SPATIAL GENETIC STRUCTURE IN MOUNTAIN HEMLOCK (*TSUGA MERTENSIANA*)

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PREFACE

Materials presented in Chapter 2 have been published in Forest Genetics 7(2): 97-108, 2000. Co- authors on this paper include Dr. Yousry A. El-Kassaby and Dr. Kermit Ritland. Shirley Barnes, a technician, generated the isozyme data set; and the thesis author performed all analyses and wrote the manuscript. Editorial assistance was provided by the co-authors.

Dr. Yousry A. El-Kassaby

Dr. Kermit Ritland:

ABSTRACT

The spatial distribution of related and unrelated alleles at a geographic, population, or local scale can unravel the relative roles of random genetic drift, mutation, and natural selection in the maintenance of genetic variation. Characterizing spatial and temporal patterns of genetic variation, and the underlying mechanisms, is central to understanding evolutionary and ecological processes like speciation, succession, and the spread and maintenance of a species. Equally important, is knowing how these patterns of variability change with physical scale and how the same processes differentially affect distinct spatial scales. The first part of this study examines mating systems, level of genetic diversity and the distribution of that genetic variation within and among populations of mountain hemlock (*Tsuga mertensiana*) across British Columbia. The second half of the thesis examines the effects of fragmentation on the demographic structure at the local scale, that is, within a single old growth stand of mountain hemlock. Levels of genetic diversity, inbreeding and isolation by distance are also examined in the natural regeneration surrounding the clearcut.

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 overall populations, which is approximately half the heterozygosity found in other conifers. Outcrossing rates did not significantly differ from 100%. Overall, populations of mountain hemlock across British Columbia 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 suggested gene flow was not restricted. 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. The low genetic diversity suggests that during a northward post-glacial range expansion, more northerly mountain hemlock populations suffered a loss in genetic variation due to this migration.

In an old growth mountain hemlock population in southern British Columbia, genetic diversity and relatedness were examined using two microsatellite loci. Levels of inbreeding were found to be significantly different from zero, and increased exponentially to an asymptote as mean diameter increased. High levels of inbreeding may have resulted from family clustering, the presence of null alleles, and spatial or temporal Wahlund effects. The difference in inbreeding levels between trees established before and after fragmentation may be suggestive of a temporal shift in breeding system. Seedlings from the 0-2cm diameter class (post-fragmentation) were found to have the highest genetic diversity and lowest levels of inbreeding. Seedlings and adults also differed in their allelic distribution suggesting that seedlings were not simply a subset of the neighbouring adult gene pool. The 1976 clearcut of 43.3 ha surrounding the old growth patch may have substantially altered the density of trees causing a change in wind currents, subsequently enhancing gene flow. Significant relatedness was detected among adult trees 5m apart and in the clearcut between seedlings 2m apart. Both natural regeneration and adult trees exhibited significant isolation by distance when pairwise estimates of relatedness were plotted as a function of increasing pairwise distance. The local genetic structure in mountain hemlock can be attributed to limited seed dispersal, seedling recruitment over a long period of time and long distance founding events.

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CHAPTER 1: GENERAL INTRODUCTION

The spatial distribution of related and unrelated alleles at a geographic, population, or local scale can unravel the relative roles of random genetic drift, mutation, and natural selection in the maintenance of genetic variation. Characterizing spatial and temporal patterns of genetic variation, and the underlying mechanisms, is central to understanding evolutionary and ecological processes like speciation, succession, and the spread and maintenance of a species (Wright 1943, Levin 1992). Equally important, is knowing how these patterns of variability change with scale and how the same processes differentially affect distinct scales. In plant populations, this evolutionary dynamic is initiated by the mating system and the mechanisms of dispersal, which ultimately decide the spatial scale over which forces like genetic drift and natural selection can act (Kalisz 1999). In the first part of this study I examine: the mating systems, level of genetic diversity, and the distribution of that genetic variation within and among populations of mountain hemlock (*Tsuga mertensiana* (Bong.), Carr.: Pinaceae) across British Columbia. As a backdrop to Chapter two, I review geographic patterns of genetic variation in conifers by examining F_{ST}, a measure of differentiation, and the potential ecological or evolutionary forces responsible for the distribution of genetic variation. The second half of the thesis examines genetic structure at the local scale, that is, within a single old growth stand of mountain hemlock. Hence, as a framework for the third chapter, I review local patterns of genetic structure in conifers, explain the measure of relatedness used in this study, and examine potential forces responsible for the observed patterns of relatedness.

Geographic patterns of genetic structure

Geographic spatial structure can be defined as the non random distribution of alleles or genotypes in space or time (Epperson 1992, Loveless and Hamrick 1984). Structure or

differentiation results when allele frequencies differ among populations. These differences in allele frequencies among populations may be the result of a combination of limited gene flow, stochastic processes during inheritance of genes from generation to generation, small population sizes during the initial founding of the population, or diversifying selection favouring different genotypes (Hartl and Clark 1997).

A measure of geographic structure, Fst

It is common practice to quantify genetic differentiation using an estimate of the parametric value F_{st} introduced in the island model of Wright (1921, 1943). This model assumes that a species or population is subdivided into an infinite number of randomly breeding islands, each with an effective population size N_e. Each of these 'islands' exchanges migrants at a rate of m every generation and all populations are equally likely to give and receive migrants from all other populations. Populations are assumed to have reached equilibrium between gene flow and random genetic drift with no selection, and the mutation rate, μ, is assumed to be much smaller than the migration rate, m. To explain the relationship between the number of migrants a population receives per generation and the correlation between randomly chosen alleles in a subpopulation relative to alleles in the whole population, Wright (1943) found upon attainment of a steady state that $F=(1-m)^2/[(2N-(2N-1)(1-m)^2]$. This simplifies to the elegant and well-known equation, $F_{st}=1/(4Nm + 1)$ for small values of m and large values of N. The quantity F_{st} is the ratio of the actual variance in allele frequency, V(q), among subpopulations to its maximum value q (1-q) and is affected primarily by intermediate allele frequencies (Wright 1969). Recently, Whitlock and McCauley (1999), and Bossart and Prowell (1998), reviewed the fallacies associated with applying the island F_{st} model to determine indirect estimates of gene flow hence to avoid repetition, I will not review these here.

Geographic patterns of variation in conifers

A review of the plant literature (Hamrick et al. 1979, Hamrick et al. 1992) concluded that long-lived woody plants maintain high levels of variation within species and within populations, but relatively less genetic diversity among populations. A later study examined which life history and ecological features are associated with differences in levels of genetic variation, and the distribution of that variation in different plant species (Hamrick and Godt 1996). Of the ecological traits examined by Hamrick and Godt (1996) breeding system and seed dispersal mechanisms were found to explain most of the differences among species. Outcrossed species with wind-mediated seed dispersal had lower values of G_{st} (0.101) than predominantly self-fertilized species with a gravity-mediated seed dispersal (G_{st}=0.533). This pattern clearly demonstrates the homogenizing effect of migration and illustrates one of the seminal predictions of Wright's F_{st} theoretical model. One immigrant per generation ($Nm \geq 1$) is sufficient to prevent fixed differences in allele frequencies (Hartl and Clark 1997). Additionally, it was found that families of long-lived perennials, like the Pinaceae, had a lower level of among population differentiation (Gst=0.073) than families of predominantly herbaceous species (Asteraceae: Gst=0.204) (Hamrick and Godt 1996). This points to key features in temperate conifers that may account for the generally low levels of among population variation. High fecundities, low rates of selfing, and widespread distributions potentially lead to large stable effective population sizes resistant to chance fluctations in allele frequencies (Hamrick et al. 1992b). Long-lived organisms exhibit non-overlapping generations which may retard the loss of genetic variation because of the continued survival of polymorphisms from one generation into the next. Under the drift model, there is a loss of genetic variation at a rate of 1/Ne per generation, but in species with overlapping generations heterozygosity is lost at a slower rate (Hartl and Clark 1997).

Local patterns of genetic structure

Fewer studies examine family structure or the spatial patterning of related individuals within a single population or single stand (Linhart et al. 1981, Furnier et al. 1987). Results seem to suggest that long-lived, predominantly outcrossed, and wind-pollinated woody species show relatively little subpopulation structuring. However, observed patterns of spatial genetic variation indicate average total kinship between individuals is a decreasing function of distances of separation (Epperson 1992).

Characterization of the spatial distribution of related genotypes over the two-dimensional space of a stand includes examination of genealogical between individual trees relationships (Thompson 1975, Meagher and Thompson 1986), correlation of uniting alleles or fixation indices (Wright 1943), spatial autocorrelation (Epperson 1992), and number of alleles in common (Surles et al. 1990, Hamrick et al. 1993). Historically the estimation of relationship between pairs of individuals has been based on genetic likelihoods of parent-offspring pairs, full-sibs and half-sibs pairs, these measures are difficult to apply to field populations where parentage cannot be directly observed (Lynch and Ritland 1999). The practice of reconstructing parent-offspring, full-sib or half-sib pairs using likelihood requires large numbers of polymorphic loci because the estimate is biased when only a few loci are used (Thompson 1975). Recently, Ritland (1996), and Lynch and Ritland (1999) have developed pairwise estimators of relatedness, which are employed in the third chapter and thus will be examined here.

A measure of relatedness, r_{xy}

Relatedness is based on the concept of identity by descent, alleles are *identical by descent* (IBD) if they are copies of a common ancestral gene. This is unlike genes *identical in*

state (IBS) where although the alleles possess exactly the same nucleotide sequence, they are derived from different copies of the same gene. If alleles are identical by descent, they must also be the same allele or *identical in state* as it is assumed mutations do not occur (Lynch and Walsh 1998). The relationship between two individuals has been characterized by the probabilities of identity by descent of their genes (Jacquard 1974). These probabilities are represented by nine condensed coefficients of identity which provide a comprehensive description of all possible ways that two alleles can be identical by descent between loci in two individuals. These probability distributions are then summarized in a measure called the 'coefficient of coancestry', Θ_{xy}, (a.k.a coefficient of consanguinity, kinship). The coefficient of coancestry is defined as the probability that the two alleles will be identical by descent if they are randomly drawn from the same locus in two different individuals. Defined as such, the coancestry of two individuals is the same as the inbreeding coefficient of their progeny (Jacquard 1974). The relatedness coefficient, r_{xy}, is described as $2\Theta_{xy}$ and is a linear function of two other coefficients Φ_{xy}; the probability that a single allele in one individual *x* is IBD with another allele in the second individual *y*, and Δ_{xy}; the probability that each of the two genes in *x* is IBD with those in *y* (Lynch and Ritland 1999). It is these last two relationship coefficients, Φ_{xy} and Δ_{xy}, which are estimated by the Lynch and Ritland regression model (1999). Since the model is clearly described in Lynch and Ritland (1999), I will briefly discuss the properties of this model in relation to two different types of molecular markers used in the current study - isozymes and microsatellites.

Relatedness is determined by the sharing of gene identity and since identity is determined by the probabilities of a model, assumptions of that model must be met in order to apply it to real data. Although the regression estimator of relatedness is unbiased regardless of the numbers of loci, sampling variance is affected by numbers of unlinked loci, number of

alleles per locus, and the type of allele frequency distributions in the data (Lynch and Ritland 1999). Since confidence limits are influenced by the magnitude of the variance, the intrinsic properties of isozymes and microsatellites can affect the reliability of the sample estimate of relatedness. Unlike the few published investigations of conifer microsatellites (e.g: Smith and Devey 1994, Pfeiffer et al. 1997, Echt et al. 1999), isozyme studies are numerous and can be found for almost every organism. The genetic and structural background of a number of enzymes has been well characterized and there are currently more than 300 enzyme loci resolved (Hillis et al. 1996). The single locus sampling variance has been found to decline with increasing numbers of unlinked loci (Lynch and Ritland 1999), an advantage that isozymes provide. However, due to higher mutation rates the level and degree of polymorphism in microsatellites is far greater than allozymes. The higher number of alleles per locus reduces the sampling variance and makes the relatedness estimate more reliable. Additionally, the triangular nature of the allele frequency distributions in microsatellites includes a wide range of frequencies, and rare alleles which when identical in state, are more faithful predictors of identity by descent (Lynch and Ritland 1999).

Local patterns of relatedness in conifers

Some studies indicate that weak family structure (some neighbouring trees are genetically similar) can arise in conifers despite wind-mediated seed dispersal (Sakai and Park 1971, Linhart et al. 1981, Furnier et al. 1987, Shea 1990, Tani et al. 1998, Ueno et al. 2000) while others have found no strong evidence of structure (Epperson and Allard 1984, Knowles et al. 1992, Leonardi et al. 1996). The studies which detected genetic structure examined variation at smaller spatial scale (e.g. Shea 1990). At this smaller spatial scale, familial clumping of seedlings is largely governed by the mechanism and amount of dispersal per generation, but is

also influenced by sib-competition, microenvironmental heterogeneity, density, morphological characteristics of seeds and pollen, phenology, and proportion of sexual to asexual propagation. Inferring both the degree and distribution of genetic relatedness among individuals in a natural population can provide insight on short term drift, local dispersal, and microhabitat selection.

Wright's model of isolation by distance (1942, 1946), which examines the dependence of mating probabilities on the distance between individuals, has been frequently employed to ascertain effects of short term genetic drift on genetic structure in forest communities. Under this model, pairwise estimates of coancestry or relatedness should decline gradually for individual pairs located at increasing distances from each other. For the most part, previous studies (Streiff et al. 1998, Tani et al. 1998, Ueno et al. 2000) which have observed weak to moderate levels of spatial genetic structure, detected these genetic correlations at very short distance classes, usually within the first 10m, with declining relatedness as the distance interval increased. Higher density populations can reduce pollen and seed dispersal and lead to near neighbour relationships giving rise to semi-isolated patches (El-Kassaby and Jaquish 1996). Additionally, non-random associations of alleles within short distances could also be attributed to local genetic drift if only a few reproductively dominant trees contributed to the next generation's gene pool.

