GENETIC DIVERSITY AND GENE CONSERVATION OF PACIFIC SILVER FIR (*ABIES AMABILIS*) ON VANCOUVER ISLAND, BRITISH COLUMBIA[1]

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ABSTRACT

Levels of inbreeding and patterns of genic diversity of eight Vancouver Island Pacific silver fir (*Abies amabilis* (Dougl.) Forbes) populations were determined using genetic variation at 13 allozyme loci. With the exception of one locus, the segregation analyses for all polymorphic loci exhibited distinct, co-dominant expression and simple Mendelian segregation in their mode of inheritance. Estimates of inbreeding varied among populations and ranged from zero to as much as 27%. A highly positive correlation of population mean seed size and multilocus outcrossing rate was observed ($r = 0.712$, $0.05 < P < 0.10$). Similar to most studied conifers, a low level of population differentiation was observed. Nei's genetic diversity analysis produced a G_{ST} value of 0.051 indicating that the vast majority of allelic variation (95%) resides within individual populations. The average genetic distance among all populations was low (0.0112) and the correlation between genetic and geographic distances was not significant, with geographical distance, explaining only 1.4% of the variation in genetic distance among pairs of populations. A novel approach for determining the relative importance of genetically unique populations for genetic conservation purposes was presented. Finally, a proposal for combining utilization (the establishment of seed production areas) and conservation (gene conservation of unique populations) was presented.

Key words: *Abies amabilis*, allozyme, mating system, genetic diversity, gene conservation.

INTRODUCTION

Pacific silver fir (*Abies amabilis* (Dougl.) Forbes) is one of seven "true" firs found in the Pacific northwest region of North America (FOILES 1959). It has a wide geographical range that extends from the southern end of the Alaska panhandle (56 °N) to northwestern California (42 °N). It is restricted in its eastward distribution to a relatively narrow band and is seldom found more than 300 km from the Pacific Ocean (SCHMIDT 1957, FOWELLS 1965, PACKEE *et al.* 1982, WORRALL 1983) (Fig. 1). In British Columbia, Pacific silver fir is found at elevations from sea level to 1500 m at the 49th parallel and to 300 m at its northern limit (SCHMIDT 1957). The species' reaches its greatest development and commercial productivity on the west sides of the Olympic and Cascade Mountains, the foothills of the Columbia River and the west coast of Vancouver Island (HANDLEY 1982).

Pacific silver fir is considered to be a slow migrator and the time lapse since glacial recession may have been too short for the species to fully occupy its potential range (SCHMIDT 1957). It is the most shade tolerant of all forest tree species in British Columbia, this in turn contributes to relatively dense stocking because of the low space requirement of the spire-shaped crown (KRAJINA *et al.* 1982). The mating system of Pacific silver fir is of interest given its particular silvical and ecological characteristics. The species' high shade tolerance is expected to permit the development of family structure within populations, thus increasing the chance of inbreeding, particularly mating among relatives which in turn shapes the genetic architecture of the species.

The importance of Pacific silver fir as a commercial species in British Columbia has been overshadowed by the desirability and availability of other species such as Douglas-fir (*Pseudotsuga menziesii*). The role of

[1]Parts of this paper represent the senior author's Ph.D. dissertation. Robin lost a heroic battle with cancer and prematurely departed the scientific community.

Pacific silver fir in future forests of British Columbia appears promising, as indicated by the species recent sharp rise in artificial regeneration planting stocks.

Given the increased utilization of Pacific silver fir for reforestation and the apparent lack of information regarding its genetics, the generation of some knowledge regarding the nature and magnitude of the species mating system and genetic structure is a prerequisite to any effective management plan. This study reports on: (1) estimates of the mating system parameters, (2) the nature and extent of genic variation, and (3) a proposed genetic conservation strategy for this species Vancouver Island populations. Although we realize that the populations studied represent only a small part of the species natural range, the study should be considered as exploratory, preliminary information on the species genetics.

MATERIAL AND METHODS

Material

Cones were collected from eight populations of Pacific silver fir on Vancouver Island, British Colombia (Table 1, Fig. 1). Within each population, cone samples were collected from individual trees using a cone rake suspended from a helicopter (five populations) or were collected from the ground after tree felling (three populations) (Table 1). Cones were collected from individual trees and the parent-tree identity of cones and seeds was maintained throughout the study. Given the fact that Pacific silver fir was present in these populations as a co-dominant species with western hemlock (*Tsuga heterophylla*), and that the nature of individual tree cone collection was by helicopter or by hand, it is likely that sufficient distance (*i.e.*, no co-ancestry between sampled trees) was obtained. The cone collections were made over a four week period following cone/seed ripeness checks by field personnel. Cones were kept in mesh bags at 4 °C until all collections were made, then air-dried at 12–20 °C for two weeks. Seeds were extracted in a commercial cone-processing facility following standard methods used for the species. Seeds were hand-dewinged and filled seed samples were obtained using a vacuum separation apparatus (EDWARDS 1979). Filled seeds were x-rayed for verification and the percentage of filled seeds determined and seed size was indirectly estimated using 1000-seed weight measures based on samples of filled seeds from individual trees.

Electrophoretic methods

Maternal trees' genotype for 13 allozyme loci was deduced from the allozyme phenotypes/genotypes of 20 megagametophytic tissues ($1n$) per tree. Additionally, the corresponding embryo genotype of these 20 seeds was also determined. The probability of incorrectly classifying a heterozygote at any one locus is $(0.5)^{k-1}$ where k = number of seeds (TIGERSTEDT 1973), and thus for a sample of this size, very close to zero. Electrophoresis methods and extraction buffer are listed in EL-KASSABY *et al.* (1982) and CONKLE *et al.* (1982). Megagametophytic and embryonic tissues were electrophoresed on 12.5% w/v horizontal starch-gels using two buffer systems: tris-citrate pH 7.0 and sodium borate pH 8.0. The enzyme systems studied were aspartate aminotransferase (AAT) (2.6.1.1) and malate dehydrogenase (MDH) (1.1.1.37) each with three loci, glucose-6-phosphate dehydrogenase (G6P) (1.1.1.49), glutamate dehydrogenase (GDH) (1.4.1.3), isocitrate dehydrogenase (IDH) (1.1.1.42), 6-phosphogluconate dehydrogenase (6PG) (1.1.1.44), phosphoglucose isomerase (PGI) (5.3.1.9), phosphoglucomutase (PGM) (2.7.5.1), and superoxide dismutase (SOD) (1.15.1.1) all with one locus. Six out of the 13 loci were polymorphic (*Idh, Mdh–1*, and *Aat–2* with two alleles, *Pgi–2* with three alleles, and *G6p* and *Pgm* with five alleles).

Table 1. Sources of populations of Pacific silver fir cones (populations are listed by latitude).

Collection site	Code	Latitutde	Longitude	Average elevation (m)	# of trees sampled
Fleet River	F	48° 39'	124° 06'	710	9
Mystery Creek	W	48° 44'	128° 09'	625	17
Taylor River	A	49° 18'	125° 22'	300	8
Sebalhall Creek[1]	B	49° 57'	126° 25'	300	8
Maquilla Creek[1]	C	50° 04'	126° 21'	500	8
Hathaway Creek[1]	H	50° 35'	127° 33'	212	13
Ronning Creek[1]	R	50° 37'	128° 11'	275	13
Holberg Inlet[1]	N	50° 44'	126° 00'	215	11

