (*Larix lyalii*), whitebark pine (*Pinus albicaulis*) and lodgepole pine (*Pinus contorta*) (FARRAR 1997). With a relatively short growing season (frost free period) ranging from 95 to 148 days in southwestern British Columbia, mountain hemlock can withstand a temperature range of -29° to 38°C and a snowpack of up to 750 cm that often persists until August or September (MEANS 1990). The coastal range of mountain hemlock extends from Sequoia National Park in California north to Cook Inlet in Alaska, while interior populations extend east as far as the northern Rocky Mountains in Idaho and western Montana (MEANS 1990). The interior, southern and northern edges of its geographic distribution are characterized by disjunct populations. It has been previously suggested that disjunct populations may be the outcome of changing environmental conditions effecting a change in the distribution of a species (ZABINSKI 1992). The fragmented mountain hemlock populations at the southern edges of its range, and in the coastal islands of British Columbia, may represent refugial populations with greater genetic differentiation, while the northern and interior isolates could be recently colonized sites with lesser amounts of genetic variation.

Two contrasting factors may operate in determining the patterning of genetic variation in this species. Mountain hemlock, like all conifers, is wind-pollinated and monoecious, but of the North American hemlocks,

it has the second largest seeds, second only to Carolina hemlock (*T. caroliniana* Engelm.). This large seed size may decrease dispersal distance, increasing the opportunity for family structure and local differentiation of populations (EDWARDS & EL-KASSABY 1996).

Second, like many other conifers in British Columbia, recent deglaciation has resulted in extensive migration and the potential for bottlenecks. Pollen fossil records suggest that *T. mertensiana* had a southern refugium during the last glacial maximum, 18, 000 years ago. Following the end of the last Ice Age, the species migrated northward along the coast appearing in SW British Columbia 12,400-10,500 B. P. (MATHEWES 1973, WAINMAN & MATHEWES 1987) and in SE Alaska ca. 6,700 years B. P. (CWYNAR 1990).

The objectives of the present study were to: (1) describe genetic variation and population differentiation among mountain hemlock populations in British Columbia, (2) evaluate the role of mountain hemlock's migrational history in structuring this variation, and (3) determine the level of outcrossing in two Vancouver Island populations. This study is part of a larger investigation into the genetic structure of mountain hemlock, in which adaptive and quantitative attributes as well as germination ecology have been evaluated for the purpose of developing a suitable conservation strategy. Although a minor commercial species, used in small-dimension lumber and pulp, mountain hemlock serves to protect steep slopes against erosion, and is a component of wildlife habitat. Previous studies have found significant relationships between geography (latitude and elevation) and physiology (growth rate, frost hardiness, biomass and gas exchange, BENOWICZ & EL-KASSABY 1999).

MATERIALS AND METHODS

Seeds from a total of 19 populations, representing the range of distribution in British Columbia, were collected. Their locations are given in Figure 1 and elevations are given in Table 1. Sampling was performed according to the International Union of Forest Research Organization (IUFRO) regulations, which suggest that a sampled population or locality be uniform in terms of climate, landform, soil and vegetation (LINES, 1973). From the bulked seedlots, 40 haploid megagmetophyte tissues (1*n*) were then randomly sampled for each of the 19 populations. Although not ideal, enzyme variation has been historically examined by sampling bulked seedlots and using haploid tissues to interprete allele differences (YEH & EL-KASSABY 1980). Seeds were removed after seed hydration for 24 hrs and proteins were extracted using a slightly modified extraction buffer of CHELIAK & PITEL (1984).

Table 1. Population codes, elevations and geographic coordinates.

Population Name	Code	Elevation	Latitude	Longitude
Meade Crk.	A	1067	48.55	124.05
Wakeman High	B	1100	51.10	126.25
HannaRidge	C	700	56.18	129.20
Wakeman Low	D	600	51.17	126.17
Garbage Crk.	E	850	48.33	124.06
Mission	F	900	49.18	122.24
Lyon Lk.	G	1005	49.39	123.54
Parksville	H	824	49.16	124.33
Mayo Crk.	I	683	54.47	129.02
Zeballos	J	700	50.10	126.47
Kearsley Crk	K	1280	49.19	122.22
Blue Ox Crk	L	660	50.18	127.16
Hkusam Mt.	M	950	50.20	125.50
Port Alice	N	750	50.24	124.27
Sale Mt.	O	1700	51.10	118.10
Hodoo Crk.	P	1250	51.20	125.32
Copper Canyon	Q	1100	48.56	124.13
Ashly Crk.	R	1000	50.01	123.33
Woss Lk.	S	900	50.07	126.35

Horizonal starch gel electrophoresis was then conducted on 11 % horizontal starch gels and 19 loci assayed across 11 enzyme systems: flourescent esterase (FEST), isocitrate dehydrogenase (IDH), glucose-6-phosphate dehydrogenase (6PGDH), glutamate dehydrogenase (GDH), aconitase (ACO), phophoglucomutase (PGM), phosphoglucose isomerase (PGI), shikitimate dehydrogenase (SKD), leucine-aminopeptitase (LAP), malate dehydrogenase (MDH), and aspartate aminotransferase (AAT). FEST, IDH, 6PGDH, GDH and ACO each had one locus, PGM, PGI, SKD and LAP each had two loci and, MDH and AAT each had three loci. Buffer systems used were: lithium borate pH 8.3, 250 volts (RIDGEWAY *et al.* 1970); morpholine citrate pH 8, 200 volts (CLAYTON & TRETIAK 1972); sodium borate pH 8.6, 260 volts (POULIK 1957). Staining methods followed those of CONKLE *et al.* (1982) and O'MALLEY *et al.* (1980).