Many forest species exhibit stronger genetic structure in seeds or seedlings with a significant decrease in subsequent age classes. It is hypothesized that either inbred offspring die or sibling competition results in the weak genetic structure found in adults. One notable example is demonstrated by the conifer *Pinus sylvestris* where the inbreeding coefficient was found to be 0.124 in seeds, 0.007 in 10-20yrs old trees and -0.046 in individuals greater than 20 years of age (Yadzani et al. 1985). Short-distance spatial genetic structure is also thought to be maintained by microhabitat heterogeneity. One study found pronounced genetic heterogeneity

within 2 ha. among family units of ponderosa pine and was strongly associated with cone and pollen production, aphid distribution, and deer browsing (Linhart et al. 1981).

CHAPTER 2: GENETIC DIVERSITY, DIFFERENTIATION AND MATING SYSTEM IN MOUNTAIN HEMLOCK (*TSUGA MERTENSIANA*) ACROSS BRITISH COLUMBIA

Most species of conifers are long-lived, predominantely outcrossing, wind-pollinated, wind-dispersed, and usually have large geographic ranges. Levels of genetic variation are high, in conifers and populations show little genetic differentiation (Hamrick et al. 1992, Hamrick and Godt 1996), conforming to expectations under equilibrium 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 as a result of repeated long distance founding events during post-glacial expansion (Cwynar and MacDonald 1987). Thus, against a background of life-history and mating system attributes of a species, population history can significantly contribute to the genetic structure of a species.

Mountain hemlock (*Tsuga mertensiana* (Bong.) Carr.) grows in the subalpine coastal and interior forests of British Columbia and Alaska. Found within a region between 300 to 1000m 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*), Engelmann 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 750cm 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 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 diversity, 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. Firstly, mountain hemlock is wind-pollinated and monoecious, but of the North American hemlocks, it has the second largest seeds, second only to Carolina hemlock (*T. carolianana* (Engelm.)). This large seed size may decrease dispersal distance, increasing the opportunity for family structure and local differentiation of populations (Edwards and El-Kassaby 1996). Secondly, 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 southwestern British Columbia 12,400-10,500 B.P. (Mathewes 1973, Wainman 1987) and in southeastern Alaska ca. 6,700 years B.P.(Cwynar 1990).

The objectives of the present study are 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 (Sooke and North San Juan). 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.

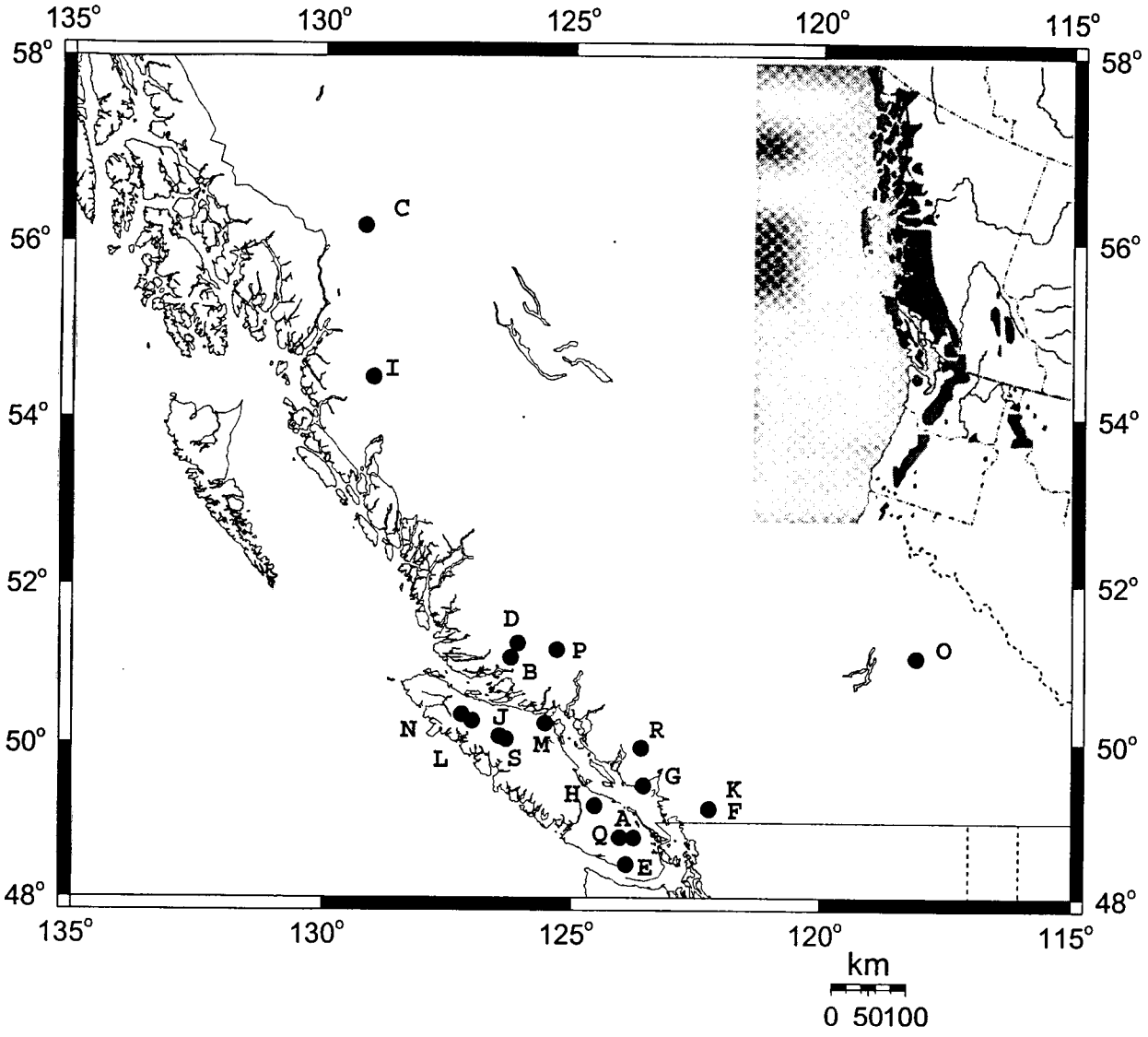
Materials and Methods

Seeds from 19 populations, representing the range of mountain hemlock in British Columbia, were collected. Their locations are given in Figure 1, and elevations are noted 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 megagametophyte tissues (1n) were then randomly sampled for each of the 19 populations. Enzyme variation has been historically examined by sampling bulked seedlots and using haploid tissues to interpret allele differences (El-Kassaby 1991). Seeds are harvested from a sample of trees in a single location and then pooled to form a bulked seedlot. From this seed pool, representative of the population, a random sample of 40 seeds are taken. Seeds were removed after seed hydration for 24 hrs and proteins were extracted using a slightly modified extraction buffer of Cheliak and Pitel (1984). Horizontal 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 (G6PDH), glutamate dehydrogenase (GDH), aconitase (ACO), phosphoglucomutase (PGM), phosphoglucose isomerase (PGI), shikitimate dehydrogenase (SKD), leucine-aminopepitase (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

Table 1. Population codes, elevations and geographic coordinates for nineteen populations sampled across British Columbia.

Population Name	Code	Elevation (m)	Latitude (N)	Longitude (W)
Meade Creek	A	1067	48.55	124.05
Wakeman High	B	1100	51.10	126.25
Hanna Ridge	C	700	56.18	129.20
Wakeman Low	D	600	51.17	126.17
Garbage Creek	E	850	48.33	124.06
Mission	F	900	49.18	122.24
Lyon Lake	G	1005	49.39	123.54
Parksville	H	824	49.16	124.33
Mayo Creek	I	683	54.47	129.02
Zeballos	J	700	50.10	126.47
Kearsley Creek	K	1280	49.19	122.22
Blue Ox Creek	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 Creek	P	1250	51.20	125.32
Copper Canyon	Q	1100	48.56	124.13
Ashly Creek	R	1000	50.01	123.33
Woss Lake	S	900	50.07	126.35

Figure 1. Locations of 19 sampling populations of mountain hemlock from British Columbia. See codes in Table 1. (*Inset*) The range of mountain hemlock.



were: lithium borate pH 8.3@250 volts (Ridgeway et al. 1970); morpholine citrate pH 8@200 volts (Clayton and 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 and North San Juan). In each population, seeds were collected from 20 individual trees spaced approximately 2-3 tree heights apart. In the two populations sampled for progeny arrays, 40 seeds per mother tree were extracted and six loci (PGI, IDH, PGM, 6PG, MDH2, MDH3) were examined.

Allozyme variation was analyzed using BIOSYS-2 (Swofford 1981, Black and Krafsur 1985), and GDD (Ritland 1989) with the following values computed: allele frequencies, average number of alleles per locus (N_a), percent polymorphic loci (%P), and expected heterozygosity under Hardy-Weinberg equilibrium (H_e). To investigate the extent of population structuring and differentiation, F_{st} (Wright 1969) and Nei's (1973) G_{st} were computed from BIOSYS-2 for individual loci of the 19 populations. Nei's genetic distance (1978) was computed between all populations. A dendrogram of genetic relationships among populations was constructed from these distances using the unweighted pair-group method, UPGMA (Sneath and Sokal 1973). The statistical significance of branches was 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 the computer program BOTTLENECK (Cornuet and Luikart 1996), which tests whether the observed number of alleles fits the heterozygosity expected under mutation-drift equilibrium. A bottleneck is expected to reduce the number of rare alleles and

thus 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 and Luikart 1996). Data were analyzed using the infinite-allele model (IAM).

The relationship between geographic distance, gene flow, and genetic differentiation was evaluated by regression of Nei's genetic distance on physical distance and by a regression of pairwise G_{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) were used to determine 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 1990b). Estimates were obtained of single locus (t_s) and multilocus (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-1-2). The number of alleles over all

Table 2. Genetic diversity measures for all 19 populations of mountain hemlock across British Columbia. Measures calculated were: mean number of alleles per locus (N_a), percent polymorphic loci (%P), expected average heterozygosity (H_e).

Population	Letter Code	N_a	%P	H_e
Meade Creek	A	1.9	36.8	0.092
Wakeman High	B	1.7	42.1	0.097
Hanna Ridge	C	1.5	31.6	0.065
Wakeman Low	D	1.6	42.1	0.084
Garbage Creek	E	1.6	31.6	0.106
Mission	F	1.7	31.6	0.122
Lyon Lake	G	1.4	26.3	0.068
Parksville	H	1.6	31.6	0.102
Mayo Creek	I	1.7	36.8	0.088
Zeballos	J	1.3	21.1	0.050
Kearsley Creek	K	1.7	42.1	0.106
Blue Ox Creek	L	1.2	10.5	0.056
Hkusam Mt.	M	1.4	31.6	0.075
Port Alice	N	1.4	26.3	0.086
Sale Mt.	O	1.2	10.5	0.050
Hoodoo Creek	P	1.6	31.6	0.095
Copper Canyon	Q	1.8	47.4	0.120
Ashly Creek	R	1.7	36.8	0.109
Woss Lake	S	1.6	36.8	0.082
Overall average		**1.58**	**32.0**	**0.087**

Table 3. Estimates of gene diversity for all populations, regional and elevational grouping for mountain hemlock. The measures used were the total heterozygosity (H_t), heterozygosity within populations (H_s), and among population genetic variation (G_{st}).

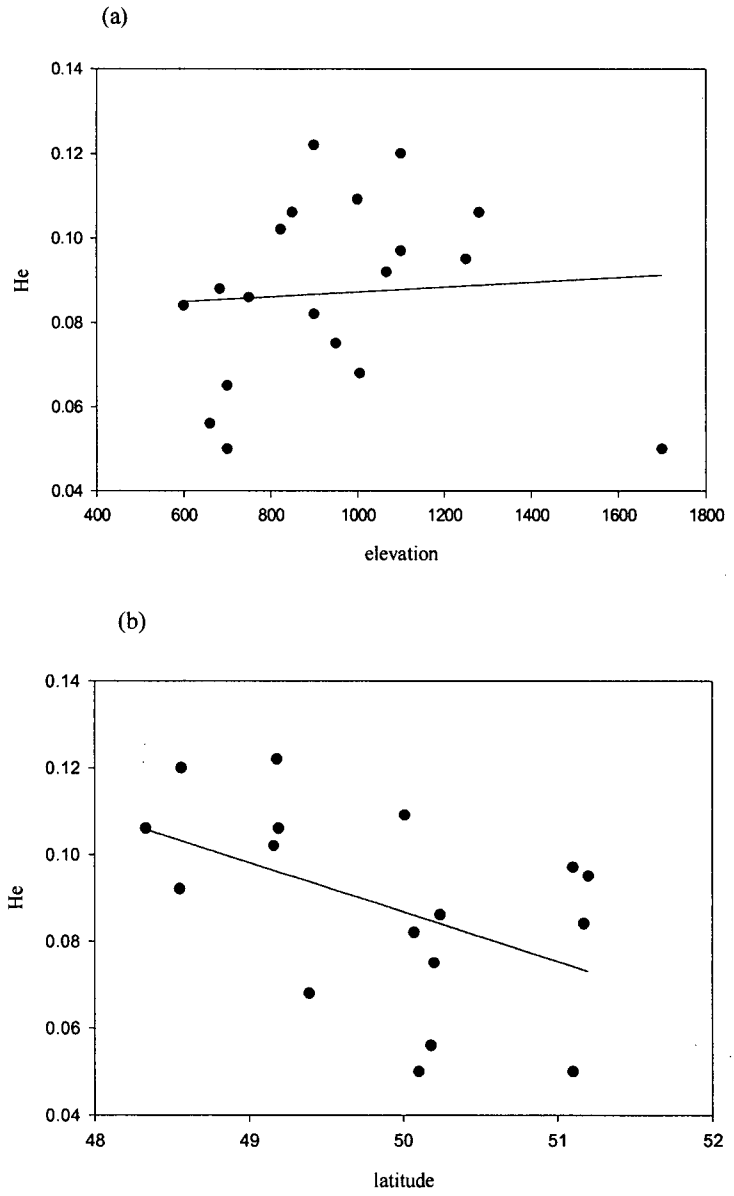
	H_t	H_s	G_{st}
All populations	0.0923	0.08553	0.07681±0.004
Island	0.09320	0.08429	0.09562±0.005
Mainland	0.09189	0.08655	0.05812±0.003
Elevations >1000m	0.09536	0.08976	0.05877±0.003
Elevations <1000m	0.09005	0.08208	0.08855±0.005