[1] Helicopter collection

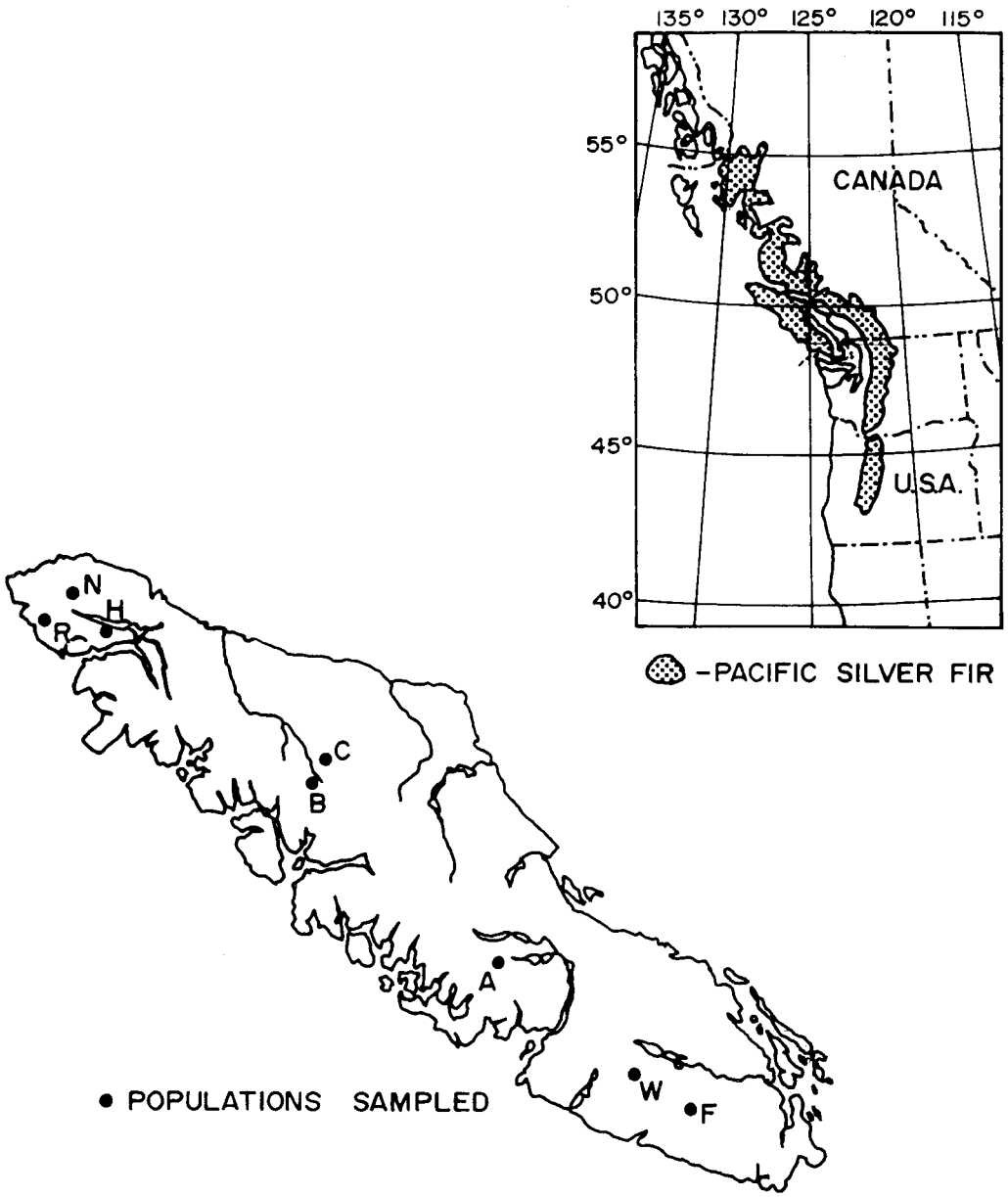

Figure 1. Distribution of the Pacific silver fir in western North America showing the location of the eight populations sampled on Vancouver Island (source: DAVIDSON 1990; FOWELLS 1965).

Genetic analysis

Single-locus (t_s) and multilocus (t_m) estimates of outcrossing rate were estimated using the maximum likelihood procedure of RITLAND and EL-KASSABY (1985). Populations' allozyme variation was analyzed using BIOSYS–1 (SWOFFORD & SELANDER 1981) and GENESTAT (LEWIS & WHITKUS 1989) with the following values computed: allele frequencies and average number of alleles per locus. Average number of alleles per locus is considered to reflect allelic richness, but as indicated by MARSHALL and BROWN (1975) inflates the contribution low-frequency alleles make to variation. Hence, a more informative measure is that of Crow and KIMURA (1970) known as the "effective" number of alleles per locus (n_e). N_e is greatest when alleles are of equal frequency and is close to one if only one allele is in very high frequency. Thus, n_e reflects both presence and frequency of alleles (HEDRICK 1983). The effective number of alleles per locus and the average expected heterozygosities (H_e) were derived for each population.

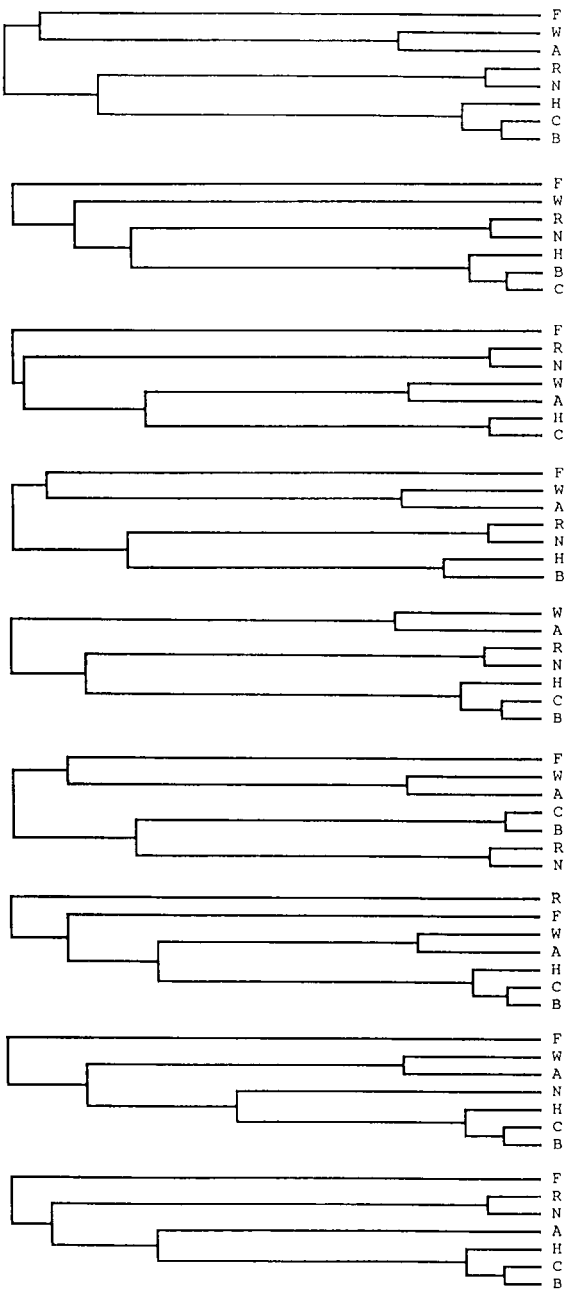

Figure 2. Dendrograms depicting hierarchical structure of genetic relatedness among the eight Pacific silver fir populations (ALL) and after the removal of each population one at a time from the original data set (for example, **-A** represents an analysis that contained all populations after the removal of the **A** population from the data).

In addition, NEI's (1973) coefficient of genetic differentiation G_{ST} was calculated for the natural populations to obtain estimates of the distribution of genetic variation.

The genetic relationship among the eight populations is presented in a hierarchical structure (genetic distance dendrograms) using the electrophoretic data following the method of NEI (1973) and the UPGMA algorithm (SNEATH & SOKAL 1973). In this hierarchical structure, the populations that are most genetically similar will cluster together forming a branch (Fig. 2).

The genetic uniqueness of a specific population (x_i) was determined as follows: (1) removing population's x_i data from the original data set (*i.e.*, $-x_i$), (2) estimating the average genetic distance $(\bar{D})$, and G_{ST}, for the new data set (*i.e.*, the original eight populations' data set minus population's x_i data), and (3) comparing the G_{ST} estimate obtained from the original analysis to that of the new eight data sets (*i.e.*,$-x_1$,$-x_2$, ...,$-x_8$). Thus, the same analysis was repeated eight times. A similar approach was used by SLATKIN (1985) in determining the magnitude and extent of gene flow among specific *Hyla arborea* populations and EL-KASSABY and YANCHUK (1995) in determining the genetic uniqueness of specific Pacific yew (*Taxus brevifolia*) populations.