In addition, seed progenies (progeny arrays) from individual trees were sampled from two natural populations at the southern tip of Vancouver Island (Sooke & San Juan). In each population, seeds were collected from 20 individual trees spaced approximately 2-3 tree heights apart. In the two populations (Sooke & North San Juan) sampled for progeny arrays, 40 seeds per mother were extracted and six loci (PGI, IDH, PGM, 6PG, MDH2, MDH3) were examined.

Allozyme variation was analyzed using BIOSYS-2 (BLACK & KRAFSUR 1985, SWOFFORD & SELANDER 1981) and GDD (RITLAND 1989) with the following

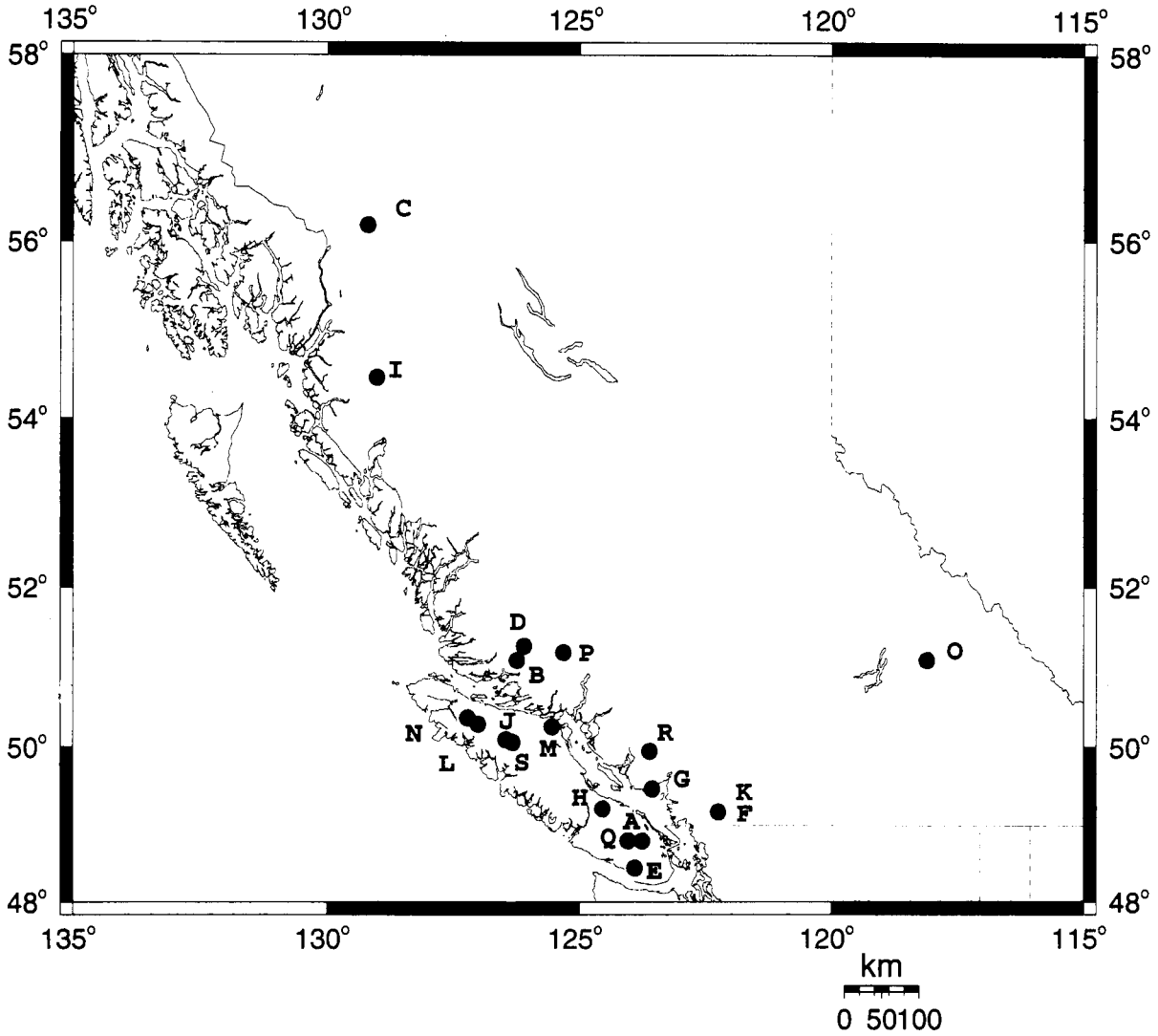

Figure 1. Locations of 19 sampling populations of mountain hemlock from British Columbia (see table 1 for coding of populations).