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 (Meade Creek) had a single private allele, while the second private allele was found in Blue Ox Creek, the population with the lowest number of alleles. The average number of alleles per locus was 1.6 and ranged from 1.2 to 1.9 (Table 2). 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. 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). Island populations (G_{st} = 0.096±0.005) showed more among-population variation than mainland populations (G_{st} = 0.058±0.003). Similarly, 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 illustrates the relationship between diversity and geography (latitude and elevation). There was no significant correlation between latitude and elevation with a Pearson's correlation coefficient of -0.24096 (p value=0.3203). By excluding the far outlying, interior population Sale Mt., the assocation between expected heterozygosity and elevation (p=0.028) is significant and explained 26.7% of the variation in heterozygosity. The outlier population (Sale Mt.) was found to be statistically influential with an absolute DFFITS value of 3.0952. A nearly statistically significant relationship was also found between elevation and percent polymorphic loci (r^2=0.208, p=0.0571), and elevation and average number of alleles per locus (r^2=0.255, p=0.0327). Expected heterozygosity showed a negative assocation with latitude (r^2=0.230, p=0.051), but two geographically distant outlier populations -- Mayo Creek and Hanna Ridge --

Figure 2. The relationship between geography and average expected heterozygosity (H_e) for coastal southwestern British Columbia. (a) A plot of H_e and elevation (m) with the inclusion of the interior population Sale Mt. This population was found to be a statistically influential outlier and was excluded from a simple linear regression analysis. The relationship was significant $p=0.028$, $r^2=0.267$. (b) A plot of latitude and H_e excluding Mayo Creek and Hanna Ridge. The simple linear regression was significant, $p=0.051$, $r^2=0.230$.



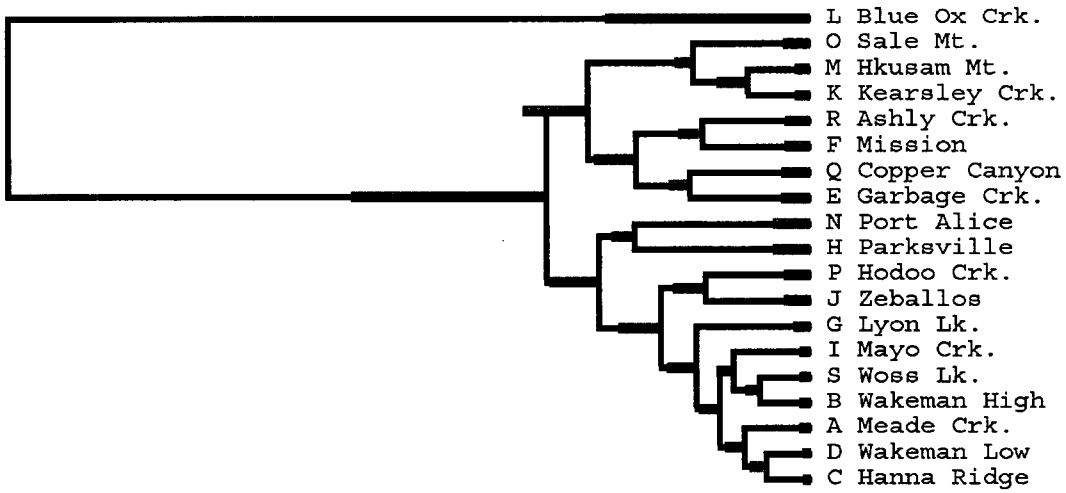
were omitted. In none of the regression analyses performed was there any evidence that the assumptions of simple linear regression were violated. The error terms were found to be normally distributed with no deviation from constant variance detected.

In the bottleneck analysis, two populations, Blue Ox Creek and Sale Mt., were left out because they did not have the required 5 polymorphic loci. Under the infinite alleles model, 9 of 18 populations showed a 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.

The dendrogram of genetic relationships among the 19 populations is shown in Figure 3. 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), which also exhibits the lowest gene diversity (Table 2). Clustering at the lowest level (pairs of populations) was significant in several cases (e.g.: Ashly Creek and Mission, Copper Canyon and Garbage Creek, Woss Lake and Wakeman High), and a somewhat geographically widespread group of three populations had a very strong cluster (Sale Mt., Hkusam Mt. and Kearsley Crk.). There is no significant 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 matrices (r =0.016) for all populations. However, the Mantel test did show a statistically significant relationship between genetic and geographic distance within the southern coastal populations (r = -0.214, p=0.010). Mantel tests conducted on island populations only found no significant relationships between physical distance and

Figure 3. A dendrogram depicting the relationships among all sampled populations of mountain hemlock in British Columbia. Significant clusters of populations in the dendrogram occur when branch length is at least twice the standard error bar, as indicated by the thicker line (Ritland 1989). The average genetic distance was 0.008, indicative of relatively low genetic differentiation. The distance matrix was calculated using Nei's (1978) standard genetic distance using a UPGMA algorithm to generate the dendrogram.



genetic distance matrices.

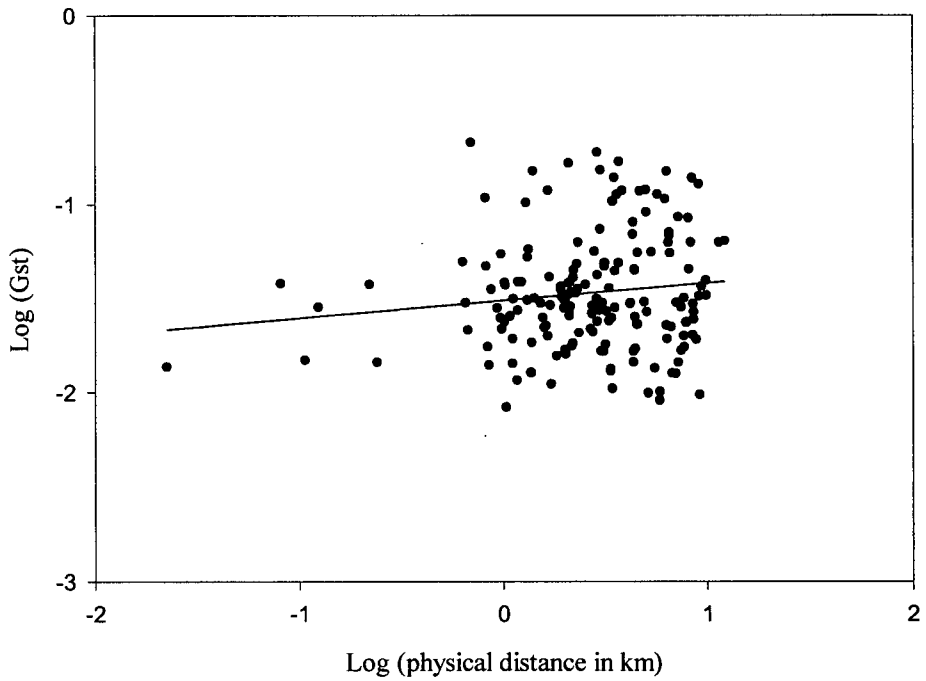
Inferred gene flow (Nm) between a pair of populations was not regressed against physical distance as proposed by Slatkin (1993), instead isolation by distance was determined by plotting log (G_{st}) against log (physical distance in km). As detailed by Whitlock and McCauley (1999), F_{st} is a function of a nonlinear relationship to Nm and hence in spatially heterogenous environments average values of Nm are understimated. No significant support was found for higher genetic differentiation as distance increased between pairs of populations for all 19 populations ($r^2 = 0.160$, $p=0.0988$) as shown in Figure 4. However, the power or probability that the model correctly describes a relationship between G_{st} and physical distance, was quite low (Power=0.3785; $\alpha=0.05$).

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). There was no evidence of biparental inbreeding, as single-locus estimates were essentially identical to the

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) for two populations of mountain hemlock. Standard errors for each estimate are presented in parantheses.

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)

Figure 4. The relationship between genetic differentiation (log G_{st}) and physical distance (log distance) for 19 sampled populations of mountain hemlock.



multilocus estimates (Table 4). Likewise, parental inbreeding coefficients were low and did not significantly differ from zero. Estimates of correlated matings obtained for both populations were quite low (Table 4) and did not differ significantly from zero (i.e., progeny were half-sibs). All sixteen Chi-squared tests performed on the progeny of heterozygous parents (inferred maternal genotypes) were not significant, confirming that isozymes in mountain hemlock are inherited in a Mendelian fashion.

Discussion

The level of genetic diversity in mountain hemlock, H_e=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 (H_e=0.04) was also found (Zabinski 1992). In mountain hemlock, the mean number of alleles (N_a=1.6) was slightly lower than what was expected from conifers as a taxonomic group (N_a=1.8) and much lower than species that show a widespread range (N_a=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. 1992a).

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 to 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. Cwynar and MacDonald (1987) explained a progressive decline in allelic diversity toward the northern periphery of lodgepole pine as the

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

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)

result of repeated long distance founding events. Although this pattern is suggested by our data, (Figure 2) larger sample sizes are needed to differentiate the effects of true bottleneck. The moderately low diversity in mountain hemlock might also be partly attributed to genetic depauperacy of southern refugial populations (Cwynar and MacDonald 1987).

Unlike *Tsuga canadensis*, where 14% of the variation was found among populations (Zabinski 1992), genetic differentiation in mountain hemlock was only 7.7%, a value similar to that 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. 1992b). Indirect estimates of gene flow derived from G_{st} values appear quite high, suggesting a value of approximately 3.8 migrants per generation. 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 and McCauley 1999).

While a southern refugium seems the most likely colonizing source for mountain hemlock, the Queen Charlotte Islands in coastal British Columbia have been suggested as a glacial refugium for Sitka spruce (*Picea sitchensis*, Soltis 1997) and many other species. 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 (Figure 3), suggesting a single refugium for mountain hemlock or a high degree of mixing following the last Ice Age.

We found no evidence of a recent bottleneck, as most populations showed significant *deficiencies* of heterozygosities instead of *excesses* as expected with bottlenecks using the test of Cornuet and Luikart (1996). Interestingly, Cornuet and 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 favour 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 may be an indication of local adaptation and selection. Geographical trends were found in a previous study of mountain hemlock where adaptive and quantitative traits were investigated (Benowicz and El-Kassaby 1999). As well, significant correlations between latitude and seed weight and germination capacity were found in another study (Edwards and El-Kassaby 1996). Increasing average heterozygosity and total number of alleles with increasing elevation may be a consequence of 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 because these populations are effectively sink populations and migrants make up a disproportionately large fraction of the gene pool.

The mating system observed for mountain hemlock in this study is predominantly outbreeding. Outcrossing rates were close to unity, and progeny descended from the same mother almost always had different fathers. 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 widespread pollen dispersal. Even without high levels of pollen flow, the low differentiation in most conifers can be explained by considering the effects of post-glacial colonization processes given the life cycle. Results from simulation studies on long-lived organisms with delayed reproduction have shown that differentiation among populations is small because the founder effect is limited. Given that mountain hemlock has a long non-reproductive juvenile period (20 –30 years), growth of a mountain hemlock population in the first years of establishment may be due to seed migration and not the reproduction of current individuals. This extensive seed migration increases the number of founders in the population decreasing the effect of genetic drift (Austerlitz et al. 2000).

However, despite low values of G_{st}, localized seed migration has the potential to create local spatial patterns of genetic structure (El-Kassaby and Yanchuk 1994, Namkoong and Gregorius 1985). Unlike its counterpart, western hemlock (*T. heterophylla*), whose seeds travel 1.6 km in a strong wind (Issac 1930), seed flight of mountain hemlock is shorter and more likely to promote local family structure and the accumulation of local genetic differences (Edwards and El-Kassaby 1996, see Chapter 3). Our isolation-by-distance analysis indicated that there were no effects of restricted gene flow with increasing geographic distance. However, at the local level, among adjacent populations or within individual forest stands, where individual

relatedness is more dynamic and affected by just a few generations of dispersal, traditional isolation-by-distance methods (Slatkin 1993, Rousset 1997) may not detect obvious microspatial genetic structure. Methods for estimating isolation-by-distances using local patterns of relatedness in conifer populations may provide insight into the role of local dispersal in the maintenance of genetic structure.