RESULTS AND DISCUSSION

Genetics of allozyme markers

The mode of inheritance of the six polymorphic allozyme loci was determined using data generated from the haploid megagametophyte of each heterozygous mother tree. A χ^2 goodness-of-fit test was employed to verify the expected 1:1 segregation. Similar allelic combinations were pooled mother trees prior to the segregation analysis. With the exception of the *Aat–2* locus, the segregation analyses for all the remaining observed pooled allelic combinations were not significantly different from the expected 1:1 ratio (Table 2), indicating that these allozymes exhibited distinct, codominant expression and simple Mendelian segregation in their mode of inheritance. The heterogeneity G test among trees' segregation ratios was significant for three allelic combinations (G6P and PGI) (Table 2); however, close examination of these allelic combinations on an individual-tree basis indicated a lack of systematic bias towards any specific allele (data not given). Conversely, the *Aat–2* locus did not exhibit the expected classical Mendelian inheritance pattern and homogeneity test among trees showed a consistent bias towards allele "1" (Table 2). This pattern supports the existence of some genetic mechanism that acts to increase its own frequency. Based on the above segregation analyses, it was concluded that the *Aât–2* locus was not suitable for the mating system estimation (CHELIAK *et al.* 1984), but was applicable for the genetic diversity analyses (ADAMS 1983).

Table 2. Log-likelihood G test on segregation ratios of seven polymorphic loci in Pacific silver fir seeds.

Locus	Genotype	Observed ratio	Pooled G[1]	Heterogeneity G[2]
Aat –2	1:2[3]	224:53	113.57**	22.25^{ns} (13)
G6p	1:2	12:8	0.81^{ns}	–
	1:3	44:36	0.80^{ns}	16.75^{ns} (13)
	1:5	315:321	0.06^{ns}	64.79^{ns} (32)
	2:5	8:12	0.81^{ns}	–
Idh	1:3	22:18	0.40^{ns}	3.69^{ns} (1)
Mdh–1	1:2	116:102	0.90^{ns}	28.02^{ns} (10)
Pgi–2	1:2	409:404	0.03^{ns}	63.09^{ns} (39)
Pgm	1:2	8:12	0.81^{ns}	–
	1:3	227:210	0.66^{ns}	44.02^{ns} (21)
	1:5	21:19	0.10^{ns}	0.10^{ns} (1)

* significant at 5% level; ** significant at 1% level;: ns – not significant;
[1] – Pooled G values indicate the overall deviation from 1:1 ratio;
[2] – Heterogeneity G values indicate the amount of heterogeneity in the segregation ratio among trees.
[3] – Allozymes were numbered starting with "1" for most common alleles; faster and slower alleles were given even and odd numbers, respectively

Table 3. Allelic frequencies (most common allele)and their 95% confidence intervals for the maternal (ovule, O) and outcrossing pollen (P) gene pools for the seven Pacific silver fir populations on Vancouver Island.

Locus	Gene pool	Population[1]						
		W (34/355)[2]	**A** (16/159)	**B** (16/160)	**C** (16/159)	**H** (26/258)	**R** (26/258)	**N** (22/219)
Pgi-2	O	0.559±0.167	0.750±0.212	0.812±0.191	0.750±0.212	0.769±0.162	0.538±0.192	0.773±0.175
	P	0.750±0.212	0.786±0.064	0.825±0.059	0.887±0.049	0.868±0.051	0.605±0.060	0.694±0.061
G6p	O	0.676±0.157	0.750±0.212	0.875±0.162	0.812±0.191	0.769±0.162	0.923±0.102	0.727±0.186
	P	0.803±0.043	0.736±0.067	0.775±0.065	0.817±0.060	0.822±0.047	0.818±0.047	0.827±0.050
Pgm	O	0.647±0.161	0.812±0.191	0.937±0.119	1.000±0.000	0.808±0.151	0.923±0.102	0.909±0.120
	P	0.716±0.048	0.672±0.073	0.944±0.036	0.905±0.046	0.837±0.049	0.845±0.044	0.808±0.052
Idh	O			1.000±0.000		0.962±0.073		
	P			0.994±0.012		1.000±0.000		
Mdh-1	O					0.962±0.073	0.923±0.102	0.964±0.078
	P					0.996±0.008	0.988±0.013	1.000±0.000

[1] see table 1 for population designation
[2] First and second numbers represent the number of genes sampled from the maternal and pollen gene pools, respectively.

Mating system

The nature and extent of genetic variation in a species is largely determined by its pattern of breeding. Its system of mating constitutes the link between successive generations whereby genetic information is transferred, organized and distributed among progeny (CLEGG 1980). Mating system studies have shown most conifers to be high, although not obligate, outcrossers (see ADAMS & BIRKES 1991 for a review). Because the reproductive process of many economically important conifers is characterized by some natural self-fertilization and most exhibit large amounts of growth depression as a result of inbreeding (FRANKLIN 1970, SORENSEN 1982), accurate estimates of levels of inbreeding become important in planning and implementing of tree improvement programs. Both environmental and genetic factors have been shown to influence mating systems (CLEGG 1980). Outcrossing rates have been reported to vary with stand density (FARRIS & MITTON

Table 4 Estimates of single-locus (t_s) and multilocus (t_m) outcrossing rates for seven populations of Pacific silver fir from Vancouver Island, B.C. (95% confidence intervals)

Locus	Population[1]						
	W	**A**	**B**	**C**	**H**	**R**	**N**
Pgi–2	1.204 (0.152)	0.478 (0.206)*	0.446 (0.213)*	0.669 (0.234)*	0.647 (0.175)*	0.892 (0.167)	0.461 (0.154)*
G6p	1.066 (0.151)	0.740 (0.174)*	0.906 (0.209)	0.913 (0.234)	0.903 (0.178)	0.947 (0.153)	0.803 (0.190)*
Pgm	1.001 (0.107)	0.853 (0.206)	0.999 (0.220)	0.842 (0.289)	0.870 (0.179)	0.999 (0.173)	0.555 (0.212)
Idh	–[2]	–	0.900 (0.515)	–	0.999 (0.175)	–	–
Mdh-1	–	–	–	–	0.899 (0.269)	–	–
t^3	1.068 (0.076)	0.696 (0.112)*	0.787 (0.120)*	0.803 (0.144)*	0.859 (0.083)*	0.946 (0.087)	0.684 (0.091)*
t_m[4]	1.089 (0.073)	0.762 (0.115)*	0.888 (0.130)*	0.798 (0.148)*	0.869 (0.091)*	0.993 (0.062)	0.725 (0.107)*
t_{mc}[5]	1.089 (0.073)	0.762 (0.115)*	0.867 (0.137)	0.798 (0.148)*	0.847 (0.100)*	0.876 (0.080)	0.650 (0.116)*

* significant at 5% level
[1] see Table 1 for population designation
[2] monomorphic locus
[3] single-locus minimum variance mean
[4] multilocus outcrossing rate
[5] multilocus outcrossing rate based on the three common loci

Table 5. Pollen allele frequencies for six variable loci in eight populations of Pacific silver fir on Vancouver Island, B.C. (populations are arranged in order of increasing latitude and followed by sample size).