values computed: allele frequencies, average number of alleles per locus ($\bar{A}$), percent polymorphic loci (%P) and expected heterozygosities (H_e). To investigate the extent of population structuring and differentiation F_{st} (WRIGHT 1969) was computed from BIOSYS-2 for individual loci of the 19 populations. NEI's (1973) G_{st} was calculated and Nei's genetic distance (1978) computed between all populations. A dendrogram of genetic relationships among populations was constructed from these distances using the unweighted pair group method (SNEATH & SOKAL 1973), and the statistical significance of branches estimated with the computer program GDD which uses the method described in RITLAND (1989).

The relationship between genetic variation (expected heterozygosity and number of alleles) and geographic variables (latitude and elevation) was examined by regression of the former on the latter. To detect any recent reductions in effective population size, the allele frequency data were analyzed with a the computer program BOTTLENECK (CORNUET & LUIKART 1996), which tests whether the observed number of alleles fits the heterozygosity expected under mutation-drift equilibrium. The effect of a bottleneck is that the expected heterozygosity (H_e) computed from a sample of genes is larger than the heterozygosity expected from the number of alleles found in the same sample assuming the population is at mutation drift equilibrium (CORNUET & LUIKART 1996). Data were analyzed first with the infinite allele model (IAM) then with the stepwise mutation model (SMM).

The relationship between geographic distance, gene

Table 2. Genetic diversity measures for all 20 populations of mountain hemlock from British Columbia.

Population	A_T	A	$\%P$	H_e
Meade Crk	36	1.9	36.8	0.092
Wakeman High	32	1.7	42.1	0.097
Hanna Ridge	28	1.5	31.6	0.065
Wakeman Low	30	1.6	42.1	0.084
Garbage Crk	31	1.6	31.6	0.106
Mission	33	1.7	31.6	0.122
Lyon Lk	27	1.4	26.3	0.068
Parksville	30	1.6	31.6	0.102
Mayo Crk	32	1.7	36.8	0.088
Zeballos	24	1.3	21.1	0.050
Kearsley Crk.	32	1.7	42.1	0.106
Blue Ox Crk.	22	1.2	10.5	0.056
Hkusam Mt.	27	1.4	31.6	0.075
Port Alice	27	1.4	26.3	0.086
Sale Mt.	22	1.2	10.5	0.050
Hoodoo Crk.	31	1.6	31.6	0.095
Copper Canyon	35	1.8	47.4	0.120
Ashly Crk.	32	1.7	36.8	0.109
Woss Lk.	30	1.6	36.8	0.082
Overall average	29.5	1.58	32.0	0.087

flow and genetic differentiation was evaluated by regression of Nei's genetic distance on physical distance, and regression of SLATKIN'S (1993) M (=Nm as estimated pairwise F_{st}) on physical distance. For this, three groups of populations were separately evaluated: (1) southwestern populations excluding Sale Mt., Hanna Ridge and Mayo Creek, (2) island only and (3) mainland only. Mantel tests (MANTEL 1967) determined the significance of associations.

Mating system analysis was conducted on the two populations sampled for individual tree progenies using the computer program MLTR, which is based on maximum likelihood (RITLAND 1990). Estimates were obtained of single locus (t_s) and multiculocus (t_m) outcrossing rates, correlated matings (r_p), inbreeding coefficients (F), and gene frequencies. Significance was determined by the bootstrap method, where the progeny array was the unit of sampling. For the North San Juan population, sixteen separate chi-squared tests were performed on each progeny array of heterozygous parents (inferred maternal genotypes) to determine if inheritance was Mendelian.

RESULTS

Seventeen of the 19 loci investigated (89.5 %) were polymorphic; AAT-1 and SKD-2 were monomorphic in all populations (Appendix A). Two populations possessed private alleles: Meade Creek (AAT-2-2) and Blue Ox Creek (LAP-1-3). The number of alleles over all loci ranged from 22 (Blue Ox Creek and Sale Mt) to 36 (Meade Creek), with an overall average of 30. The population with the highest number of alleles had a single private allele, while surprisingly, the second private allele was found in Blue Ox Creek, the population with the lowest number of alleles. Table 2 shows the average number of alleles per locus was 1.6 and ranged from 1.2 to 1.9. On average, 32 % of the loci were polymorphic within populations, and this ranged from 10 % for Blue Ox Creek and Sale Mt to 47.4 % for Cooper Canyon.

Expected heterozygosity within populations ranged from 0.122 (Mission) to 0.050 (Blue Ox Creek and Sale Mt), and averaged 0.087 (Table 2). For the 19 populations, total genetic diversity (H_t) was 0.092. A significant amount of variation is found among populations, as reflected by a G_{st} value of 0.077 (SE = 0.004).