CHAPTER 3: THE EFFECTS OF FRAGMENTATION ON DEMOGRAPHIC SPATIAL PATTERNS OF GENETIC RELATEDNESS

Gene flow, as an evolutionary force has the potential to change the spatial dynamics and structure of a species. Migration directly impacts the distribution of genetic variation in a species, influences colonization, and can prevent extinction of populations through rescue events (Ouborg et al. 1999). Ultimately, gene flow plays a significant role in maintaining the integrity of a species. Historically and more recently the development of agricultural and forestry practices dramatically fragment continuous habitats of many species and alter patterns of gene flow. In the literature preference has been given to the ecological consequences of fragmented terrain (Agee and Smith 1984, Lovejoy et al. 1986, Wilcove et al. 1986, Young 1988, Kapos 1989, Saunders et al. 1991), but over the past decade more studies are beginning to focus on how changes in mating systems, pollen movement, and seed dispersal can impact spatial genetic structure (Fore et al. 1992, Murawski et al. 1994, Hall et al. 1996, Nason and Hamrick 1997, Adams et al. 1998, Dayanandan et al. 1999).

The reduction of habitats to small, isolated remnants will theoretically lead to the erosion of genetic variation by random drift at a geometric rate and to an increase in divergence among populations. However, empirical data on forest habitat fragmentation is not consistent with theoretical predictions on the loss of allelic diversity as a result of genetic drift. Prober and Brown (1994) found a very significant positive correlation between remnant size and genetic diversity in *Eucalyptus albens*, with smaller and more isolated remnants possessing lower genetic diversity than less isolated and larger remnant populations. In *Pithecellobium elegans* measures of genetic variation were the lowest in smaller fragments that were furthest from the continous reserve (Hall et al. 1996). Dramatic consequences were demonstrated in two other studies: a severe genetic bottleneck was found in *Symphonia globulifera* (Aldrich et al. 1998)

where seedlings in the remnant forest were produced by only a few adults; Hamilton (1999) detected single maternal lineages in 10 ha remnants of *Corythophora alta.*

However, many other studies have failed to find evidence of a loss of genetic diversity in the tropical trees *Carapa guianensis* (Dayanandan et al. 1999), *Shorea megistophylla* (Murawski et al. 1994), and *Swieteina humilis* (White et al. 1999) or in the temperate species *Acer saccharum* (Fore et al. 1992, Young et al. 1996), *Juniperus communis* (Van der Merwe et al. 2000), *Pseudotsuga menziesii* (Adams et al. 1998), *Pinus contorta* (Thomas et al. 1999), and *Larix laricina* (Knowles et al. 1992). In fact, in one study an increased level of disturbance seemed to result in a significant increase both in the effective number of alleles per locus and expected heterozygosity (*Pterocarpus macroscarpus),* while there was no discernible effect in *Dipterocarpus oftusifolius* or *Sindora siamensis* (*pers comm.* K. Chaisurisri).

Genetic diversity can also be affected by inbreeding following changes to a species' mating system either directly by modifications to the pollination system or indirectly by a reduction in density of reproductive trees. Inbreeding can arise from either self-fertilization, as a direct consequence of lowered density, or when remaining reproductive individuals share a common ancestor, as is more likely in small populations (El-Kassaby and Jaquish 1996, Young et al. 1996). Only a few studies have unequivocally demonstrated the effect of ecosystem degradation on mating system. Selective logging was found to reduce the multilocus outcrossing rate by 16% in *Shorea megistophylla* (Murawski et al. 1994) and 8% in *Shorea siamensis* (K. Chaisurisri *pers comm.*). However, logging did not affect the mating system of *P. macrocarpus*, *Sindora siamensis*, and *D. oftusifolius* (K. Chaisurisri *pers comm*). Two studies of the temperate conifers *Larix laricina* (tamarack) and *Picea mariana* (black spruce) found significant inbreeding coefficients in the natural regeneration founded after a clearcut for the

tamarack population, and fire in the case of black spruce (Boyle et al. 1990, Knowles et al. 1992).

Although several other studies have found significant inbreeding levels associated with fragmented forests (Aldrich et al. 1998, Dayanandan et al. 1999, Thomas et al. 1999, White et al. 1999), most were unable to tease apart inbreeding from the effect of null alleles. Null alleles occur when mutations or duplication/inversion events in the flanking region of the sequence repeat prevent amplification of an allele (Pemberton et al. 1995, Westman and Kresovich 1998). The presence of null alleles, if undetected, can result in a reduced number of heterozygotes assayed and inflate the inbreeding coefficient (Aldrich et al. 1998). Furthermore, the above-noted studies found significant fine scale spatial genetic structure in the seeds or seedling (significant inbreeding) with a loss structure (no inbreeding) at later life history stages. This age related heterogeneity may be the result of random mortality through self-thinning or competition among siblings. Typically, older cohorts of trees are found to have higher observed heterozygosities because balancing selection, which favours heterozygote survival and, acts to remove inbred individuals at earlier age classes (Yadzani et al. 1985, Smouse 1986, Muona et al. 1987, and Ritland 1990a). Finally, the inbreeding coefficient can be further inflated because of a spatial or temporal Wahlund effect either through fine scale spatial structuring or through the pooling of allele frequencies from different cohorts (Hartl and Clark 1997).

It is hypothesized that temperate zone tree communities are more resistant to the effects of habitat fragmentation than tropical communities because of higher population densities, widespread distributions, and wind-mediated dispersal mechanisms (Wilcove et al. 1986, Nason and Hamrick 1997). Tight coevolution between plants and pollinators, coupled with low densities of reproducing adults, make tropical forests more vulnerable to isolating effects of fragmentation (Nason and Hamrick 1997). With the exception of a few studies (Hall et al. 1996,

Ge et al. 1998, Hamilton 1999, and Van der Merwe et al. 2000), the majority of evidence points to the fact that gene flow is not necessarily reduced after fragmentation even though remnant populations become isolated. In fact the enhanced interfragment gene flow, found primarily in juveniles, seedlings, and seeds (Boyle et al. 1990, Fore et al. 1992, Knowles et al. 1992, Ballal et al. 1994, and Nason and Hamrick 1997) indicates that long distance pollen and seed flow is significant. For species dependant on animal-mediated pollen flow, the density of plants can affect pollinator flight distance. With the reduction in density that follows fragmentation, remnants may act as stepping stones for pollinators increasing gene flow among patches (Chase et al. 1996).

Predictions about the effects of fragmentation on genetic structure must be made against the backdrop of current ecological traits and patterns of genetic variation within and among populations. Few studies (Boyle et al. 1990, Knowles et al. 1992, Ge et al. 1998) have examined the impact of fragmentation on local dispersal and microspatial genetic structuring in conifers. The current study provides a detailed examination of the effects of fragmentation on the demographic structure within a stand of the temperate conifer, mountain hemlock (*Tsuga mertensiana*).

We hypothesize that, given the ecology and mating system of the species, fragmentation may result in the short term in high levels of gene flow and the loss of local genetic structure. Seed flight of mountain hemlock is thought to be shorter than its counterpart, western hemlock (*Tsuga heterophylla*), largely due its size and weight, suggesting that at the local scale there might be strong family structure (Edwards and El-Kassaby 1996). Other environmental factors which potentially influence mating patterns in stands include reproductive phenology, and distance between mates and stand density. After a clearcut, stand density is dramatically reduced. In a wind-pollinated species the neighbourhood area remains constant but the effective

population size has been reduced increasing the chance that neighbours will mate. These outcrossing events will increase levels of inbreeding if mating occurs between adjacent individuals that share a recent common ancestor (Loveless and Hamrick 1984). An abundant and widespread species, the edges of the distribution of mountain hemlock are characterized by disjunct populations resulting in its inclusion in the world list of threatened species (Farjon et al. 1993). Disjunct populations are already naturally fragmented and a further reduction in population sizes may in the long run lead to increased genetic differentiation.

Although mountain hemlock is primarily used for watershed protection and wildlife habitat, with timber harvesting in British Columbia reaching into the higher elevation regions, habitat fragmentation for subalpine species is increasingly becoming a reality (El-Kassaby and Edwards 1998, Seidel and Cooley 1974). Only a few studies have evaluated genetic structure throughout the different life stages of tree species (Linhart et al. 1981, Hamrick et al. 1993, Alvarez-Buylla et al. 1996). Thus the specific aims of this investigation are to: (a) retrospectively assess the impact of clearcutting on genetic structure and diversity within different life history stages, and (b) examine how levels of genetic diversity and inbreeding in old growth understory seedlings compare with natural regeneration from a clearcut.

Materials and methods

Study species

Mountain hemlock (*Tsuga mertensiana* (Bong.) Carr: Pinaceae) is a predominantly outcrossed conifer (see Chapter 2). Protogyny and synchrony of female cone receptivity and pollen release have been found to occur in this species (Means 1990). Both pollen and seeds from this tree are well adapted for wind-mediated dispersal. Like many other Pinaceae, mountain hemlock pollen is saccate (Owens and Blake 1983), while the mature seed has a seed

wing 2.67 times the size of the seed itself (Owens and Molder 1975). Mountain hemlock is known to have a long nonreproductive juvenile growth phase of 20 to 30 years (Fowells 1965), although some trees may bear cones as young as 20 years of age. Previous studies have shown that after mountain hemlock forests are clearcut, they regenerate very slowly due in part to the adverse conditions at high elevations (El-Kassaby and Edwards 2000, Minore and Dubrasich 1981, Brett and Klinka 1998).

Field Site

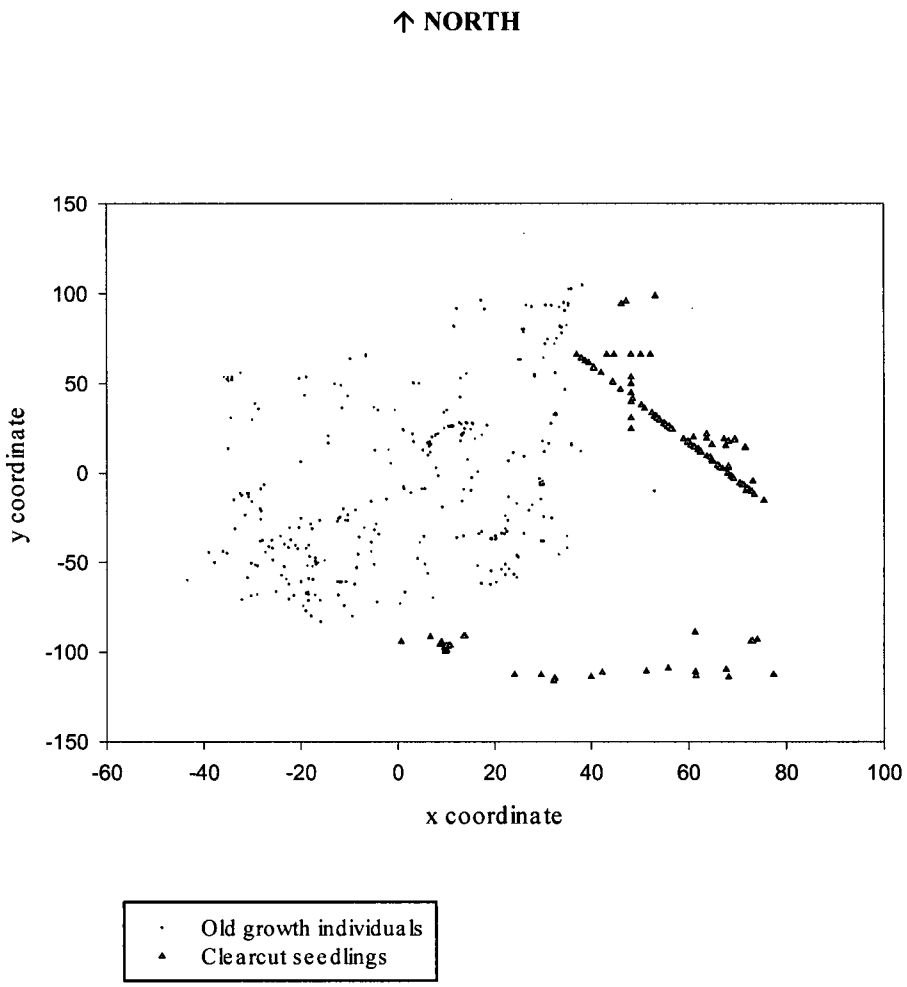
We studied a population of mountain hemlock on the south facing slope of Mount Cheam at an elevation of 1400m, bordering the Chilliwack river in southwestern British Columbia. The site is an old growth mixed-species stand dominated by mountain hemlock and pacific silver fir (*Abies amabilis*) with varying amounts of yellow cedar (*Chamaecyparis nootkatensis*). Running through the old growth stand were many slow moving creeks where the soils were moist and poorly drained. However, in some areas of the site, the soils were well drained, and the terrain was quite uneven. Adjacent to the old growth stand on the western, eastern and southern sides is a 24-year old clearcut. A total area of 43.3 ha was harvested in 1976 and is naturally regenerating. In 1993 the clearcut was surveyed for species composition and density and it was determined that there were 2,496 stems/ha of which 77% was amabilis, 19% western hemlock and 2% mountain hemlock and yellow cedar (Erik Nelson *pers comm*). The stand is part of a larger population-wide study of local genetic structure in four species of conifers in British Columbia. A previous study (S. Travis and K. Ritland, *unpublished data*) using isozymes found that mean genetic relatedness declined with increasing distance within this stand, hence it provided an ideal opportunity for a more detailed examination of the impact of disturbance on fine genetic structure and dispersal. Trees were sampled in two dimensions

within four plots approximately 3,600 m^2, covering an area of 16,200m^2 or 1.5 ha. By sampling in two dimensions the number of pairwise comparisons within a specific distance increases, and the potential for capturing complete information on a single 'neighbourhood' increases (Ritland 1996). Foliage was sampled from trees using a pole pruner; where inaccessible, bark was substituted. Trees in the old growth were mapped directly into a Cartesian coordinate system using two measuring tapes, disposable string, flagging tape, a double right-angle prism, a 2m pole and following a method modified from Reed et al. (1989). Additionally, the natural regeneration from the southern and eastern edges of the surrounding clearcut were sampled along three length transects of 100m each (Figure 5). We found more seedlings on the eastern side of the clearcut than in the southern area (*personal observation*). Only those seedlings greater than 0.33m in height were sampled. Only trees in the old growth were measured for diameter at breast height (DBH) and a subset (N=154) were cored to be used to determine tree age.