Locus	Allele	Population[1]							
		F (180)	**W** (335)	**A** (159)	**B** (160)	**C** (159)	**H** (258)	**R** (258)	**N** (219)
Pgi-2	1	0.672	0.704	0.786	0.825	0.887	0.868	0.605	0.694
	2	0.328	0.296	0.201	0.175	0.113	0.132	0.395	0.306
	n[2]	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000
G6p	1	0.572	0.803	0.736	0.775	0.817	0.822	0.918	0.827
	2	0.044	0.003	0.000	0.000	0.000	0.000	0.000	0.000
	3	0.006	0.000	0.031	0.012	0.000	0.008	0.000	0.000
	5	0.378	0.191	0.223	0.213	0.183	0.169	0.182	0.164
	n	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.009
Pgm	1	0.783	0.716	0.672	0.944	0.905	0.837	0.845	0.808
	2	0.011	0.027	0.006	0.000	0.013	0.000	0.000	0.000
	3	0.189	0.248	0.322	0.056	0.076	0.151	0.155	0.187
	5	0.006	0.009	0.000	0.000	0.006	0.004	0.000	0.000
	n	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.005
Idh	1	1.000	0.997	1.000	0.994	1.000	1.000	1.000	1.000
	3	0.000	0.003	0.000	0.006	0.000	0.000	0.000	0.000
Mdh-1	1	1.000	1.000	1.000	1.000	1.000	0.996	0.988	1.000
	2	0.000	0.000	0.000	0.000	0.000	0.004	0.012	0.000
Aat-2	1	0.972	0.788	0.074	0.938	0.924	0.984	0.965	0.945
	2	0.028	0.212	0.126	0.162	0.076	0.016	0.035	0.055

[1] see Table 1 for population designation
[2] n; null allele

1984, SHEA 1987), elevation (PHILLIPS & BROWN 1977, Neale and Adams 1985) and population substructuring (RITLAND & EL-KASSABY 1985).

The mating system of Pacific silver fir is of interest given its particular silvical and ecological characteristics. The high shade tolerance of Pacific silver fir permits the development of family structure within populations. The presence of family structure is conducive to mating among relatives while the cone-bearing 30% of the canopy, most male cones in the lower portion of the crown) promotes cross-fertilization (FRANKLIN & RITCHIE 1970, OWENS & MOLDER 1977). Although no controlled mating studies have been reported for Pacific silver fir, the species is characterized by very low yields of filled seed (FRANKLIN 1974, OWENS & MOLDER 1977).

Single- (t_s) and multilocus (t_m) population estimates of outcrossing rate and outcrossed pollen allele frequencies (p) for five polymorphic loci (*Pgi–2, G6p, Pgm, Idh* and *Mdh–1*) were determined. A multilocus estimate of outcrossing was considered more accurate and less sensitive to violations of model assumptions because a greater number of outcrosses may be identified with certainty (SHAW *et al.* 1981). Population **F** was originally included in the study but no estimates for outcrossing rates could be obtained using the model of RITLAND and EL-KASSABY (1985). The procedure for estimating t and p is iterative and, in the case of population **F**, failed to produce convergent values. The nonconvergence of estimates may be indicative of a failure of one or more model assumptions (SCHOEN 1988, RITLAND, pers. comm). The mixed mating model assumes that "successive outcross events within a family arise from independent draws of pollen from the total population of male plants" (SCHOEN & CLEGG 1984). Phenological and/or spatial variation in pollen distribution may modify the fertilization probabilities of pollen genotypes such that pollen genotypes received by females are correlated to some degree. This scenario is most likely to occur when relatively few males are contributing to the pollen cloud (SCHOEN 1988). Population **F** was the highest elevation sample in this study, where high amounts of precipitation and/or a short growing season may act to reduce the number of parents contributing to the pollen cloud. FRANKLIN and RITCHIE (1970) observed close synchrony in pollen dispersal and seed cone receptivity in Pacific silver fir.

Differences between gene pools (maternal and outcrossing pollen) at any one locus were determined by comparing the overlap of confidence intervals ($P < 0.05$; JONES & MATLOFF 1986, EL-KASSABY *et al.* 1987). With the exception of the *Pgm* locus in population **C**, no significant differences between the two gene pools were observed (Table 3), indicating that the outcrossed pollen pool is representative of the maternal population, or conversely, that the maternal trees are representative of the stands in which they were collected (BROWN *et al.* 1975, EL-KASSABY *et al.* 1987). It is also noteworthy that the confidence intervals of pollen allele frequencies are smaller than that of ovule allele frequencies. This is expected because of variation in sample size (BROWN *et al.* 1975) and reinforces use of the pollen pool (with larger 'n') estimates in population genetic studies (see genetic diversity section below) where the sample of maternal parents is limited (EL-KASSABY 1991). The penetrance of alleles across populations is also apparent from Table 3. Populations **R** and **W** tend to be the most variable in allelic composition for the *Pgi–2* locus and population **W** for *G6p* and *Pgm.* These two stands differ by nearly 2 degrees latitude, with **W** being one of the most southerly collections and **R** the most northern. The remainder of the populations possess quite similar allele frequencies. This pattern has been described in other *Abies* species (NEALE & ADAMS 1985, JACOBS *et al.* 1984, SHEA 1987).

Table 3 also reveals that not all loci are variable across all seven populations. However, allelic variation exists at least in the pollen pool, at *Pgi–2, G6p* and *Pgm* in all stands. VAQUERO *et al.* (1989) note that loci with relatively rare alleles create a large number of empty genotypic classes in arrays of progeny from a single mother, which in turn, cause difficulties in estimating outcrossing rates. Precision of single locus outcrossing rate estimates depend on allele and maternal genotype frequencies. Marker loci that are virtually monomorphic ($p_i > 0.970$; SHAW & ALLARD 1982) add very little information on outcrossing (because so few heterozygotes are recovered in the progeny) and produce large variances (SHAW & ALLARD 1982, RITLAND 1983). BROWN *et al.* (1975) point out that the variance of a given single locus t value is minimized when allele frequencies are equal. Given these considerations, multilocus outcrossing rate estimates were calculated using all variable loci in each population (t_m) and only the three most polymorphic loci (*Pgi–2, G6p* and *Pgm*) which were also common to all populations (t_{mc}) (Table 4).

Single locus estimates were significantly different from $t = 1.0$ for at least one locus in five of seven populations (Table 4). Estimates of outcrossing varied from as low as 45% to 100% and the *Pgi–2* locus gave consistently lower estimates than other loci (Table 4). Variation in single locus estimates of outcrossing is common among conifers. This variation is most likely statistical because even if allele frequencies (p's) are the same there will be varying genotypes among mother trees sampled in each population, which will possess

different powers of detection of outcrossing events. Whether the variation is statistical or loci do not fit assumptions of the mating model, the greater degrees of freedom provided by multilocus estimates make them a less biased estimate of outcrossing (SHAW *et al.* 1981). It can be seen from Table 4 that outcrossing rates based on multilocus estimates differ from 1.0 in all populations except **B**, **R** and **W**. SHAW and ALLARD (1982) suggest that where forms of inbreeding (i.e. mating among relatives) in addition to selfing occur, then t_m is expected to be higher than single-locus estimates. The minimum variance mean of single-locus estimates were compared to corresponding multilocus estimates for each population of Pacific silver fir. Multilocus estimates exceeded means of t_s for all populations except for population **C**. Differences range from 1% to nearly 6% but all fall within the limits of the 95% confidence intervals. NEALE and ADAMS (1985) found a similar range of differences in four populations of balsam fir (*Abies balsamea*) and concluded that mating other than selfing was not a factor. In Pacific silver fir, high shade tolerance and heavy seed (FRANKLIN 1974) are conducive to the development of family clusters and very likely render the assumption that all inbreeding is due to self-fertilization invalid. Differences between t_m and t_s, while not great in magnitude, are observed in 6 of 7 sampled populations, which suggests that some mating among relatives may be occurring and some assortative mating may be practiced even when complete outcrossing is apparent (populations **B**, **R** and **W**). Table 4 also gives multilocus estimates for each population based on the three commonly variable loci (t_{mc}). These differences are generally small and t_m's with a reduced number of loci are always smaller, probably as a result of reduction in sample size. The significance of deviation from complete outcrossing does not change. Estimates of outcrossing obtained by single- and multilocus methods exceed 1.0 in one of seven populations (**W**).