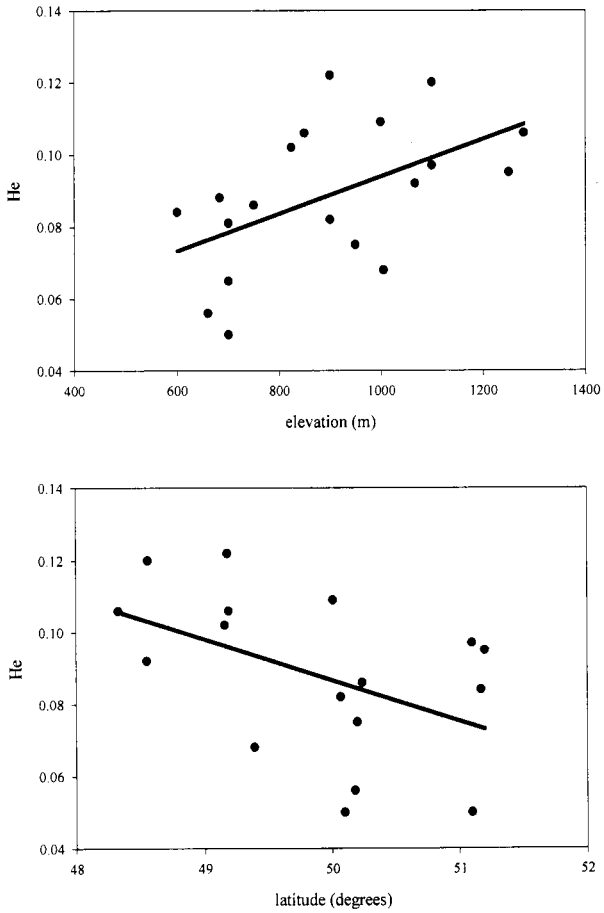

Figure 2. (**a**) Relationship between elevation (m) and average expected heterozygosity after the removal of the interior population Sale Mt. The regression was significant $p < 0.05$, $r^2 = 0.267$. (**b**) Relationship between latitude and average expected heterozygosity for coastal southwestern British Columbia. The regression was significant $p < 0.05$, $r^2 = 0.227$.

Island populations (G_{st} = 0.096±0.005) showed more among population variation than mainland populations (G_{st} = 0.058±0.003). High elevation populations (> 1000m) had a greater proportion of the total genetic diversity among populations (G_{st} = 0.089±0.003) than those at lower elevations (G_{st} = 0.059±0.005).

Figure 2 portrays the relationship between diversity and geography (latitude and elevation). Without including the far outlying, interior population Sale Mt., the association between expected heterozygosity and elevation (p = 0.028) and elevation explained 26.7 % of the variation in heterozygosity. A similar relationship was observed for total numbers of alleles and elevation (r^2 = 0.295, p = 0.020). Expected heterozygosity showed a negative association with latitude (p = 0.051), but two geographically distant outlier populations – Mayo Creek & Hanna Ridge – were omitted.

In the bottleneck analysis, two populations, Blue Ox Creek and Sale Mt., were left out of because they did not have the required 5 polymorphic loci. Under the IAM, 9 of 18 populations showed significant ($p < 0.05$) heterozygosity *deficiency* ($H_e < H_{eq}$), based on the Wilcoxon sign-rank test for significance; none showed the excess expected with a recent bottleneck. Under the SMM model, 17 of the 18 populations showed a significant heterozygosity deficiency. Eight populations showed statistically significant deficiencies under both the IAM and the SMM.

The dendrogram of genetic relationships among the 19 populations is shown in Figure 3 (significant clusters of populations in the dendrogram occur when branch length is at least twice the error standard error bar, as indicated by the thicker line, c.f. RITLAND 1989). The average genetic distance was 0.008, indicative of relatively low genetic differentiation. The most genetically distinct population is Blue Ox Creek (branch length of 0.024). This population also ranks among the lowest gene diversity (Table 2). Clustering at the lowest level (pairs of populations) were significant in several cases (e.g., Ashly Creek & Mission; Copper Canyon & Garbage Creek; Woss Lake & Wakeman High), and a somewhat geographically widespread group of three populations had a very strong cluster (Sale Mt., Hkusam Mt.& Kersley Crk.). There is weak support for two overall clusters – one cluster involving SW British Columbia and the Interior (this includes the strong cluster of the above three populations), and the other cluster involving the North Coast of B.C. and Northern Vancouver Island.

A Mantel test conducted on the genetic and physical distance matrices found no significant correlation between the two (r =0.016). SLATKIN's (1993) regression analysis also showed no apparent pattern of isolation by distance for the 19 populations. However, after the removal of three physically distant, outlier populations (Sale Mt., Hanna Ridge and Mayo Creek), the Mantel test became statistically significant (r = –0.214, p = 0.010). Mantel tests conducted on island populations only and mainland populations only found no significant relationships between physical distance and genetic distance matrices.

The regression of $\log_{10}(M)$ on $\log_{10}$(distance) is shown in Figure 4. The regression coefficient was negative (b = –0.19) but explained only 4.6 % of the variation. The intercept of the regression line was 0.88 (±0.33), giving an estimate of Nm of 7.7 (e.g., $10^{0.88}$). Although the regression was found to be significant, a plot of the residuals showed heterogeneity of variances, violating a basic assumption of linear regression models.