Microsatellite Analysis

DNA was isolated from 514 leaf and 5 bark samples following a modification of the CTAB protocol of Doyle and Doyle (1990). Leaf tissue (0.15-0.20g) and/or bark (0.20g) was homogenized with liquid nitrogen and incubated in 1800uL CTAB isolation buffer (2% CTAB, 1.4M NaCl, 20mM EDTA, 100mM TRIS-HCL pH=8.0, 1% 2-beta-mercaptoethanol) at 65-70°C waterbath for 45 minutes. In conifers, polysaccharides, polyphenolics, and tannins are a significant problem, particularly in woody tissues, and can interfere with PCR amplification (Murray and Thompson 1980). Hence 1% polyvinylpyrrolidine was used to optimize the purity and yield of DNA (De Filippis and Magel 1998). After centrifuging the solution, the protein-CTAB complex was removed with equal volume of a chloroform:isoamyl (24:1) solution. The

Figure 5. A spatial map of individual mountain hemlock trees sampled on the south-facing slope of Mount Cheam (1400m) which borders the Chilliwack River. Old growth trees (N=320) were sampled in two dimensions within four plots approximately 3,600 m^2, covering an area of 16,200m^2 or 1.5 ha. The natural regeneration surrounding the old growth on the southern and eastern edges were sampled along three length transects of 100m each (N=194).



supernatant was removed and the mixture was incubated with 10mg/ml RNAase A at 37°C for30 minutes and then the solution was mixed twice with equal parts phenol and chloroform. After removing the residual proteins with a final chlorform:isoamyl solution, the nucleic acids were precipitated out with 5M NaCl and 100% isopropanol. The pellet was then washed with chilled 70% ethanol to remove the salt, air dried and dissolved in dH_2O.

Twenty-two primers (John Carlson *pers comm.*) designed for western hemlock (*Tsuga heterophylla*) were tested on mountain hemlock DNA. Microsatellite alleles were consistently detected *T. mertensiana* following cross-species PCR amplification of DNA at three loci: HS10, HS26 and HS29. Only two loci (HS29 and HS26) were found to be polymorphic and the other was found to be monomorphic (HS10). Samples were assayed for genotypes at the two polymorphic loci HS29 and HS26. Polymerase chain reactions (PCR) amplifications were performed using 10 µl total reaction volumes. The reaction mixture contained 1.0 il of 10X Erica Haddelberg buffer (100 mM Tris-HCl pH=8.0, 500 mM KCl, 25 mM $MgCl_2$, 0.1% gelatin and 1.6 mg/mL BSA), 1.0 il of 2.0 mM dNTP, 0.5 pmol each of forward and reverse primers, 0.5 pmol M13 IRD-labeled primer, 1Unit Taq DNA Polymerase, and between 30-50 ng of DNA template. Samples were amplified in a PTC - Programmable Thermal Cycle with each primer pair having its own cycle profile. Following amplification, 3 µl of loading dye (100% formamide, 1 mg/ml pararosaniline basic red) was added to each reaction. The microsatellites were then visualized on 6% polyacrylamide gels on a LI-COR 4200 automated electrophoresis unit. Twenty-one alleles were detected at HS26 ranging from 182 bp to 222 bp, and thirty alleles were detected at HS29 ranging from 203 bp to 265 bp.

Statistical analyses

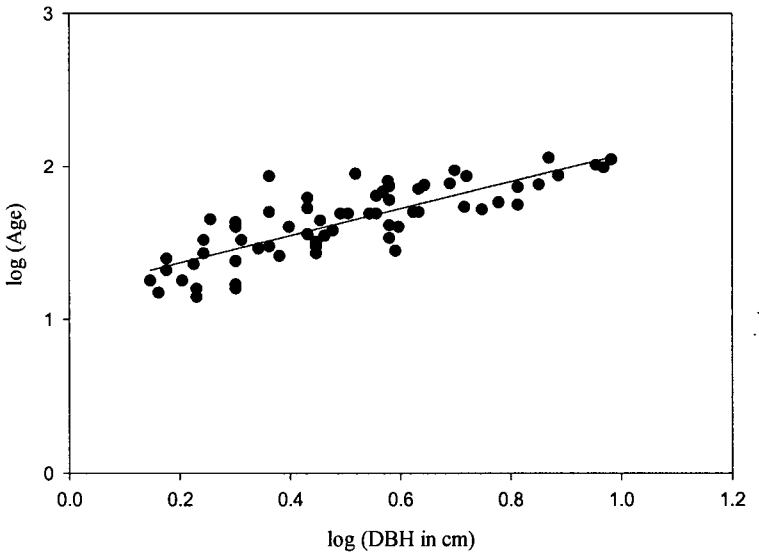
Two basic comparisons underlay the statistical analyses conducted: 1) the old growth stand was contrasted to the clearcut, and 2) genetic diversity and structure of life history stages (seedlings, saplings and adults) were assessed within the old growth stand. Trees were classified into life-history stages rather than age classes because in many long lived species individuals of the same age can be in the same or very different demographic states (Caswell 1982). Fitness components like fecundity in these organisms are stage specific rather than age specific because movement from one demographic state to another is density dependent (Orive 1995). Hence, for my analyses, individuals were divided into size classes using the convention of diameter-at-breast height (DBH), and the terms demographic and stage class are used interchangeably (Vandermeer 1978). This was performed after a significant relationship between age and DBH was established using a regression analysis in SAS version 6.12 (SAS Institute Inc. 1988).

A simple linear regression (N=154) of the log-transformed variables age and diameter at breast height (DBH) was significant ($p<0.0001$), but did not meet all the assumptions of the linear regression model. Although errors were found to be independent and normally distributed, the variance of the error terms increased with age. The results of the regression imply that for trees in the bigger diameter classes DBH is not a good predictor of age. The primary reason for performing the regression analysis was to be able to distinguish between seedlings established before and after the date of the clearcut (1976). Hence, to determine if DBH is a good predictor of age for the seedling classes, another linear regression analysis (N=64) was performed on the log-transformed variables. The results indicate that the linear model was significant ($r^2= 0.656$, $p<0.0001$) with a mean-square error of 1.38 (Figure 6). There was no evidence to indicate that the error terms violated the assumptions of a linear regression model. For the seedlings, DBH was a reasonably good predictor of age enabling us to classify seedlings into two diameter

categories 0-2 cm and 3-10 cm, saplings 11-29 cm and adults 30 cm plus. Those seedlings with a diameter between 0-2 cm were taken to represent post-fragmentation seedlings while those with diameters between 3-10 cm were categorized as pre-fragmentation seedlings.

Allelic diversity was calculated for each of the different stage classes: seedlings, saplings, and adults. As well, genetic diversity in the old growth stand was contrasted to that of the clearcut using GENEPOP (Raymond and Rousset 1995b). Inbreeding coefficients (F_{is}), expected heterozygosities (H_e), observed heterozygosity (H_o), and allele frequencies were calculated. Inbreeding coefficients were calculated according to Weir and Cockerham (1984) and significance was determined using an exact Hardy-Weinberg test with the alternative hypothesis of heterozygote deficiency (Rousset and Raymond 1995). The ratio of observed number of alleles per locus, A_o, over the effective number of alleles per locus, A_e, was also calculated ($A_e = 1/\Sigma p_i^2$ where p_i is the frequency of the ith allele). This standardized measure of allele eveness was used to compare relative abundance of allele in each stage class because it is less influenced by sample size than A_o. In addition, to determine if the genotypic and allelic distributions between the old growth and the clearcut as well as among the different life stages were identical, a Markov-chain estimation of Fisher's exact probability test was performed (Raymond and Rousset 1995a, Goudet et al. 1996). FSTAT was used to estimate R_{st}, a measure of genetic differentiation within each age class, in which each microsatellite locus is weighted by the amount of allelic variance (Rousset 1996). Although geographic patterning was suggested by the small moving creeks, sample size limitations permitted subdivision of the old growth stand into only four demes of 40 m x 80 m and the clearcut into demes of 50 m x 20 m. Finally null allele frequencies were estimated at both loci for all life stages, a method developed by Chakraborty et al. (1992), which determines the frequency from heterozygote deficiency and null homozygotes.

Figure 6. A plot of the simple linear regression of log (Age) against log (DBH cm) for seedlings that were found to be 24 years old and younger (N=64). The regression was significant ($p<0.0001$) with a coefficient of determination (r^2) of 0.656. There was no evidence to indicate that the assumptions of the simple linear model were violated.



Mean genetic relatedness was calculated for distance intervals 2, 5, 10, 20, 50 and 100 m for all individuals in the old growth as well as within each of the diameter classes using the regression estimator of Lynch and Ritland (1999). Distance intervals were cumulative and represented the maximum distance between two trees upon which a relatedness estimate was calculated. Mean genetic relatedness at cumulative distance intervals was calculated by averaging pairwise relatedness estimates at that distance interval over 100 bootstraps. The standard error of mean genetic relatedness was calculated using the standard deviation and significance tested using 95% confidence intervals (Sokal and Rohlf 1995). As well, estimates of pairwise relatedness were regressed on pairwise distance for discrete but varying distance classes to examine the impact of isolation by distance.

Finally, using SPATIAL GENETIC SOFTWARE (SGS; Degen 2000), the spatial distribution of individual trees was tested to determine if it exhibited a clumped, random, or regular spatial pattern using the Clark and Evan's aggregation index (Clark and Evans 1954). This measure examines the distance from an individual to its nearest neighbour regardless of direction. The expected mean distance to nearest neighbours if individuals in the population were randomly distributed is calculated and then a ratio of the observed mean distance to the expected is used as a meaure of deviation from randomness. Product-moment correlations between DBH and location, represented by position along the x coordinate or y coordinate, in the stand was calculated within each stage class of the old growth using SAS version 6.12 (SAS Institute Inc. 1988).

Results

Life history stages

A total of 324 individuals from the 1.5 ha old growth stand were assayed at the two loci HS26 and HS29. Seventeen individuals of the old growth stand were not scorable at both loci

and were dropped from the genetic analysis leaving a sample size of 307 individuals. These individuals represent potential null homozygotes but were not included because they were blank at both loci. Of these 307 individuals assayed 100 were seedlings, 94 saplings and 113 adults. A total of 21 alleles for all 307 individuals in the old growth were found at HS26 while at the second microsatellite a total of 25 unique alleles were found. At the first locus, HS26, the allele frequency distribution was unimodal for all classes of trees, whereas post-fragmentation seedlings at the second locus showed a unimodal distribution, while all other stages had a multimodal distribution.

Across both loci, there were slight differences in diversity measures for all four life history stages as evident in Table 6. There was an increase in expected heterozygosity (H_e) between pre-fragmentation and post-fragmentation trees, with post-fragmentation seedlings possessing an expected heterozygosity of 0.907 and the prefragmentation seedlings, saplings and adults with heterozygosities of 0.915, 0.932 and 0.922 respectively. Observed heterozygosities appeared to decrease across age classes at both loci with with the highest value (0.852) found in the post-fragmentation seedlings and the lowest in the saplings and adults (0.762 and 0.765). All stage classes, except those seedlings established after fragmentation, were found to have approximately 1.6 times more observed alleles per locus (A_o) than effective numbers of alleles per locus (A_e).

There was no evidence of statistically significant inbreeding in the post-fragmentation seedlings but in the other three stage classes the inbreeding coefficients were found to be significantly different from zero. The adults and saplings had inbreeding coefficients of 0.17 and 0.182, while the pre-fragmentation seedlings had an F_{is} of 0.161. Inbreeding coefficients were regressed against mean diameter to determine if there was an increase in F_{is} with stage class. A non-linear regression analysis found a statistically significant relationship between inbreeding

and diameter (p=0.0312; Figure 7). The function that best fit this relationship was an exponential increase to an aysmptote represented by the regression equation was $Y_{hat}= a (1-e^{-bX})$. The value for the constant, *a* was found to be 0.1790 (p=0.0037) while *b* was 0.2984 (p=0.0478).

There was some evidence for weak structuring within the adult and pre-fragmentation classes with positive values of R_{st} of 0.034 and 0.036 respectively. Estimates of standard deviations were not obtained through jacknifing over loci as only two loci were available. Genotypic and allelic composition across both loci was significantly different between adults and post-fragmentation seedlings (p value=0.012, p value=0.0019), and adults and pre-fragmentation seedlings (p value=0.0032, p value=0.0007). Pairwise R_{st} values calculated for all pairwise comparisons between the four different age classes and presented in Table 7 indicate a slight differentiation between post-fragmentation seedlings and the other three stages, with the largest pairwise value of R_{st} (0.0649) between the two types of seedlings.

The null-allele frequency at locus HS26 was quite low and on average was 0.0557. The second locus had a null-allele frequency substantially higher at 0.1214. There was a difference between post- fragmentation seedlings and the rest of the old growth at both loci; post-fragmentation seedlings showed no occurrence of null alleles at the first locus and a lower frequency (0.0828) at the second.

On average two adults were found to be a mean distance of 4.54 m, saplings were found to be on average 4.93 m apart and two pre-fragmentation seedlings were found to be 2.96 m. The aggregation index indicated that the spatial distribution of trees in all diameter classes was clumped. The product-moment correlation between DBH and the y coordinate were significant in the adult class (r=-0.2005, p=0.033). The measurement of diameter in pre-fragmentation seedlings was found to be significantly correlated with position along the x coordinate or the

east-west axis (r=-0.415, p=0.0006) and along the y coordinate or the north-south axis (r=-0.307, p=0.013).