Given that some amount of inbreeding (as indicated by outcrossing rates < 1.0 in five of seven populations) is apparent, it is pertinent to ask whether '*t*' reflects the actual amount of inbreeding taking place. RITLAND (1983) describes '*t*' as the "effective" outcross rate, a summary variable representing the net effect of deviation from panmixis caused by correlations of maternal and paternal genotypes, variation in self-compatability, gametic selection and early zygotic lethality. ADAMS and BIRKES (1991) caution that s (=1– t) represents the proportion of viable progeny due to selfing, but is not a measure of the actual frequency of self-pollination, which may be considerably greater. SORENSEN (1982) maintains that self-incompatability mechanisms appear to be lacking in conifers and crossing experiments in noble fir (*Abies procera*) by SORENSEN *et al.* (1976) revealed that relative self-fertility is high in that species. Embryo abortion is common in Pacific silver fir (OWENS & MOLDER 1977), but it is not known to what extent embryos resulting from selfing are aborted. The species is known to produce large numbers of otherwise normal-appearing empty seeds (FRANKLIN 1974). Selfing reduced numbers of filled seed by 31% in noble fir (SORENSEN *et al.* 1976). It may be expected then, that a positive relationship would exist between percentage of filled seed and outcrossing rate, with high seed yields correlated with high levels of outcrossing. In this study, simple correlation between mean seed yield per population and t_m was very weakly negative (r = –0.165) and not significant. The relationship has intuitive appeal and had sample sizes permitted outcrossing rate estimates on an individual tree level then the correlation could have been based on individual variation rather than population means. However, EL-KASSABY *et al.* (1987) found the correlation between percent filled seed per tree and individual tree outcrossing rate in a western white pine (*Pinus monticola*) population to be non-significant. Seed yields are subject to a number of environmental factors (climate, insects, etc.) and a clear relationship, although theoretically plausible, may not be detectable from collections in natural stands. Seed size, however, is considered to be one of the least plastic of plant characters (SORENSEN & FRANKLIN 1977) and known to be under a high degree of genetic control (KHALIL 1986, STOEHR & FARMER 1986, CHAISURISRI *et al.* 1992). A highly positive correlation of population mean seed size and multilocus outcrossing rate was observed in Pacific silver fir (r = 0.712, 0.05 < P < 0.10). Seed size was represented by mean thousand-seed weight estimates. This is the reverse of that found in subalpine fir (*Abies lasiocarpa*) by SHEA (1987) where higher than average outcrossing rates were obtained from trees with smaller seeds.

Although variation in allele frequencies is not extensive over the range of Pacific silver fir sampled, there is some apparent variation in the rate of outcrossing among the seven populations of Pacific silver fir in this study. The mating system is known to be dynamic (HAMRICK 1982) and given Pacific silver fir's ecological status as a climax species (KRAJINA *et al.* 1982), it is not surprising that population estimates of inbreeding were variable (from zero to as much as 27 percent). Differences in the magnitudes of outcrossing obtained by single- and multilocus estimation procedures suggest that some related matings are occurring, a result which is not unexpected in light of the high shade tolerance and limited seed dispersal exhibited by this species. At the sampling intensity available to this study, deviation

from panmixs could not be associated with mating behavior, however, seed size was strongly related to the extent of apparent outcrossing in Pacific silver fir.

Genetic diversity

As HEDRICK (1983) describes, "our fundamental interest in determining the extent of genetic variation is to document the variation that results in selective differences among phenotypes". Because natural populations of forest trees are not likely to behave as ideal, panmictic breeding units, efficient sampling strategies depend upon some estimate of the degree to which populations are subdivided genetically (GURIES & LEDIG 1977, GREGORIUS & ROBERDS 1986).

Most conifers exhibit similar life histories. They are long-lived, often occupy heterogeneous environments and necessarily must possess the capacity for adaptations which enable them to remain in subsequent generations (REHFELDT & LESTER 1969, MÜLLER-STARCK & GREGORIUS 1986). Genetic variation is maintained by high levels of outcrossing and mechanisms to promote outcrossing, such as inbreeding depression and self-incompatibility, are expected to be characteristic of outbreeding species (LOVELESS & HAMRICK 1984, LANDE & SCHEMSKE 1985).

As indicated above, the biology and ecology of Pacific silver fir are unique among western North American conifers. Its extremely high shade tolerance and late successional status (KRAJINA *et al.* 1982) coupled with production of heavy seeds which are often cached by frugivores (FRANKLIN 1974) suggest at least the potential for some reproductive isolation and population substructure within the species. Further, the ability of Pacific silver fir to occupy a variety of sites (SCHMIDT 1957) suggests exposure to variable selection regimes. Narrow distribution (east-west) and island inhabitance may also act to restrict gene flow and promote differentiation (LOVELESS & HAMRICK 1984).

Adequate sample size for estimating allele frequencies should exceed $nk = 100$ (BROWN & MORAN 1981) where '*n*' is the number of maternal trees in the population and '*k*' equals the number of progeny per tree. Because the number of maternal trees sampled in the present study is low (eight to 17), pollen allele frequency data were chosen to represent the eight populations in analyses of allelic variation and in determining the diversity and genetic distance measures of NEI (1972, 1973, 1977 and 1978). Pollen allele frequencies are derived by "subtracting" the maternal genotype (known with virtual certainty from a sample of 20 seeds per tree) from the diploid embryo genotype. Pollen allele frequencies have been used in several studies of electrophoretic variation in conifers because a larger portion of the population gene pool is represented (FINS & LIBBY 1982, STEINHOFF *et al.* 1983, MILLAR 1983, LI & ADAMS 1989, SURLES *et al.* 1989). Where between generation comparisons were desired, maternal tree and embryo allele frequencies were utilized, acknowledging the dependency of these two sets of data. Statistical bias introduced by small sample sizes (less than 20 individuals) is found to be reduced by applying a correction factor of $2N/(2N-1)$ to H_e (NEI & ROYCHOUDHURY 1974). This correction was applied to estimates of H_e for all maternal gene pools in this study.

Pollen allele frequency estimates for individual loci across eight populations are shown in Table 5. The inclusion of *Idh* and *Mdh–1* as polymorphic reflect individual frequencies where the incidence of the most common allele was less than 0.99 but when averaged over all individuals exceeds the 99% criterion (HARTL 1980). There are several geographic trends apparent in

Table 6. Estimation of heterozygosity parameters for eight populations of Pacific silver fir from Vancouver Island, B.C.

Population[1]	$\bar{A}$	n_e	H_e (adult)	H_e (embryo)
F	1.73	1.23	0.156	0.108
W	1.82	1.22	0.177	0.125
A	1.64	1.21	0.097	0.091
B	1.55	1.11	0.068	0.054
C	1.55	1.09	0.889	0.052
H	1.73	1.10	0.124	0.072
R	1.45	1.16	0.109	0.073
N	1.55	1.16	0.081	0.073
Average	**1.64**	**1.83**	**0.118**	**0.085**

[1] See Table 1 for population designations

$\bar{A}$ – asverage number of alleles per locus; n_e – effective number of alleles per locus

H_e – expected heterozygosity for the adult and embryo populations

Table 7. Estimates of Nei's (1973) genetic distances (below the diagonals) and geographic distance in kilometers (above the diagonals) between pairs of populations of Pacific silver fir.

Population[1]	F	W	A	B	C	H	R	N
F	–	28	110	222	225	335	368	363
W	0.01848	–	83	195	199	308	341	336
A	0.01420	0.00480	–	112	116	225	258	254
B	0.01696	0.01283	0.01433	–	9	113	147	145
C	0.02133	0.01517	0.01316	0.00178	–	111	145	142
H	0.01745	0.01390	0.00889	0.00504	0.00192	–	33	32
R	0.01173	0.00596	0.00783	0.00830	0.00843	0.00617	–	15
N	0.01165	0.01080	0.01589	0.01332	0.01681	0.01359	0.00201	–