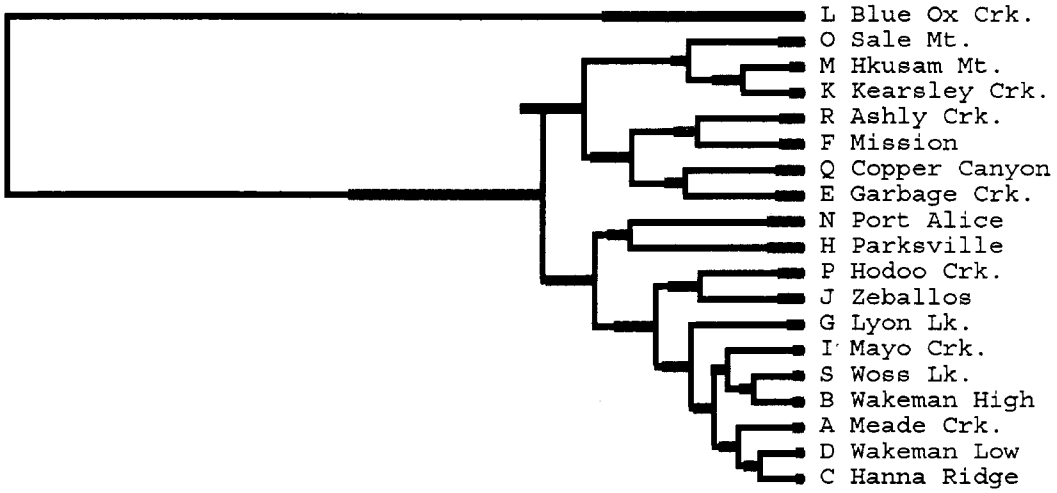

Figure 3. (**a**) A dendrogram depicting the relationship among all sampling populations in British Columbia. (**b**) A dendrogram depicting the relationship among the coastal southwestern populations of British Columbia. The distance matrix was calculated using NEI'S (1978) standard genetic distance.

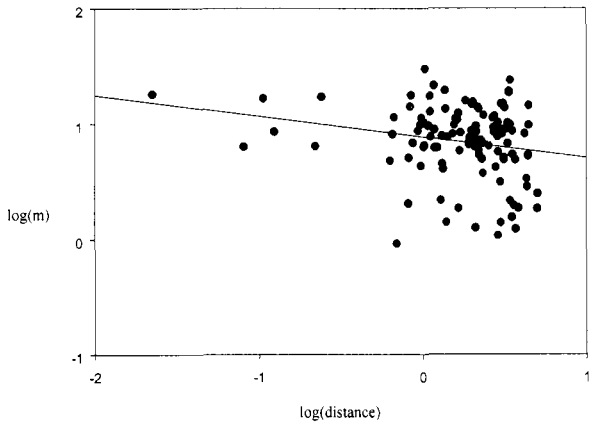

Figure 4. Relationship between gene flow and simple physical distance for 16 sampling populations following SLATKIN'S (1993) method of isolation by distance.

Table 3. Estimates of gene diversity for all populations, regional and elevational groupings.

	H_t	H_s	G_{ST}
All populations	0.0923	0.08553	0.07681
Island	0.09320	0.08429	0.09562
Mainland	0.09189	0.08655	0.05812
Elevations > 1000m	0.09536	0.08976	0.05877
Elevations < 1000m	0.09005	0.08208	0.08855

Multilocus estimates of outcrossing rate were high (0.99 for Sooke and 0.96 for North San Juan) and did not differ significantly from unity (Table 4; ovule and pollen pool allelic frequencies did not differ at the 95 % level, and were pooled for the final analysis). However, the estimate for North San Juan suggests a small amount of selfing. There was no evidence of biparental inbreeding, as single-locus estimates were essentially identical to the multilocus estimates (Table 4). Likewise, parental inbreeding coefficients were low and did not significantly differ from zero, although again, there is a suggestion of inbreeding in North San Juan. Estimates of correlated matings obtained for both populations were quite low (Table 4) and did not significantly differ from zero (e.g., progeny were all half-sibs). All sixteen Chi-squared tests were found not to be significant confirming that isozymes in mountain hemlock are inherited in a Mendelian fashion.

DISCUSSION

The level of genetic diversity in mountain hemlock, H = 0.093, is low compared to other late-successional, wind-pollinated, long-lived woody perennials that have an outcrossing breeding system and a widespread distribution (Table 5). The only other published isozyme study of a *Tsuga* species was of eastern hemlock (*T. canadensis*), where a very low level of genetic variation (0.04) was found (ZABINSKI 1992). In mountain hemlock, the mean number of alleles (1.6) was lower than what was expected from conifers as a taxonomic group (1.8) and much lower than species which show a widespread range (2.6; HAMRICK *et al.* 1992). The same is true for the percentage of polymorphic loci in this species (32 %), which although much higher than its eastern counterpart *T. canadensis* (10 %; ZABINSKI 1992), is lower than what was reported for long lived woody perennials (49 %; HAMRICK *et al.* 1992).

