PATTERNS OF ALLOZYME VARIATION IN TAMARACK

(*LARIX LARICINA* (DU ROI) K. KOCH) FROM NORTHERN ONTARIO

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ABSTRACT

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Key Words: tamarack, eastern larch, Larix laricina, allozyme, isozyme, population, genetic variation, northern Ontario.

Roots from approximately 30 trees from each of 44 populations across the range of tamarack (Larix laricina (Du Roi) K. Koch) in northern Ontario were analyzed electrophoretically for allozymic variation in 14 enzyme systems coded by 20 loci. A low level of variability was found in this conifer. On average, 22.7 - 28.8% of the loci per population were polymorphic depending on the criterion of polymorphism, with a mean of 2.60 alleles per polymorphic locus. Expected and observed heterozygosity per population were 0.091 and 0.087, respectively. G-tests for allelic homogeneity among populations indicated genetic heterogeneity ($p < 0.05$) at four loci. Approximately 4% of the total genetic diversity resided among populations. The mean genetic distance over all pairs of tamarack was 0.0045. Genetic distance was significantly related to geographic distance, and the latter accounted for 9% of the variation in genetic distance. Seven significant ($p < 0.05$) canonical discriminant functions accounted for 67.4% of the total variation at the polymorphic loci. Genetic variation in tamarack appeared to be affected by the environmental variables. An evolutionary bottleneck might be responsible for the low variability. The relatively short colonization since glaciation seemed the most likely factors causing the relatively low differentiation among populations.

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INTRODUCTION

Most tree species are divided into relatively small breeding populations because of limited pollen or seed dispersal. Other factors such as physical isolation, breeding system, natural selection and random genetic drift accentuate such subdivision (Guries and Ledig 1981). The sum total of such ecological and genetic relationships among individuals and the populations they comprise is termed "population structure" (Jain 1975).

Of special interest to geneticists and tree breeders are the genetic consequences of population structure. It is generally recognized that forest tree species, especially conifers, are characterized by considerable genetic variation, both across their native range, and from tree to tree within stands. Allozyme analysis has become popular during the last decade for elucidating population genetic structure of forest trees. Unlike complex morphological and physiological traits, allozymes are products of single genes and provide direct measures of genetic variation. Furthermore, they are relatively easy to assay (Conkle 1972, 1981; Guries and Ledig 1982).

Much information has accumulated about electrophoretically detectable isozyme polymorphisms of various coniferous tree species. With a few exceptions (Fowler and Morris 1977; Simon et al. 1986), the occurrence of isozyme polymorphism in natural populations of species is universal. The majority of genetic variation has been found to be distributed within populations as compared to that among populations (Hamrick 1979; O'Malley et al. 1979; Yeh and O'Malley 1980; Guries and Ledig 1982; Dancik and Yeh 1983; Furnier and Adams 1986). Some authors (e.g. Yeh and O'Malley 1980) reported that in some cases the patterns of allelic frequencies and heterozygosities were clinal with respect to environmental factors, such as latitude, longitude and elevation. Such data can help us to get a deeper insight into the relationships between relevant environmental factors, optimal adaptation, and the genetic variation pattern of a tree species.

Tamarack (eastern larch, Larix laricina (Du Roi) K.Koch) is one of the most widely distributed north American coniferous species. Scientific and economic interest in this species has recently increased in eastern Canada (Coles 1979; Park and Fowler 1982) as well as the northeastern United States (Carter and Simpson 1985). This is due in part to the species' potential to alleviate the impending wood supply shortages in parts of eastern North America (Corriveau and Vallee 1981; Carter and Simpson 1985).

Tamarack's life history and ecological characteristics are known in a general way, but its population biology and

genetics have been little investigated. Almost nothing is known about the allozyme variation within and among populations. The objectives of the present study were to describe the genetic structure of tamarack within and among populations in northern Ontario using allozyme variation and to evaluate the relationship of these variation patterns to environmental gradients.