[1] see Table 1 for population designation

the table. A private allele, found only in population **A** was detected at the *Pgi–2* locus, whereas all other alleles occurred in at least two populations. Allele '2' in *G6p* was detected only in the two southernmost populations (**F** and **W**). The null allele ('n') was detected at the *Pgm* locus in only two of the northernmost populations, **N** and **H**. Variation at *Mdh–1* was found only in the northern samples (**R** and **H**). Populations had either four or five varying loci and three of the of 13 loci assayed were commonly variable (*Pgi–2, G6p* and *Pgm*). The average actual and effective number of alleles per locus are listed in Table 6. The greatest disparity in these two measures occurs in population **H** where, although a larger number of alleles were detected (1.73 alleles per locus on average), some of these alleles occurred at relatively low frequencies, reducing the effective number to 1.10. Actual allele counts did not show any trend with geography, however effective numbers of alleles were negatively correlated (r = –0.719, $P < 0.05$), indicating that, in general, allelic variation decreased with increasing latitude. Overall, populations possessed relatively low levels of allelic diversity with n_e values showing a tendency for one allele to dominate. No population was devoid of variation and n_e estimated suggest that population **W** was the most genetically diverse and population **C** the most depauperate. These populations also had the largest and smallest samples, respectively, however Spearman's rank correlation (ZAR 1984) failed to show any strong relationship between sample size and diversity (r = 0.241), as theoretically predicted by GREGORIUS (1987). Average expected heterozygosities (H_e) are listed in Table 6 for maternal tree and embryo gene pools with their weighted mean values. These measures of diversity showed a similar geographic trend to n_e, the correlation with latitude being negative and just significant at $\alpha = 0.05$ (r = –0.643) for the maternal gene pool and also significant for the embryos gene pool (r = –0.755). This was not unexpected since both quantities were derived from similar data structures. Average H_e's differed between gene pools, and in all populations maternal tree heterozygosity was greater than that expected for the embryo population.

Estimates of NEI's (1978) genetic distance between pairs of Pacific silver fir populations along with their geographic distances (measured in kilometers) are presented in Table 7. All values $\bar{D}$ of were very close to zero. Overall, the average genetic distance was 0.0112. The smallest value joined populations **B** and **C** which were also in the closest geographic proximity (9 km). Another two stands (**N** and **R**) which are physically near each other were also shown to be genetically very similar (D = 0.00201). Population **F** was distinguishable from the rest of the stands by being the most remote genetically. This trend is evident in the graphical representation shown in Figure 2. Two other subgroups appeared in the cluster diagram but groupings do not fit any strong geographic trend, as two of the northernmost samples (**N** and **R**) were grouped more closely with two of the southern populations (**A** and **W**) than with the other northern samples the two middle latitude stands (**B** and **C**). The correlation among genetic and geographic distances was not significant, with geographical distance, explaining only 1.4% of the variation in genetic distance among pairs of populations.

NEI's (1977) G_{ST} was estimated using only the polymorphic loci in order to emphasize the extent of population differentiation (BROWN 1979). A low level of population differentiation was observed and was manifest by the average G_{ST} value of 0.051, or close to 5% of the total diversity detected in all the samples being attributed to genetic differences among populations of Pacific silver fir (Table 8, All). Thus, the vast majority of allelic variation (95%) resides within individual stands.

The variation in G_{ST} among related species parallels results for most other conifer genera (reviewed by EL-

Table 8. Estimates of genetic distance and G_{ST} values for the eight original populations (ALL) and the eight different runs where populations were removed one at a time from the analysis (-A indicates a run without population A). The G_{ST} value of the eight populations is in bold and G_{ST} values that are higher than the original G_{ST} are in italics.

Genetic parameter	Populations								
	All	–A	–B	–C	–F	–H	–N	–R	–W
$\bar{D}$	0.007	0.007	0.007	0.007	0.006	0.007	0.007	0.006	0.007
G_{ST}	**0.05112**	0.05080	*0.05196*	0.04965	0.04719	0.05110	*0.05203*	0.04683	*0.05135*

KASSABY 1991). NEI (1973) stresses that G_{ST} values are population-specific and non-comparable unless breeding systems are similar. Given that variation in mating systems among populations of Pacific silver fir was detected (see mating system section above) and is often encountered in other conifers (ADAMS & BIRKES 1991) the concept of a species average G_{ST} may be inappropriate, yet NEI (1975) reports these values himself.

ROBERDS and CONKLE (1984) maintain that even though allele frequencies may not differ substantially, population structure may still be present. The mating system can affect population structure by promoting or restricting hybridization (RITLAND 1983). Mating systems characterized by some degree of inbreeding will exhibit reduced recombination and increased homozygosity which ultimately restricts gene flow and decreases the effective population size (RITLAND 1983, LOVELESS & HAMRICK 1984). Results of the present study suggest that some evolutionary forces in addition to the mating system may be acting in populations of Pacific silver fir.

The expectation of a high degree of population differentiation based on the biology of Pacific silver fir was not borne out in the analysis of electrophoretic variation, but this result may not be that surprising considering the limits to detection placed on it by the nature of the variables being estimated. Nonetheless, there are several lines of evidence which point to some limited heterogeneity among populations. Heterozygosity appears to be reduced in northern latitudes and in embryo gene pools. Heterozygosity appears to be higher in extant populations, suggesting selection is acting to eliminate inbred seeds in nature. Greater genetic diversity, as measured by n_e or H_e, was seen in populations sampled in southern Vancouver Island. Interestingly, a more southerly population, **F**, was also the most genetically differentiated, as revealed by its position in the genetic distance dendrogram (Fig. 2).

Genetic conservation

The understanding of the mating system, genetic structure of a species (*i.e.*, distribution of genetic variation within- and among-populations), and the search for genetically unique populations are important for genetic management and conservation programs. If the species' mating system and gene flow produce a homogeneous pattern of variation and adaptation across its range, then it is expected that forestry activities will result in a minimal impact on its genetic structure. On the other hand, if heterogeneous levels of genetic variation and adaptation are present across the landscape, then forestry practices should be conducted in such a way that the present level of heterogeneity is maintained. If species' genetic variation and its distribution are considered during forest management, then the goal of combining the utilization and conservation of unique populations can be achieved. This is a fundamental concept that modern forestry practices should strive to embrace.

The data collected from the electrophoretic assessment of genetic variation and its apportionment within and among the eight Pacific silver fir populations, indicated that only 5% of the total allelic variation was attributed to differences among populations (*i.e.*, 95% of the variation resided within-populations). Although this value is typical of most conifers investigated (HAMRICK & GODT 1989), the analysis did not equivocally identify populations with unique genetic attributes. Although the dendrogram of the genetic distance analysis indicated that population **F** was the most genetically differentiated (Fig. 2). It should be stated at this juncture that the mean genetic distance among the eight populations was $\bar{D}$ = 0.007 (Table 8).

The genetic relatedness among the eight populations could be presented in a hierarchical structure (dendrogram) using the electrophoretic data following the method of NEI (1973) (coefficient of gene differentiation, G_{ST}) (Fig. 2; All). In this hierarchical structure, the populations that are genetically most similar will cluster together forming a branch (Fig. 2). Populations that are genetically less similar will be located on separate branches (Fig. 2). The genetic uniqueness of a population can be determined using the same ap-

proach after the removal of that population from the data, and the same analysis is repeated and new dendrogram and average genetic distance ($\bar{D}$) as well as coefficient of gene differentiation (G_{ST}) for that run are calculated. The hierarchical relationship among the remaining populations relative to the original dendrogram can be used as an indication of the genetic uniqueness of the removed population. This approach was used by EL-KASSABY and YANCHUK (1995) in determining the genetic uniqueness of British Columbia's Pacific yew (*Taxus brevifolia*) populations.

The electrophoretic data from the eight populations were used to determine the presence or absence of genetic uniqueness among these populations. A total of eight analyses were conducted and their corresponding dendrograms, $\bar{D}$, and G_{ST} values were produced (Fig. 2 and Table 8). Changes in the dendrogram relative to the original (All) (*i.e.*, the relative order among the remaining seven populations) were only observed in cases where the G_{ST} value of that specific run was higher than the original analysis (Fig. 2 and Table 8). Lower G_{ST} values than the original estimate could only be produced after the removal of a population that is unique (*i.e.*, genetically different from the remaining populations). SLATKIN (1985) applied the same concept in the determintion of gene flow among *Hyla arborea* populations. In his analyis, higher gene flow estimates were observed when isolated populations were removed from the data. Thus, including remote populations in the analysis resulted in lowering the estimate of gene flow among all populations.