In the other conifers for which low levels of allozyme variation have been reported (*Pinus torreyana* – Torrey pine, *Pinus resinosa* – red pine, and *Thuja plicata* – western red cedar), the species are hypothesized have undergone a population bottleneck or a series of bottlenecks during the Pleistocene (ZABINSKI 1992). Hence, during a northward post-glacial range expansion, mountain hemlock may have similarly undergone a loss in genetic variation due to a series of stepping stone founder events. CWNYAR & MACDONALD (1987) explained a progressive decline in allelic diversity toward the northern periphery of lodgepole pine as the result of repeated long distance founding events. Although this pattern is suggested by our data, (Figure 2) further investigation would allow the effect of sampling error to be differentiated from a true bottleneck. The moderately low diversity in mountain hemlock might also be partly attributed to genetic depauperacy of southern refugial populations (CWNYAR & MACDONALD 1987), but cannot be documented from our data.

Unlike *T. canadensis*, where 14 % of the variation was found among populations (ZABINSKI 1992), moun-

Table 4. Estimates of multilocus outcrossing rate (t_m), single-locus outcrossing rate (t_s), parental inbreeding coefficients (F) and correlation of paternity among siblings (r_p).

Population	t_m	t_s	F	r_p
Sooke	0.992 (0.021)	0.989 (0.031)	0.032 (0.326)	0.029 (0.035)
North San Juan	0.958 (0.035)	0.944 (0.043)	0.080 (0.138)	0.096 (0.075)

Table 5. Levels of genetic variation in different ecological and life history categories. (N_a = mean number of alleles, *%P* = percent polymorphic loci, H_T = total heterozygosity, G_{st} = genetic diversity among populations).

Species	N_a	*%P*	H_T	G_{ST}	Reference
Tsuga mertensiana	*1.6*	*33*	*0.093*	*0.077*	*current study*
Tsuga canadensis	–	10	0.04	0.14	(ZABINSKI 1992)
Long lived woody perennial	1.8	49	0.148	0.084	(HAMRICK *et al.* 1992)
Outcrossing, wind pollinated	1.8	53	0.154	0.077	(HAMRICK *et al.* 1992)
Widespread range	2.6	74	0.228	0.033	(HAMRICK *et al.* 1992)
Gymnosperm	1.8	53	0.281	0.073	(HAMRICK *et al.* 1992)
Late-successional status	1.7	48	0.146	0.080	(HAMRICK *et al.* 1992)

tain hemlock genetic differentiation was only 7.7 %, a value similar to found for long lived woody perennials (8.4 %) and outcrossed conifers (7.7 %) but higher than that found for widespread species (3.3 %; HAMRICK *et al.* 1992). Indirect estimates of gene flow derived from G_{st} values appear quite high, suggesting a value of approximately 3.8 migrants per generation. As a late-successional, shade-tolerant species, gene flow via pollen might be expected to be low. A recent expansion in a species' distribution may create the effect of homogenizing genetic differences over a large geographic scale even if current levels of gene flow are restricted. This violates the assumption of equilibrium between migration and drift in the gene flow estimator (WHITLOCK & MCCAULEY 1999).

While a southern refugium seems the most likely colonizing source for mountain hemlock, the Queen Charlotte Islands in coastal British Columbia has been suggested as a glacial refugium for Sitka spruce (*Picea sitchensis*, SOLTIS *et al.* 1997). It is possible that Mayo Creek and Hanna Ridge were descended from refugial populations in the Queen Charlotte Islands. However, these populations nest within other Central Coast B.C. populations in the dendrogram (Fig. 3), suggesting a single southern refugium for mountain hemlock.

We found no evidence of a recent bottleneck, as most populations showed significant *deficiences* of heterozygosities instead of *excesses* as expected with bottlenecks using the test of CORNUET & LUIKART (1996). Interestingly, CORNUET & LUIKART (1996) noted that populations increasing in size from small N_e tend to have loci with the opposite expectation – a heterozygote *deficiency*, as the relaxed genetic drift tends to favor the accumulation of rare alleles. Indeed, we did find such deficiency in the majority of populations, and many of these populations have rare alleles.

The correlation of population variability with latitude and elevation (Figure 2) may be an indication of local adaptation and selection. Geographical trends were found in two previous studies of mountain hemlock where adaptive and quantitative traits were investigated (BENOWICZ & EL-KASSABY 1999). As well, significant correlations between latitude and seed weight and germination capacity were found in another study (EDWARDS & EL-KASSABY 1998). Increasing average heterozygosity and total number of alleles with increasing elevation may be a consequence the more variable environments of higher elevations. Climatic changes are more extreme with increasing elevation, and topography more complex; higher levels of heterozygosity may serve an adaptive role allowing mountain hemlock to grow in geographically diverse habitats.