For all old growth trees (N=307) overall mean relatedness was found to be significantly different from zero for two individuals separated by less than 5m. However, within none of the life history stages was mean relatedness found to be significantly different from zero when 95% confidence intervals were calculated. Because the number of pairwise comparisons was small

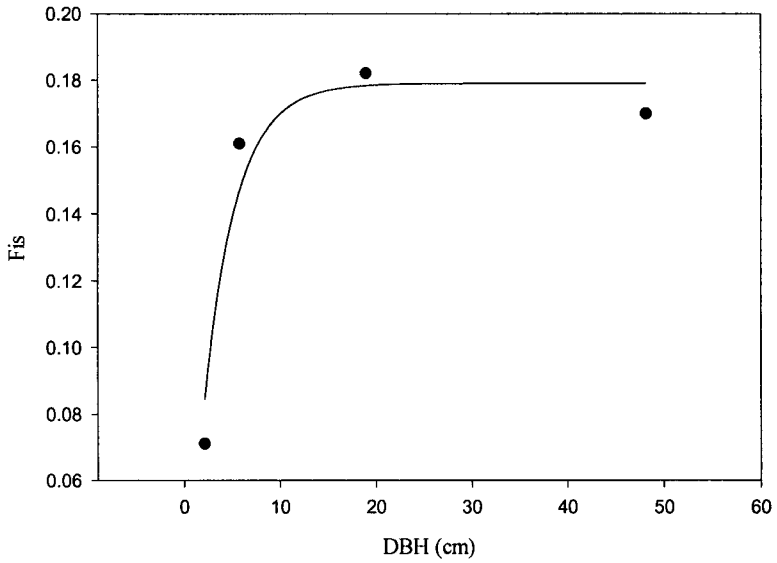
Table 6. Genetic diversity measures for the different life stages in an old growth of mountain hemlock and seedlings found in surrounding clearcut. Standard errors and p-values (given in parantheses) are given for F_{is}.

Life stages	DBH (cm)	N	A_o /A_e	H_e	F_{is}	R_{st}
OLD GROWTH						
Post-fragmentation seedlings	0-2	36	2.639	0.907	0.071±0.033 (n.s.)	-0.013
Pre-fragmentation seedlings	3-10	64	1.633	0.915	0.161±0.008 (p=0.040)	0.036
Saplings	11-29	94	1.582	0.932	0.182±0.0001 (p=0.0001)	-0.005
Adults	30 plus	113	1.681	0.922	0.170±0.0013 (p=0.0038)	0.034
Clearcut	not measured	194	1.627	0.934	0.119±0.008 (p=0.0504)	0.005

Table 7. Estimates of genetic differentiation (R_{st}) among life history stages in an old growth mountain hemlock stand.

R_{st} estimates	0-2cm seedlings	3-10cm seedlings	Saplings
3-10cm seedlings	0.0649	-	-
Saplings	0.0466	-0.0084	-
Adults	0.0392	-0.0068	-0.0064

Figure 7. A nonlinear regression analysis found a statistically significant relationship between inbreeding and mean diameter in each life history class (p=0.0312). The function that best fit this relationship was an exponential increase to an asymptote represented by the regression equation, $Y_{hat}=0.1790(1-e^{-0.2984X})$.



for adults, saplings and seedlings at the 2m and 5m distance intervals, sampling error magnified the variance of the relatedness estimate resulting in large standard errors (Table 8).

Nonetheless, a clear pattern emerges from the old growth: a decline in genetic relatedness occurs with increasing distance (Figure 8a). Overall mean genetic relatedness in the adult stage was the highest followed by the pre-fragmentation seedlings and then saplings. In the adults two individuals found 2 m apart had an average relatedness coefficient of 0.103, 35.5 times higher than pre-fragmentation seedlings where mean genetic relatedness at 2m was nearly zero (0.0029) and only 3.3 times higher than saplings (0.0314; Table 8). Mean genetic relatedness in adults drops off by 34% to 0.0354 when the pairwise distance interval is increased to 5m but remained positive (at 10m: 0.0157) until 20m at which point it effectively became zero (-0.0025).

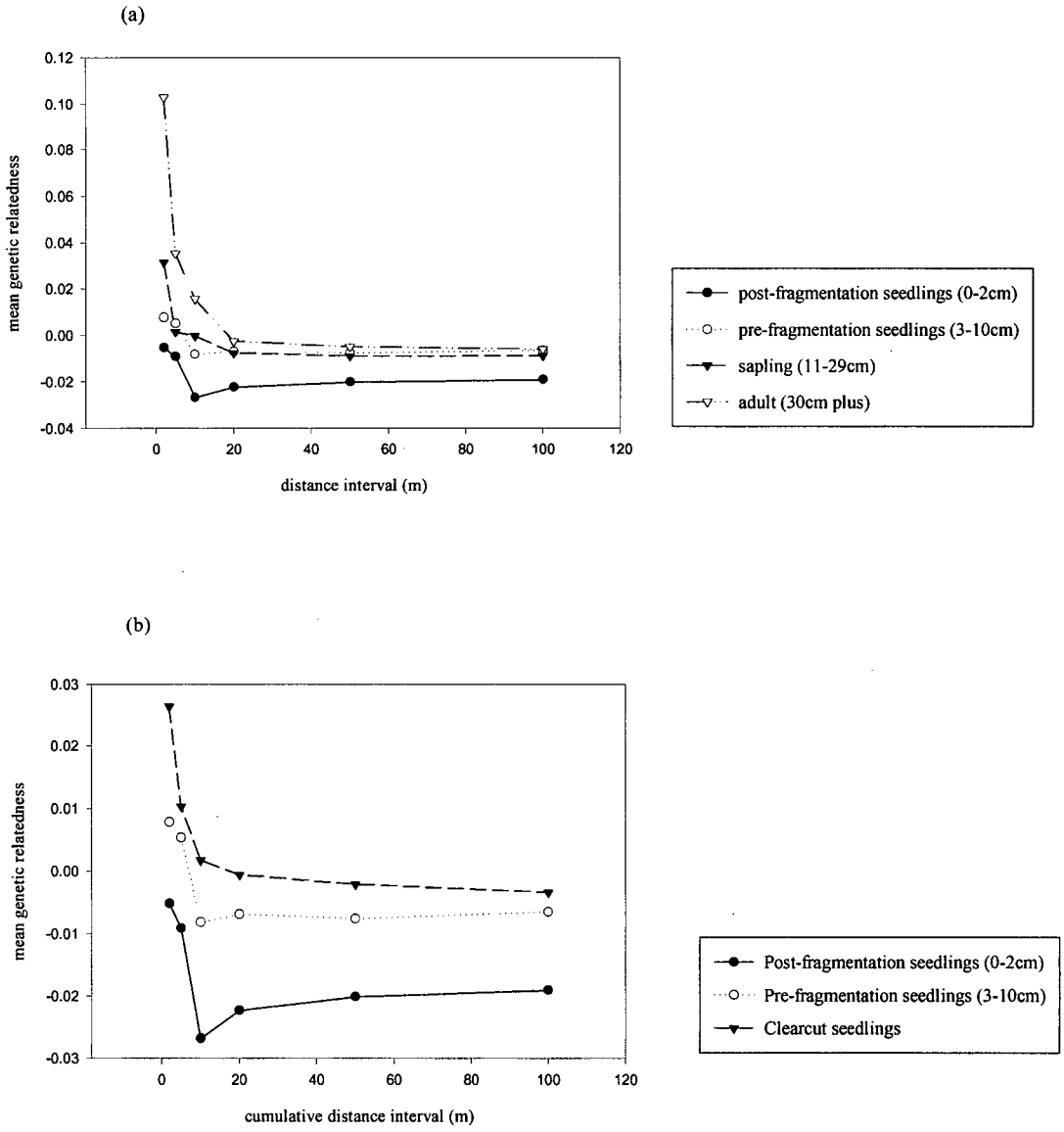
In the saplings and pre-fragmentation seedlings, mean genetic relatedness was positive until about 5m (saplings: 0.0015, seedlings: 0.0053) after which it became effectively zero. Relatedness estimates for post-fragmentation seedlings were effectively zero regardless of the distance interval.

Using the regression estimator, we examined relatedness for pairs of trees separated by a distance between 0-3.99 m, 4-10 m, and >10 m. These pairwise estimates were then regressed against pairwise distance to examine the effects of isolation by distance. As a non-linear relationship between distance and relatedness for old growth trees is suggested by Figure 8a, a non-linear function was tested. A significant regression was found only for pairs of adult trees that were no more than 4m apart (p value=0.0005; Figure 9a). An exponential decay function best fitted the data with the equation of $Y_{hat}=0.927e^{-1.7118X}$ representing the function. However, only one coefficient was significant 1.712±0.609 (p=0.0078), while the other was not 0.927±0.523 (p=0.0845). In addition, the variance in errors at smaller pairwise distances was

Table 8. Estimates of mean genetic relatedness for the different life history stages; and clearcut seedlings and their associated standard errors calculated across 100 bootstraps and based on the Lynch and Ritland (1999) regression estimator. Average number of comparisons for each stage is also calculated. * indicate significance at an α=0.05. Since mean relatedness is effectively zero after 10m, no other distance intervals are given.

SIZE CLASS	MEAN RELATEDNESS					
DISTANCE CLASS	2m	mean # of comparisons	5m	mean # of comparisons	10m	mean # of comparisons
Old Growth - ALL	0.024±0.014	233	0.016±0.006*	703	0.006±0.004	1783
Adults	0.103±0.067	23	0.035±0.033	50	0.016±0.014	140
Saplings	0.031±0.037	20	0.002±0.015	70	-0.0001±0.010	173
Pre-fragmentation seedlings	0.008±0.017	73	0.005±0.009	176	-0.008±0.004	387
Post-fragmenation seedlings	-0.005±0.031	14	-0.009±0.016	30	-0.027±0.011	52
Clearcut seedlings - ALL	0.026±0.007*	495	0.010±0.0043	1046	0.002±0.0026	2332
<10m from edge of old growth	0.011±0.020	38	-0.011±0.0103	96	-0.014±0.0057	203
>20m from edge of old growth	0.020±0.008*	417	0.008±0.0050	845	0.001±0.0031	1711

Figure 8. Mean genetic relatedness against a cumulative distance interval for mountain hemlock trees at Mount Cheam. (a) A comparison of mean genetic relatedness in the four life history stages. (b) A comparison of mean genetic relatedness in clearcut seedlings, post-fragmentation seedlings (0-2cm), and pre-fragmentation seedlings (3-10cm).



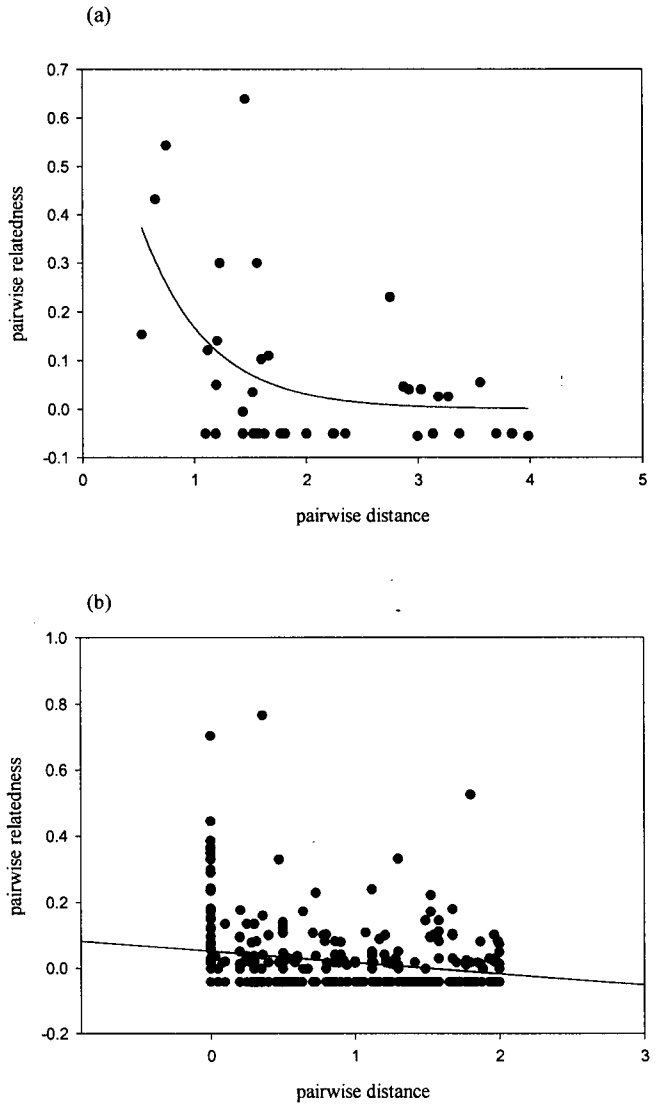
large but decreased with increasing pairwise distance and the assumption of normality was not met (p=0.0004).

Old growth vs. Clearcut

A total of 194 seedlings from the surrounding clearcut were assayed at both loci, 21 unique alleles were found at HS26 and 31 at HS29. Locus HS26 had a unimodal allele frequency distribution, while the alleles at locus HS29 exhibited a multimodal frequency distribution, with 5 unique alleles found in the clearcut. The null allele frequency at HS26 and HS29 was estimated to be 0.0293 and 0.0995, respectively. Genetic diversity measures for all clearcut seedlings are given in Table 6. Although expected heterozygosity of the clearcut was quite high, 0.934, it was not different in magnitude from any of the old growth age classes. However, observed heterozygosity was found to be similar in magnitude to the post-fragmentation seedlings, H_o=0.823 and much higher than prefragmentation seedlings, saplings, and adults. The relative abundance of alleles per locus (A_o/A_e) was 1.626 similar to values found in both the adult, sapling and pre-fragmentation seedling classes.