The observed changes could be interpreted as follows: the removal of any genetically unique population from the data resulted in the production of a stable dendrogram because its removal did not affect the calculations of the hierarchical genetic relatedness among the remaining populations (*i.e.*, this population is not important in "anchoring" the dendrogram). Conversely, the removal of any of the genetically similar population affected the remaining populations, thus resulting in changes in the relative order of the remaining populations. Following this concept, the eight analyses conducted produced a consistent association between the changes in the dendrogram order and the G_{ST} estimates indicating that populations **A**, **C**, **F**, **H**, and **R** all harbour some unique genetic attributes, at least for the isozyme systems considered. Therefore, these populations should be considered for conservation purposes. It is noteworthy to mention that the average genetic distances of the eight analyses did not differ from the original $\bar{D}$ = 0.007 (Table 8), indicating that when the genetic distances among populations are very small, average estimates are not sensitive enough to detect the observed differences in G_{ST} and genetic den-drogram orders.

Pacific silver fir is not under any genetic improvement program in British Columbia and our seed orchard experience have indicated that seed orchards do not represent a secure source for seed production. It should also be stated that all current planting programs are dependent on natural-stand seed collections. The knowledge gained from the species' mating system and the genetic uniqueness of some of its populations could be used to guide our effort in establishing several seed production areas. In this case, some of the seed production areas should be established in genetically unique populations. This approach will provide an opportunity to combine conservation and utilization efforts in a constructive fashion. Estimates of inbreeding levels will be important in determining the way these populations should be managed to minimize inbreeding. Furthermore, the establishment of seed production areas in genetically unique populations will secure their removal from short-term harvesting plans.

REFERENCES

ADAMS, W. T. 1983: Applications of isozymes in tree breeding. *In:* Isozymes in Plant Genetics and Breeding, Part A. Elsevier Sci. Publishing Company. (S.D. Tanksley and T.J. Orton, eds). pp. 381–400.

ADAMS, W.T. & BIRKES D. S. 1991: Estimating mating patterns in forest tree populations. *In:* Biochemical Markers in the Population Genetics of Forest Trees (Hattemer, H.H., S. Fineschi, F. Cannata, and M.E. Malvolti, eds.). 1991 SPB Academic Publishing bv, The Hague, The Netherlands. pp. 127–172.

BROWN, A. H. D. 1979: Enzyme polymorphisms in plant populations. *Theor. Pop. Biol.* **15**:1–42.

BROWN, A. H. D., MATHESON, A.C. & ELDRIDGE, K.G. 1975: Estimation of the mating system of *Eucalyptus obliqua* L'Herit. using allozyme polymorphisms. *Aust. J. Bot.* **23**:931–949.

BROWN, A. H. D. & G.F. MORAN, G. F. 1981: Isozymes and the genetic resources of forest trees. *In:* Proc. Symp. Isozymes of N.A. For. Trees and For. Insects (M.T. Conkle, ed.) USDA For. Serv. Gen. Tech. Rep. PSW–48. pp. 1–10.

CHAISURISRI, K., EDWARDS, D. G. W. & EL-KASSABY, Y. A. 1992: Genetic control of seed size and germination in Sitka spruce. *Silvae Genet.* **41**:348–355.

CHELIAK, W. M., MORGAN, K., DANCIK, B. P. STROBECK, C. & YEH, F. C. H. 1984: Segregation of allozymes in megagametophytes of viable seed from a natural population of jack pine, *Pinus banksiana* Lamb. *Theor. Appl. Genet.* **69**:145–151.

CLEGG, M. T. 1980: Measuring plant mating systems. *BioSci.* **30**:814–818.

CONKLE, M.T., P.D. HODGKISS, L.B. NUNELLY AND S.C. HUNTER. 1982: Starch gel electrophoresis of conifer seeds: a laboratory manual. Gen. Tech. Rep. PSW–64.

Pac. S.W. For and Range Exp. Sta. USDA For. Serv. Berkeley, Calif. 18pp.

CROW, J. F. & KIMURA, M. 1970. An Introduction to Population Genetics Theory. Harper and Row, N.Y.

DAVIDSON, R. H. 1990: Patterns of variation in Pacific silver fir (*Abies amabilis* (Dougl.) Forbes) on Vancouver Island. Ph.D. Thesis. University of British Columbia, Vancouver, B.C.

EDWARDS, D. G. W. 1979: An improved air seed-sorter for laboratory use. Env. Can., For. Serv. Rep. BC–X–188, 11pp.

EL-KASSABY, Y. A. 1991: Genetic variation within and among conifer populations: Review and evaluation of methods. *In:* Biochemical Markers in the Population Genetics of Forest Trees (Hattemer, H.H., S. Fineschi, F. Cannata, and M.E. Malvolti, eds.). 1991 SPB Academic Publishing bv, The Hague, The Netherlands. pp. 61–76.

EL-KASSABY, Y. A., MEAGHER, M. D., PARKINSON, J. & PORTLOCK, F. T. 1987: Allozyme inheritance, heterozygosity and outcrossing rate among *Pinus monticola* near Ladysmith, British Columbia. *Heredity* **58**: 173–181.

EL-KASSABY, Y.A., YEH , F. C. & SZIKLAI, O. 1982: Inheritance of allozyme variants in coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*). *Can. J. Genet. Cytol.* **24**:325–335.

EL-KASSABY, Y.A. & YANCHUK, A. D. 1995: Genetic variation of Pacific yew in British Columbia and its conservation (Baradat, Ph., W.T. Adams, and G. Müller-Starck, eds.). SPB Academic Publishing bv, The Netherlands. pp. 227–235.

FARRIS, M. A. & MITTON, J. B. 1984: Population density, outcrossing rate, and heterozygote superiority in ponderosa pine. *Evolution* **38**:1151–1154.

FINS, L. & LIBBY, W. J. 1982: Population variation in Sequoiadendron: Seed and seedling studies, vegetative propagation and isozyme variation. *Silvae Genet.* **31**:102–110.

FOLLES, M. W. 1959: Silvics of grand fir. U.S. For. Serv. Gen. Tech. Rep. PNW–8.

FOWELLS, H. A. 1965: Silvics of forest trees of the United States. USDA Agric. Handb. No. 271. pp. 546–556.

FRANKLIN, E. C. 1970: Survey of mutant forms and inbreeding depression in species of the family *Pinaceae*. USDA For. Serv. Res. Pap. SE–61, 21pp.

FRANKLIN, J. F. 1974: *Abies* Mill. Fir. *In:* Seeds of Woody Plants of the United States. (C.S. Schopmeyer, Tech. Coord.) U.S.D.A. Agric. Handb. No. 450:168–183.

FRANKLIN, J. F. & RITCHIE, G. A. 1970: Phenology of cone and shoot development of noble fir and some associated true firs. *Forest Sci.* **16**:356–364.

GREGORIUS, H.-R. 1987: The relationship between the concepts of genetic diversity and differentiation. *Theor. Appl. Genet.* **74**:397–401.

GREGORIUS, H. -R. & ROBERDS, J. H. 1986: Measurement of genetical differentiation among subpopulations. *Theor. Appl. Genet.* **71**:826–834.

GURIES, R.P. & LEDIG, F. T. 1977. Analysis of population structure from allozyme frequencies. *In:* Proc. 14th South. For. Tree Improv. Conf. Gainsville, Fla. June 14–16, 1977. pp. 246–253.

HAMRICK, J. L. 1982: Plant population genetics and evolution. *Amer. J. Bot.* **69**:1685–1693.

HAMRICK, J. L. & GODT, M. J. W. 1989: Allozyme diversity in plant species. *In:* Differentiation patterns in higher plants (Urbanska K, ed.). Academic Press, N.Y. pp. 53–67.

HANDLEY, D. L. 1982: The British Columbia industry's interest in the true firs. *In:* The Biology and Management of True Fir in the Pacific Northwest. (C.D. Oliver and R. M. Kenady, eds.) USDA For. Serv. Pac. Nor. West Range and Exp. Sta. and Univ. of Wash. Seattle, WA. pp. 261–262.

HARTL, D. L. 1980: Principles of Population Genetics. Sinauer Assoc. Sunderland, Mass.