An alternative explanation to the altitudinal trend of heterozygosity and allelic diversity may be related to a predicted shift in the upper and lower boundaries of the mountain hemlock zone, within which new hemlock forests become established after disturbance (MEANS 1990). Recent increases in growing season temperature have resulted in an increase in productivity by 60 % in four high-elevation stands in Washington which contain 48–96 % mountain hemlock. It has been hypothesized if mean annual temperature increases 2.5 °C, the mountain hemlock zone will shift upwards in elevation (>570 m) but experience a decrease in area from 9 to 2 percent (GRAUMLICH *et al.* 1989).

The mating system observed for mountain hemlock in this study is remarkably outbreeding. Outcrossing rates were close to unity, and progeny descended from the same mother almost always had different fathers (Table 4). This suggests wide dispersal of pollen, as well as effective mechanisms for preventing selfing, such as differential timing of maturation for male vs. female reproductive structures. Indeed, the low values of G_{st}, are compatible with this evidence for wide pollen dispersal.

However, despite low values of G_{st}, localized seed migration has the potential to create local spatial patterns of genetic structure (EL-KASSABY & YANCHUK 1994, Namkoong and GREGORIUS 1985). Unlike its counterpart, western hemlock (*T. heterophylla*), whose seeds travel 1.6 km in a strong wind (ISAAC 1930), seed

flight of mountain hemlock is shorter and more likely to promote local family structure and the accumulation of local genetic differences (EDWARDS & EL-KASSABY 1996, ALLY & RITLAND *unpublished.*). Even at the regional level, our isolation-by-distance analysis indicates the effects of localized gene flow, namely a decrease of relatedness with distance. However, at the local level, among adjacent populations or within individual forest stands, where individual relatedness is more dynamic and affected just a few generations of dispersal, traditional isolation-by-distance methods (SLATKIN 1993, ROUSSET 1997) may not detect obvious genetic structure. We are currently developing methods for estimating isolation-by-distances using extremely local patterns of relatedness in conifer populations (ALLY & RITLAND *unpublished.*).

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Appendix A. Allozyme frequencies for the eighteen coastal and one interior, Sale Mt. Mountain hemlock populations from British Columbia.

Locus	Allele	Population									
		A	B	C	D	E	F	G	H	I	J
Fest-2	1	0.775	0.825	0.775	0.725	0.650	0.700	0.950	0.700	0.725	0.725
	3	0.225	0.175	0.225	0.275	0.350	0.300	0.350	0.300	0.275	0.275
Idh	1	0.900	0.775	0.850	0.925	0.550	0.625	0.875	0.875	0.800	0.975
	2	–	–	–	–	0.100	–	–	–	–	–
	3	0.100	0.225	0.150	0.075	0.350	0.350	0.125	0.125	0.200	0.025
Pgm-1	1	0.900	0.825	0.950	0.927	0.850	0.825	0.875	0.875	0.900	0.950
	2	0.025	0.050	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000
	3	0.025	0.100	0.050	0.073	0.050	0.150	0.000	0.125	0.100	0.050
	5	0.050	0.025	0.000	0.000	0.100	0.000	0.075	0.000	0.000	0.000
Pgm-2	1	0.975	0.975	1.000	0.925	1.000	1.000	0.950	0.975	0.975	1.000
	3	0.025	0.025	0.000	0.075	0.000	0.000	0.050	0.025	0.025	0.000
Pgi-1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.975	1.000
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000
Pgi-2	1	0.925	0.925	0.950	0.975	1.000	1.000	0.900	1.000	0.975	1.000
	2	0.025	0.025	0.000	0.025	0.000	0.000	0.050	0.000	0.025	0.000
	3	0.050	0.050	0.050	0.000	0.000	0.000	0.050	0.000	0.000	0.000
6pgd-2	1	1.000	0.925	0.950	0.875	1.000	1.000	1.000	1.000	1.000	0.850
	2	0.000	0.075	0.050	0.125	0.000	0.000	0.000	0.000	0.000	0.150
Gdh	1	0.875	1.000	1.000	1.000	0.975	1.000	1.000	0.975	1.000	1.000
	2	0.050	0.000	0.000	0.000	0.025	0.000	0.000	0.025	0.000	0.000
	3	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	5	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Skd-1	1	1.000	1.000	1.000	0.975	0.925	0.800	1.000	1.000	0.875	0.925
	2	0.000	0.000	0.000	0.000	0.000	0.150	0.000	0.000	0.075	0.075
	3	0.000	0.000	0.000	0.025	0.025	0.025	0.000	0.000	0.000	0.000
	5	0.000	0.000	0.000	0.000	0.050	0.025	0.000	0.000	0.050	0.000
Skd-2	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Lap-1	1	0.975	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Lap-2	1	0.975	0.950	1.000	0.950	0.975	1.000	0.975	0.925	0.950	1.000
	2	0.025	0.050	0.000	0.050	0.025	0.000	0.025	0.075	0.050	0.000
Mdh-1	1	1.000	1.000	1.000	1.000	1.000	0.975	1.000	0.975	1.000	1.000
	3	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.025	0.000	0.000
Mdh-2	1	0.825	0.850	0.900	0.875	0.800	0.700	1.000	0.650	0.925	1.000
	2	0.075	0.100	0.025	0.050	0.025	0.200	0.000	0.000	0.000	0.000
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	4	0.100	0.050	0.075	0.075	0.175	0.100	0.000	0.350	0.075	0.000
Mdh-3	1	0.925	0.900	0.925	0.900	0.925	0.975	0.900	0.875	0.975	1.000
	2	0.000	0.000	0.000	0.025	0.075	0.000	0.100	0.000	0.000	0.000
	3	0.075	0.100	0.075	0.075	0.000	0.025	0.000	0.125	0.025	0.000

Appendix A. Allozyme frequencies for the eighteen coastal and one interior, Sale Mt. Mountain hemlock populations from British Columbia.