The inbreeding coefficient was calculated across both loci and was found to be significantly different from zero (F_{is}= 0.119). The seedlings in the surrounding clearcut had an inbreeding coefficient higher than post-fragmentation seedlings but lower than those individuals in the pre-fragmentation class. Fisher's exact tests showed that the clearcut and old growth were significantly different from each other both genotypically and in allelic composition, results are given in Table 9. However, when genetic differentiation was calculated using R_{st}, weak differentiation between only the clearcut and post-fragmentation seedlings was found. This may be due to how the estimator R_{st} is calculated. Based on the stepwise mutation process, R_{st} considers two alleles differing by one or two repeats as related. As R_{st} is the fraction of the total

Figure 9. Local genetic structure in a mountain hemlock stand. (a) A significant non-linear regression was found only for pairs of adult trees that were no more than 4m apart (p value=0.0005). An exponential decay function best fitted the data with the equation of $Y_{hat}=0.927e^{-1.7118X}$ representing the function. (b) Significant isolation by distance was found for seedlings at 20m or more from the edge of the old growth that were no more than 2m apart. The relationship was best represented by the linear regression equation $Y_{hat}= 0.0516-0.0349X$ ($p<0.0001$).



variance in allele sizes between two populations, it will be affected by modal differences in the allele distribution. In the goodness of fit statistic, which evaluates the null hypothesis of absence of population differentiation, each allele regardless of its size is counted as different (Goudet et al. 1996). Overall there was very weak genetic structuring within the clearcut (R_{st}=0.005). Again, standard errors for R_{st} could not be calculated because of a limitation in the number of microsatellite loci used.

Mean genetic relatedness in all clearcut seedlings was found to be significantly different from zero for seedlings up to 5m apart (Table 8). Also estimated was the mean relatedness of clearcut seedlings at 10 m or less and those at 20 m or greater from the edge of the old growth. Those seedlings at 20 m and more from the edge of the old growth had a significant mean relatedness (Table 8). The mean genetic relatedness of the clearcut seedlings at 2 m, 5 m, 10 m, 20 m, 50 m and 100 m was compared to mean relatedness of 0-2 cm and 3-10 cm diameter classes of seedlings (Figure 8b). At 2m the clearcut seedlings exhibited higher mean relatedness (0.0264) than either class of seedlings but declined rapidly to 0.0103 at 5 m and 0.0017 at 10 m, effectively exhibiting a mean relatedness of zero at 20 m.

When pairwise relatedness was plotted as a function of distance for clearcut seedlings at 20 m or more from the clearcut, slight significant isolation by distance was found for seedlings no more than 2 m apart (Table 10). The relationship was best represented by the linear regression equation Y_{hat}= 0.0516-0.0349X ($p<0.0001$) with significant coefficients ($p<0.0001$). Variation in pairwise distance at 2m explained approximately 4.13% of the variation in pairwise relatedness (Figure 9b). Although there was no evidence of heteroskedascity, the error terms were not normally distributed ($p<0.0001$). I also found a positive relationship between pairwise relatedness and pairwise distance for clearcut seedlings no more than 2 m apart and which were less than 10m from the edge of the old growth (r^2=0.107, $p<0.0420$). This positive linear

relationship is represented by the equation $Y_{hat} = -0.0045+0.0483X$. The distribution of the error terms were found to be significantly different from normal ($p<0.0001$) and errors were found to be heteroskedastic as evidenced from the residual plot.

Table 9. Genetic differentiation between clearcut seedlings and stage classes in the old growth. Differentiation was examined using R_{st} and testing whether genotypic and allelic distributions were significantly different among these comparisons.

Comparison	Genotypic differentiation p value	Allelic differentiation p value	R_{st}
Clearcut seedlings & Post-fragmentation seedlings	0.00629	0.00416	0.0490
Clearcut seedlings & Pre-fragmentation seedlings	0.02209	0.01246	-0.0065
Clearcut seedlings & Saplings	<0.0001	<0.0001	-0.0046
Clearcut seedlings & Adults	<0.0001	<0.0001	-0.0037

Table 10. Results from linear regressions performed on pairwise relatedness against pairwise distance for clearcut mountain hemlock seedlings. Significance was estimated at an $\alpha=0.05$ and indicated by *.

CLEARCUT	DISTANCE CLASS		
10m from OG (N=48)	**0-2m**	**>2m-5m**	**>5m-10m**
slope	0.0483	-0.0190	-0.0052
p value	0.0420	n.s.	n.s.
R sq	0.1071	0.0000	0.0111
pairwise comp	39	60	111
20m from OG (N=144)			
slope	-0.0342±0.008	-0.0017	-0.00004
p value	<0.0001*	n.s	n.s
R sq	0.0437	0.0006	0.0000
pairwise comp	405	444	890

Discussion

Diversity among stages

Long-lived organisms like trees are ideal for studying temporal and spatial genetic differentiation because age cohorts established at different times can be examined

simultaneously. The impact of major historical events like fragmentation, which differentially affect diversifying forces like natural selection, dispersal, and genetic drift, can be assayed retrospectively. This study has provided just such an opportunity. Previous studies of temporal variation in trees have generally found low levels of heterozygosity and high levels of inbreeding in younger age classes (Linhart et al. 1981, Roberds and Conkle 1984, Yadzani et al. 1985, Muona et al. 1987, and Shea 1990). However, the current study found seedlings from the 0-2cm diameter class (post-fragmentation) harboured the largest genetic diversity and had the lowest levels of inbreeding. These understory seedlings showed a significantly different allelic distribution than the adult class, suggesting that they are not simply a subset of the neighbouring adult gene pool. Pairwise R_{st} among the different demographic classes established that post-fragmentation seedlings were differentiated from the pre-fragmentation seedlings (R_{st}=0.0649) possibly suggesting different parental gene pools. Predominantly outcrossed, mature pollen of mountain hemlock is smooth and saccate (Owens and Blake 1983) increasing the efficiency with which it travels through air along wind currents. Furthermore, near-distance pollen flow depends on the height of the source, density of trees, and mass and shape of the pollen grain. In some cases pollen grains are expected to disperse distances greater than 10 times the height of the source (Levin and Kerster 1974). The 1976 clearcut of 43.3 ha surrounding the old growth may have substantially altered the density of trees causing a change in wind currents, subsequently enhancing gene flow (El-Kassaby and Jaquish 1996). Long distance pollen/seed dispersal from farther away in the stand or from an entirely different population would facilitate the mixing of gene pools.

Another remarkable result found by the current study was that inbreeding level, as measured by F_{is}, increased exponentially with mean diameter but then levelled off. The inbreeding coefficient is a function of relatedness or coancestry of the previous generation

because it is the probability that an individual carries a pair of alleles derived from a common ancestor (Jacquard 1974). Thus, high levels of inbreeding in the pre-fragmentation, sapling, and adult classes indicate some degree of relatedness in the previous generation. High levels of inbreeding may indirectly result from family clustering, assemblages of genetically similar individuals or family units. Family groups have been documented in other forest trees (Coles 1976, Linhart et al. 1981) and herbs (Levin and Kerster 1968) but the deficiency of heterozygotes is more pronounced in outbreeding species where pollination is animal-mediated (Brown 1979). Since mountain hemlock is highly outcrossed, it is likely that the related individuals are at most half-sibs. In some annual and conifer populations inbreeding levels are found to increase from adults to zygotes, usually as a result of self-fertilization in adults. As those zygotes become reproductively mature adults, selection acts to remove the inbred individuals and inbreeding decreases (Dole and Ritland 1993, Ritland 1990a). The result obtained for this study, the difference in inbreeding level between trees established before and after fragmentation may be suggestive of a temporal shift in the breeding system from inbreeding to outcrossing.

An alternative explanation for the high inbreeding coefficients may be that heterozygotes were scored as homozygotes when alleles were close in size (Aldrich et al. 1998). Similarly, null-alleles or non-amplifying alleles are a common occurrence when using microsatellites, particularly in cross-species amplification as was the case with the current study, and may inflate inbreeding coefficients (Pemberton et al. 1995, Paetkau and Strobeck 1995, Westman and Kresovich 1998). Estimates of null alleles suggest high allele frequencies at the most variable locus, HS29, and may explain in part the comparatively reduced observed heterozygosities at that locus for all classes. Although the estimation of null-allele frequency included null homozygotes (Chakraborty et al. 1992), without the use of controlled crosses it is not possible to

independently assess its impact on the inbreeding coefficient. The estimation of null alleles assumes panmixia, ascribing all of the heterozygote deficiency relative to Hardy-Weinberg expectation to null alleles (Brookfield 1996) and excludes the effect of population subdivision or inbreeding. Population substructuring, which leads to inbreeding, should affect all loci simultaneously resulting in a consistent reduction in observed heterozygosity across all loci (Van Treuren 1998). We found a 10% reduction in observed heterozygosity between the 0-2cm seedlings and the other demographic classes at the first locus and a similar reduction (5%) at the second locus suggesting that more than just null-alleles are causing an increase in inbreeding with stage class.

Finally a spatial or temporal Wahlund effect may also be responsible for the high levels of inbreeding found in the 3-10cm seedling, sapling and adult diameter classes (Hamrick et al. 1993, Thomas et al. 1999, Alvarez-Buylla et al. 1996). The lower inbreeding coefficient of post-fragmentation seedlings (0-2cm) may be due to a diminished temporal Wahlund effect, a result of the inclusion of fewer cohorts than in the 3-10cm seedlings, sapling, and adults (Aldrich et al. 1998). While a spatial Wahlund effect may be eliminated by subdividing individuals within each of the demographic classes by location, removal of the temporal effect is much more difficult given the small sample sizes. However, subdivision of diameter classes still resulted in significant inbreeding coefficients primarily at the the second microsatellite locus but did reduce the level of inbreeding at the first microsatellite locus. It is unlikely at such a fine scale that there is a spatial Wahlund effect.

Spatial and temporal structure

Most plant population studies suggest that spatial genetic differentiation is greater than temporal genetic differentiation (Linhart et al. 1981, Roberds and Conkle 1984, Yadzani et al.

1985, Muona et al. 1987, Fore and Hickey 1992, Schnabel and Hamrick 1995, Caujapé-Castells and Pedrola-Monfort 1997). Our finding of minimal temporal differentiation (R_{st}=0.0079) and moderate spatial differentiation particularly within the adult (R_{st}=0.034) and pre-fragmentation (R_{st}=0.036) classes is comparable to levels found by other studies. Because two measures of genetic relatedness were used, R_{st} and the Lynch and Ritland (1999) relatedness estimator, historical and current dispersal can be evaluated. R_{st} like its counterpart F_{st} is the sum of dispersal and establishment over many generations and represents the effect of mating between remote relatives due to population structuring. When the time scale of interest is large, R_{st} is determined by the accumulation of mutations, but when the time scale is relatively recent genetic drift is more important (Slatkin 1995). The Lynch and Ritland (1999) relatedness estimator examines local patterns of relatedness where most individuals are separated by only a few generations of dispersal. In pre-fragmentation seedlings R_{st} was small and there was no indication of isolation by distance when pairwise relatedness was regressed against distance indicating that the weak genetic structure may primarily be a result of a founding event from a small group of common ancestors. An example of a 'founder effect', reproductive dominance, has been previously demonstrated in the tropical tree *Symphonia globulifera* (Aldrich et al. 1998). Reproductive dominance occurs when only a few highly fecund adults produce most of the seedlings. It may be that the 3-10 cm seedlings are the outcrossed seedling progeny of the adults in the old growth.

One characteristic of old growth mountain hemlock stands is the formation of edaphic gaps and canopy openings arising from the patchy mortality of dominant trees. Seedlings that adapt to the available microsites will be successful colonists (Seidel and Cooley 1974, Lertzman et al. 1996). Edaphic gaps are usually associated with small creeks or thin soils on a rock outcrop, and successful mountain hemlock germination occurs on snow (epigeal), mineral soil,

or moist organic soil (Means 1990). The 3-10 cm seedling class exhibited a clumped distribution, as evident from the significant Clark and Evans aggregation index, which appeared to parallel closely the location of the primary creeks within the old growth (*personal observation*). Additionally, the significant correlation between DBH and location in pre-fragmentation seedlings indicates that seedling recruitment occurs over time and in stages. Overlapping seed shadows caused by the high density of adult trees may have reduced the levels of relatedness among adjacent prefragmentation seedlings (Ueno et al. 2000).

Some studies (Linhart et al. 1981, Roberds and Conkle 1984, Hamrick et al. 1993) including the current study, have shown that adult age classes can retain microspatial genetic structuring. Adults exhibited a small value of R_{st} among demes, the highest levels of overall mean relatedness (0.103±0.067), and an exponential decay of pairwise relatedness with increasing pairwise distance to a maximum of 5 m. These results imply genetic structure in adults is a consequence primarily of localized seed dispersal with perhaps some indication of a small founder effect.

The adult classes also exhibited a negative correlation between diameter and position along a north-south axis suggesting that colonization of the old growth occurred in stages. Early colonization of the site appears to have begun at the south end of the old growth and may have been the result of local dispersal events over many years from trees in a neighbouring population. Marked by a long non-reproductive juvenile period, the succession of the forest stand into one dominated by mountain hemlock would occur only through the arrival of new migrants over that period, increasing the number of founders in the population and decreasing the effect of genetic drift (Austerlitz et al. 2000). With some level of coancestry in these new migrants (as indicated by the high inbreeding level) a low density of competitive trees, and

several favourable germination sites, family groups were successfully established and persisted for several generations.