HEDRICK, P. W. 1983: Genetics of Populations. Sci. Books Int. Portola Valley, CA.

JACOBS, B. F., WERTH, C. R. & GUTTMAN, S. I. 1984: Genetic relationships in *Abies* (fir) of eastern United States: an electrophoretic study. *Can. J. Bot.* **62**:609–616.

JONES, D. & MATLOFF, N. 1986: Statistical hypothesis testing in biology: a contradiction in terms. *J. Econ. Entomol.* **79**:1156–1160.

KHALIL, M. A. K. 1986: Variation in seed quality and some juvenile characters of white spruce (*Picea glauca* [Moench] Voss). *Silvae Genet.* **35**:78–85.

KRAJINA, V. J., KLINKA , K. & WORRALL, J. 1982. Distribution and ecological characteristics of trees and shrubs of British Columbia. Faculty of Forestry, UBC. 131pp.

LANDE, R. & SCHEMSKE, D. W. 1985: The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* **39**:24–40.

LEWIS, P. O. & WHITKUS, R. 1989: GENESTAT for microcomputers. *Am. Soc. Plant. Taxon Newsletter* **2**:15–16.

LI, P. & ADAMS, W. T. 1989: Range-wide patterns of allozyme variation in Douglas-fir (*Pseudotsuga menziesii*). *Can. J. For. Res.* **19**:149–161.

LOVELESS, M. D. & HAMRICK, J. L. 1984: Ecological determinants of genetic structure in plant populations. *Ann. Rev. Ecol. Sys.* **15**:65–95.

MARSHALL, D. R. & BROWN, A. H. D. 1975. Optimum sampling strategies in genetic conservation. *In:* Crop Genetic Resources for Today and Tomorrow (J.H. Frankel and J.G. Hawkes, eds.) Cambridge Univ. Press, London. pp. 53–80.

MILLAR, C. I. 1983: A steep cline in *Pinus muricata*. *Evolution* **37**:311–319.

MÜLLER-STARCK, G. & GREGORIUS, H.-R. 1986: Monitoring genetic variation in forest tree populations. *In:* 18th IUFRO World Congress, Ljubljana Yugoslavia, 1986. Division 2, Vol. II (E. Donaubauer, ed.) Sept. 7–21, pp. 589–599.

NEALE, D. B. & ADAMS, W. T. 1985: Allozyme and mating-system variation in balsam fir (*Abies balsamea*) across a continuous elevational transect. *Can. J. Bot.* **63**:2448–2453.

NEI, M. 1972: Genetic distance between populations. *Amer. Nat.* **106**:283–292.

NEI, M. 1973: Analysis of gene diversity in subdivided populations. *Proc. Nat. Acad. Sci. USA* **70**:3321–3323.

NEI, M. 1975: Genetic variability in natural populations. *In:* Molecular population genetics and evoultion. Morth-Holland Publ. Amsterdam, Oxford. pp.127–174.

NEI. M. 1977: F-statistics and analysis of gene diversity in subdivided populations. *Ann. Hum. Genet.* **41**:225–233.

NEI, M. 1978: Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**:583–590.

NEI, M. & ROYCHOUDHURY, A. K. 1974: Sampling variances of heterozygosity and genetic distance. *Genetics* **76**:379–390.

OWENS, J. N. & MOLDER, A. A. 1977: Sexual reproduction of *Abies amabilis*. *Can. J. Bot.* **55**:2653–2667.

PACKEE, E. C., OLIVER, C. D. & CRAWFORD, P. D. 1982: Ecology of Pacific silver fir. *In:* The Biology and Management of True Fir in the Pacific Northwest. (C.D. Oliver and R.M. Kenady, eds.) USDA For. Serv. Pac. Nor. West Range and Exp. Sta. and Univ. of Wash., Seattle, WA. pp. 19–34.

PHILLIPS, M. A. & BROWN, A. H. D. 1977: Mating system and hybridity in *Eucalyptus pauciflora*. *Aust. J. Biol. Sci.* **30**:337–344.

REHFELDT, G. E. & LESTER, D. T. 1969: Specialization and flexibility in genetic systems of forest trees. *Silvae Genet.* **18**:118–123.

RITLAND, K. 1983: Estimation of mating systems. *In:* Isozymes in Plant Genetics and Plant Breeding, Part A, (S.D. Tanksley and T.J. Orton, eds.). Elsevier, Amsterdam. pp. 289–302.

RITLAND, K. & EL-KASSABY, Y. A. 1985: The nature of inbreeding in a seed orchard of Douglas-fir as shown by an efficient multilocus model. *Theor. Appl. Genet.* **71**:375–384.

ROBERDS, J. H. & CONKLE, M. T. 1984: Genetic structure in loblolly pine stands: allozyme variation in parents and progeny. *For. Sci.* **30**:319–329.

SCHMIDT, R. L. 1957: The silvics and plant geography of the genus *Abies*. *In:* The coastal forests of British Columbia. B.C. For. Serv. Tech. Publ. T46. 31pp.

SCHOEN, D. J. 1988: Mating system estimation via the one pollen parent model with the progeny array as the unit of observation. *Heredity* **60**:439–444.

SCHOEN, D. J. & CLEGG, M. T. 1984: Estimation of mating system parameters when outcrossing events are correlated. *Proc. Natl. Acad. Sci. USA* **81**:5258–5262.

SHAW, D.V. & ALLARD, R. W. 1982: Estimation of outcrossing rates in Douglas–fir using isozyme markers. *Theor. Appl. Genet.* **62**:113–120.

SHAW, D.V., KAHLER, A. L. & ALLARD, R. W. 1981: A multilocus estimator of mating system parameters in plant populations. *Proc. Nat. Acad. Sci. U.S.A.* **78**:1298–1302.

SHEA, K. L. 1987: Effects of population structure and cone production on outcrossing rates in Engelmann spruce and subalpine fir. *Evolution* **41**:124–136.

SLATKIN, M. 1985: Rare alleles as indicators of gene flow. *Evolution* **39**:53–65.

SNEATH, P. H. A & SOKAL, R. R. 1973: Numerical Taxonomy. W. H. Freeman, San Francisco, USA.

SORENSEN, F. C. 1982: The roles of polyembryony and embryo viability in the genetic system of conifers. *Evolution* **36**:725–733.

SORENSEN, F. C. & FRANKLIN, J. F. 1977: Influence of year of cone collection on seed weight and cotyledon number in *Abies procera*. *Silvae Genet.* **26**:41–43.

SORENSEN, F. C., FRANKLIN, J. F. & WOOLLARD, R. 1976: Self- pollination effects on seed and seedling traits on noble fir. *For. Sci.* **22**:155–159.

STEINHOFF, R. J., JOYCE , D. G. & FINS, L. 1983: Isoyzme variation in *Pinus monticola*. *Can. J. For. Res.* **13**:1122–1131.

STOEHR, M. V. & FARMER, R. E. JR. 1986: Genetic and environmental variance in cone size, seed yield and germination properties of black spruce clones. *Can. J. For. Res.* **16**:1149–1151.

SURLES, S. E., HAMRICK, J. L. & BONGARTEN, B. C. 1989: Allozyme variation in black locust (*Robinia pseudoacacia*). *Can. J. For. Res.* **19**:471–479.

SWOFFORD, D. L. & SELANDER, R. B. 1981: BIOSYS-I: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Hered.* **72**:281–283.

TIGERSTEDT, P. M. A. 1973: Studies on isozyme variation in marginal and central populations of *Picea abies*. *Hereditas* **75**:47–60.

VAQUERO, F., VENCES, F. J. GARCIA, P., RAMIREZ, L. & PEREZ DE LA VEGA, M. 1989: Mating system in rye: variability in relation to the population and plant density. *Heredity* **62**:17–26.

WORRALL, J. 1983: Temperature-bud burst relationships in amabilis and subalpine fir provenance tests replicated at different elevations. *Silvae Genet.* **32**:203–209.

ZAR, J. H. 1984: Biostatistical Analysis (2nd ed.) Prentice Hall. Englewood Cliffs, N.J.