Locus	Allele	Population								
		K	L	M	N	P	Q	R	S	O
Fest–2	1	0.825	1.000	0.825	0.600	0.700	0.650	0.700	0.800	1.000
	3	0.175	0.000	0.175	0.400	0.300	0.350	0.300	0.200	0.000
Idh	1	0.600	0.500	0.725	0.900	0.900	0.625	0.700	0.775	0.625
	2	0.025	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000
	3	0.375	0.500	0.275	0.100	0.100	0.375	0.275	0.225	0.375
Pgm–1	1	0.875	1.000	0.925	0.775	0.925	0.825	0.825	0.825	1.000
	2	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	3	0.100	0.000	0.075	0.200	0.025	0.050	0.150	0.125	0.000
	5	0.000	0.000	0.000	0.025	0.050	0.125	0..025	0.050	0.000
Pgm–2	1	1.000	1.000	1.000	1.000	0.975	0.925	1.000	1.000	1.000
	3	0.000	0.000	0.000	0.000	0.025	0.075	0.000	0.000	0.000
Pgi–1	1	1.000	1.000	1.000	1.000	0.975	0.900	1.000	1.000	1.000
	3	0.000	0.000	0.000	0.000	0.025	0.100	0.000	0.000	0.000
Pgi–2	1	0.925	1.000	1.000	1.000	0.925	0.975	0.925	0.950	1.000
	2	0.025	0.000	0.000	0.000	0.075	0.000	0.075	0.050	0.000
	3	0.050	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000
6pgd–2	1	1.000	1.000	1.000	0.975	0.750	0.950	1.000	0.925	1.000
	2	0.000	0.000	0.000	0.025	0.250	0.050	0.000	0.075	0.000
Gdh	1	1.000	1.000	0.975	1.000	1.000	1.000	0.975	1.000	1.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000
	3	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000
	5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Skd–1	1	0.950	1.000	0.950	0.875	0.975	0.950	0.950	0.925	1.000
	2	0.050	0.000	0.025	0.000	0.000	0.025	0.050	0.025	0.000
	3	0.000	0.000	0.025	0.000	0.025	0.025	0.000	0.025	0.000
	5	0.000	0.000	0.000	0.125	0.000	0.000	0.000	0.000	0.000
Skd–2	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Lap–1	1	1.000	0.975	1.000	1.000	1.000	1.000	0.975	1.000	1.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000
	3	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Lap–2	1	0.950	0.550	1.000	1.000	1.000	0.975	1.000	0.950	1.000
	2	0.050	0.450	0.000	0.000	0.000	0.025	0.000	0.050	0.000
Mdh–1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh–2	1	0.700	1.000	0.825	0.800	0.825	0.975	0.825	0.975	0.700
	2	0.075	0.000	0.000	0.000	0.000	0.000	0.100	0.025	0.000
	3	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.100
	4	0.225	0.000	0.175	0.150	0.175	0.025	0.075	0.000	0.200
Mdh–3	1	0.925	1.000	0.925	1.000	1.000	0.875	0.975	0.975	1.000
	2	0.000	0.000	0.000	0.000	0.000	0.100	0.025	0.025	0.000
	3	0.075	0.000	0.075	0.000	0.000	0.025	0.000	0.000	0.000

Appendix A. Allozyme frequencies for the eighteen coastal and one interior, Sale Mt. Mountain hemlock populations from British Columbia.

Locus	Allele	Population									
		A	B	C	D	E	F	G	H	I	J
Aco–1	1	1.000	0.975	1.000	1.000	1.000	1.000	0.975	1.000	0.950	1.000
	2	0.000	0.025	0.000	0.000	0.000	0.000	0.025	0.000	0.025	0.000
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000
Aat–1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Aat–2	1	0.975	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Aat–3	1	1.000	1.000	0.975	1.000	1.000	1.000	1.000	0.975	0.975	1.000
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.025	0.000
	5	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		K	L	M	N	P	Q	R	S	O	
Aco–1	1	0.975	1.000	1.000	1.000	0.975	0.950	1.000	1.000	1.000	
	2	0.025	0.000	0.000	0.000	0.025	0.050	0.000	0.000	0.000	
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
Aat-1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
Aat–2	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
Aat–3	1	1.000	1.000	1.000	1.000	0.975	0.975	0.875	1.000	1.000	
	2	0.000	0.000	0.000	0.000	0.025	0.025	0.000	0.000	0.000	
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.125	0.000	0.000	
	5										