Effect of fragmentation

Data from this study seems to indicate that fragmentation of the old growth has increased the opportunity for long distance gene flow. The significant differences in allelic and genotypic distributions between the clearcut seedlings and the old growth and high allelic diversity in the clearcut suggest there must be substantial amounts of long distance seed dispersal. Although adults may be contributing seed to the surrounding clearcut, these seedlings appear to have been founded from a different gene pool than the post-fragmentation seedlings within the old growth. Unrestricted pollen and/or seed flow may explain the high allelic diversity found in clearcut seedlings. Empirical studies which compare open-terrain dispersal to that of forested areas suggest less than 10% of pollen entering a forest edge would be airborne at 100m while at the same distance in open terrain 50% of pollen would be airborne (Levin and Kerster 1974). Without the effect of overlapping seed shadows sibling and cousin clusters could form in the clearcut explaining the significant levels of inbreeding and higher mean genetic relatedness estimate in clearcut seedlings.

Fragmented forested landscapes result in altered wind profiles allowing for higher wind speeds which can increase the transfer of material like pollen dust and seeds from the surrounding forest into open areas by up to 40% (Saunders et al. 1991). Surprisingly, isolation by distance was detected in clearcut seedlings greater than 20m from the edge of the old growth, but those seedlings at less than 10m showed an increase in pairwise relatedness with increasing distance between two individuals. This may simply be a function of the density of dispersed seeds and the probability of their survival over time relative to the distance from the parent trees

(Janzen 1970). High seed densities and high mortality near the parental tree will combine to allow only a small proportion of the nearby fallen seeds to survive relative to those dispersed at farther distances (Augspurger 1983). Franklin and Smith (1974) found that most of the mountain hemlock seedfall in a clearcut was dispersed within 38.1m of the stand edge, but central portions of a 30-acre clearcut still received 50,000 to 100,000 sound seeds per acre during a bumper seed year. The combination of significant isolation by distance in the clearcut and small values of R_{st} suggest that the accumulation of genetic structure in the surrounding natural regeneration is largely a result of localized dispersal. Using a demographic genetic framework has been important for detecting slight temporal changes in genetic diversity and structure, however further examination is required before conclusions can be drawn on the full effects of fragmentation in conifers.

The current study does provide evidence that forest fragmentation may alter patterns of spatial relatedness and the relative influence of evolutionary forces like genetic drift and gene flow in shaping genetic variation. Given the recent pressures on high elevation timber species, to guarantee sustainability of mountain hemlock populations consideration must be given to the interaction between genetic structure and life cycle characteristics. Natural regeneration of mountain hemlock after a disturbance like clearcutting and fire is slow (Seidel and Cooley 1974, Agee and Smith 1984, Minore and Dubrasich 1981, Brett and Klinka 1998). Young mountain hemlock seedlings grow substantially better in partial shade; and although increases in light intensity and day-length will augment seedling height, it prevents or delays terminal-bud formation (Means 1990). Following a shelterwood treatment of a mountain hemlock dominated stand, seedlings suffered a 90% mortality rate largely due to lethal surface temperatures (Seidel and Cooley 1974). Finally, germination in mountain hemlock requires highly specific environmental conditions, a particular heat sum accumulation, shade and warm soils, minimal

site disturbance, and a good seed supply. Mountain hemlock appears to have a narrow window of opportunity in which a seedling can become successfully established. Although natural germination can occur during July and August, these germinants will have to survive snowpacks and rapidly decreasing temperatures in the following months (El-Kassaby and Edwards 2000). Following a wide-scale perturbation, these factors suggest mountain hemlock is not capable of maintaining its own populations solely through natural regeneration. As a predominantly outcrossed species where seedling recruitment occurs over time, and localized dispersal maintains genetic structure, the conservation of its genetic resources must be considerate of locally adapted genotypes.

CHAPTER 4: CONCLUSIONS AND FURTHER AREAS OF STUDY

Mountain hemlock is a predominantly outcrossed species which exhibits half the genetic variation found in other wind-pollinated, outcrossed, and long-lived species. Other species of 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), are hypothesized to have undergone a population bottleneck or a series of bottlenecks during the Pleistocene (Zabinski 1992). Although this pattern is suggested by the data, a more extensive sampling of the range of mountain hemlock, in particular the northern and southern extremes, would substantiate this claim. Like other conifers at the broad geographic scale, mountain hemlock populations are highly undifferentiated genetically with most of the genetic variation found within populations. The near absence of among population differentiation, and the extensive pollen flow, have led some studies to conclude that conifer populations approach panmictic units (Guries and Ledig 1982, Epperson and Allard 1984). However other studies including this one have found that local genetic structure, due to short seed dispersal distances, can arise within populations even with extensive pollen flow. Hence, the smaller spatial scale has the potential to detects differences in gene flow which are cancelled out when sampling is done over a geographic or larger spatial area (Levin 1992).

The influence of local dispersal on the genetic substructuring of the old growth and clearcut seedlings is evident from our data. Gene flow patterns are affected not only at the landscape and regional scale, through habitat fragmentation by humans, but also by the microsite and ecological dynamics of an old growth stand. The current study raised the possibility that the pre-fragmentation seedlings may be the outcrossed progeny of the adults and that not only old growth adults were contributing to the clearcut. Parentage analysis, which allows for the reconstruction of a population level pedigree, would enable an investigation of these questions.

In addition, this type of analysis would further determine the relative importance of local versus long distance dispersal to genetic substructuring on an ecological time scale. Microsatellites are ideal for tracing seed flow in conifers because of their codominance and hypervariability. Moreover, because parentage analysis measures gene movement into a particular area, not only can one calculate the distribution of dispersal distances but also generate estimates of neighbourhood size through time.

Local and long distance seed dispersal are important initiators of the scale at which patterns of relatedness are generated in the clearcut. The maintenance and even creation of fine-scale differentiation can also be a result of natural selection operating over a heterogeneous environment, like that of a clearcut (Linhart and Grant 1996). Mountain hemlock stands endure climatic extremes of -29°C to 38°C, annual snowfall in excess of 2200 cm, and the persistence of the snowpack usually until August or September (Means 1990). However, micro-environmental correlations that imply selection within a stand or across a clearcut would need to be strong in order to counteract the extensive gene flow each generation (Epperson 1992). The relative roles of selection and drift can be distinguished by examining F-statistics across loci, since differentiation due to sampling drift would have a similar effect on all loci, while selection may result in differences in fixation indices across loci (Linhart et al. 1981, Roberds and Conkle 1984). Heterogeneity of F-statistics can also be explained by stochastic rates and historical patterns of gene flow. Quite often populations do not receive all alleles at the same rates or from the same locations and when migration rates are small this historical fingerprint can persist for long periods (Williams and Guries 1994). It would be interesting to examine more microsatellite loci and re-examine the F-statistics giving consideration to differential null allele inflation of the inbreeding coefficents.

Finally, the remarkable result of increasing inbreeding levels with increasing mean diameter warrants further investigation. Six quantitative traits were measured on the trees in the old growth at Mount Cheam - diameter at breast height (DBH), height, stomatal density on upper side of needle, needle length, needle area and growth rate. It would be useful examine the relationship between measured inbreeding levels (F_{is}) or difference in homozygosity level and trait values within different age classes. Outcrossed species are generally observed to retain more recessive deleterious mutations which can decrease the fitness of inbred individuals. Unlike highly selfed populations where these recessive deleterious mutations are purged, greater levels of levels of inbreeding depression are found in outcrossed species where inbreeding occurs because there is a small amount of self-fertilization or mating among relatives (Charlesworth and Charlesworth 1987). Although highly deleterious mutations will be eliminated at the embryo/seed stage, mildly deleterious mutations may be expressed at later life stages and result in reductions in growth or fecundity. The relationship between individual inbreeding coefficient and quantitative traits can be used to examine the decrease in fitness due to inbreeding. The current study suggested a shift in breeding system, it may be worth examining if this temporal shift is matched by a shift in genetic load.

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APPENDIX A

Allozyme frequencies for the nineteen populations sampled across British Columbia

Locus	allele	Population[1]																	
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	P	Q	R	S	O
Fest-2	1	0.775	0.825	0.775	0.725	0.650	0.700	0.950	0.700	0.725	0.725	0.825	1.000	0.825	0.600	0.700	0.650	0.700	0.800	1.000
	3	0.225	0.175	0.225	0.275	0.350	0.300	0.350	0.300	0.275	0.275	0.175	0.000	0.175	0.400	0.300	0.350	0.300	0.200	0.000
Idh	1	0.900	0.775	0.850	0.925	0.550	0.625	0.875	0.875	0.800	0.975	0.600	0.500	0.725	0.900	0.900	0.625	0.700	0.775	0.625
	2					0.100						0.025						0.025		
	3	0.100	0.225	0.150	0.075	0.350	0.350	0.125	0.125	0.200	0.025	0.375	0.500	0.275	0.100	0.100	0.375	0.275	0.225	0.375
Pgm-1	1	0.900	0.825	0.950	0.927	0.850	0.825	0.875	0.875	0.900	0.950	0.875	1.000	0.925	0.775	0.925	0.825	0.825	0.825	1.000
	2	0.025	0.050	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	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	0.100	0.000	0.075	0.200	0.025	0.050	0.150	0.125	0.000
	5	0.050	0.025	0.000	0.000	0.100	0.025	0.075	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.050	0.125	0.025	0.050	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	1.000	1.000	1.000	1.000	0.975	0.925	1.000	1.000	1.000
	3	0.025	0.025	0.000	0.075	0.000	0.000	0.050	0.025	0.025	0.000	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	1.000	1.000	1.000	1.000	0.975	1.000	1.000	1.000	1.000	1.000	0.975	0.900	1.000	1.000	1.000
	3									0.025						0.025	0.100			
Pgi-2	1	0.925	0.950	0.975	1.000	1.000	0.900	1.000	0.975	1.000	1.000	0.925	1.000	1.000	1.000	0.925	0.975	0.925	0.950	1.000
	2	0.025	0.000	0.025	0.000	0.000	0.050	0.000	0.025	0.000	0.000	0.025	0.000	0.000	0.000	0.075	0.000	0.075	0.050	0.000
	3	0.050	0.050	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.025	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	1.000	1.000	1.000	0.975	0.750	0.950	1.000	0.925	1.000
	2	0.000	0.075	0.050	0.125	0.000	0.000	0.000	0.000	0.000	0.150	0.000	0.000	0.000	0.025	0.250	0.050	0.000	0.075	0.000
Gdh	1	0.875	1.000	1.000	1.000	0.975	1.000	1.000	0.975	1.000	1.000	1.000	1.000	0.975	1.000	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	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000
	3	0.050												0.025						
	5	0.025																		
Skd-1	1	1.000	1.000	1.000	0.975	0.925	0.800	1.000	1.000	0.875	0.925	0.950	1.000	0.950	0.875	0.975	0.950	0.950	0.925	1.000
	2	0.000	0.000	0.000	0.000	0.000	0.150	0.000	0.000	0.075	0.075	0.050	0.000	0.025	0.000	0.000	0.025	0.050	0.025	0.000
	3	0.000	0.000	0.000	0.025	0.025	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.025	0.025	0.000	0.050	0.000
	5	0.000	0.000	0.000	0.000	0.050	0.025	0.000	0.000	0.050	0.000	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	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	1.000	0.975	1.000	1.000	1.000	1.000	0.975	1.000	1.000
	2	0.025																0.025		
	3												0.025							
Lap-2	1	0.975	0.950	1.000	0.950	0.975	1.000	0.975	0.925	0.950	1.000	0.950	0.550	1.000	1.000	1.000	0.975	1.000	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	0.050	0.450	0.000	0.000	0.000	0.025	0.000	0.050	0.000

Locus	allele	Population																	
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	P	Q	R	S	O
Mdh-1	1	1.000	1.000	1.000	1.000	1.000	0.975	1.000	0.975	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	3						0.025		0.025											
Mdh-2	1	0.825	0.850	0.900	0.875	0.800	0.700	1.000	0.650	0.925	1.000	0.700	1.000	0.825	0.800	0.825	0.975	0.825	0.975	0.700
	2	0.075	0.100	0.025	0.050	0.025	0.200	0.000	0.000	0.000	0.000	0.075	0.000	0.000	0.000	0.000	0.000	0.100	0.025	0.000
	3														0.050					0.100
	4	0.100	0.050	0.075	0.075	0.175	0.100	0.000	0.350	0.075	0.000	0.225	0.000	0.175	0.150	0.175	0.025	0.075	0.000	0.200
Mdh-3	1	0.925	0.900	0.925	0.900	0.925	0.975	0.900	0.875	0.975	1.000	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	0.025	0.075	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.025	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	0.075	0.000	0.075	0.000	0.000	0.025	0.000	0.025	0.000
Aco-1	1	1.000	0.975	1.000	1.000	1.000	1.000	0.975	1.000	0.950	1.000	0.975	1.000	1.000	1.000	0.975	0.950	1.000	1.000	1.000
	2	0.000	0.025	0.000	0.000	0.000	0.000	0.025	0.000	0.025	0.000	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	0.025	0.000	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	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	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0.025																		
Aat-3	1	1.000	1.000	0.975	1.000	1.000	1.000	1.000	0.975	0.975	1.000	1.000	1.000	1.000	1.000	0.975	0.975	0.875	1.000	1.000
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.025	0.000	0.000	0.000	0.000	0.000	0.025	0.025	0.000	0.000	0.000
	5			0.025														0.125		

Table 2. Continue

[1]see Table 1, for population codes