LITERATURE REVIEW

Ecology of Tamarack

Tamarack has one of the widest ranges of all north American conifers. It extends from Newfoundland to Alaska and from the northern limit of tree growth, south to Maryland and West Virginia (Roe 1957; Fowells 1965). Tamarack grows under extremely varied environmental conditions in both the boreal and the Great Lakes-St. Lawrence forest region. Exceptionally tolerant of high soil moisture and low temperature, it is usually confined to bogs and swamps in the southern parts of its distribution but further north it grows on much drier sites (Fowells 1965). These features have major implications for the forester in terms of management practices and tree improvement strategies.

Tamarack is one of the most rapidly growing conifers (Roe 1957). It was found to out-grow other native conifers in New Brunswick, Ontario, the Lake States and other northern areas of the United States (Littlefield 1939; Roe 1952; MacGillivray 1967; Cech et al. 1977; Mead 1978). It has been planted on only a limited scale in the past, but it is now considered as a potentially important reforestation species (MacGillivray 1969).

Chandler (1959) reported tamarack can be propagated

from cuttings taken from terminal shoots during August and September. Farmer et al. (1986) demonstrated that cuttings from greenhouse-produced seedlings and natural stands can also be rooted under certain conditions.

Genetics of Tamarack

Three basic methods are frequently used by researchers to study genetic variation in forest trees: provenance tests, progeny tests and allozyme analysis (Farmer et al. 1982). A provenance test is an experiment in which seeds are collected from a number of widely scattered stands (usually natural), and the seedlings are grown under a common environment to examine if any variation exists over its geographic range.

A progeny test compares the performance of offspring of different parents, or compares performance of offspring and parents.

Allozyme analysis reveals the genetic characteristics of organisms through analysis of enzymes. An allozyme is an enzyme that is the product of a particular allelic form of a gene, separable by electrophoretic procedure. Allozymes differ by their charge, size and shape, and by their specific properties in metabolic regulation.

After tissue preparation, protein extraction and stabilization, the allozymes are separated by electrophoresis of the protein extracts in starch, polyacrylamide, cellulose acetate or agarose gels. Most authors have used simple zone starch gel electrophoresis.

After electrophoresis, the gel can be stained for a particular enzyme by pouring on a solution containing a substrate and a dye to react with the enzyme. Dark bands appear and their relative positions are determined. These bands are then interpreted in terms of loci and alleles and their frequencies tallied.

The study of tamarack genetics has been mainly limited to a few provenance tests. The earliest work was done by Pauley (1965) in Minnesota. He reported that tree height in a nursery test, near the end of the second growing season ranged from 24 cm for an east-central Minnesota source to 59 cm for a Nova Scotia source. Bud-set occurred earliest in northern provenances. Jeffers (1975) reported significant differences in 8-year survival and height among tamarack provenances planted at two locations in the Lake States. Local provenances and some distant provenances ranked among the best for survival and height growth. Cech et al. (1977), in a test of 16 tamarack provenances in West Virginia, found significant differences among provenances in survival, height and diameter growth. Growth was relatively correlated with latitude of origin but evidently not related to elevation. They suggested that the most northern provenance tested (Ontario) was from a different base population.

Since tamarack has a more or less continuous, trans-continental distribution, genetic variation across the species' range is expected to be of a clinal nature for some characteristics along environmental gradients (Rauter and

Graham 1983). Several researchers have been concerned with geographic variation in height, date of bud set and root pattern (Rehfeldt 1970; Riemenschneider and Jeffers 1980). They found that a clinal variation pattern existed and, even where stands were isolated, the gene pool was highly variable and unsegmented. Thus, Rehfeldt (1970) suggested that tamarack was genetically highly variable at the intrapopulation level.

To understand the genetic structure of natural populations and develop effective breeding strategies, a few mating experiments have been carried out. In their controlled pollination experiment, Park and Fowler (1982) found relatively large specific combining ability variances for early seedling heights in tamarack. The authors concluded that vegetative propagation and clonal selection may have an important role to play in the genetic management of this species. Knowles et al. (1987) indicated that the mean multilocus outcrossing rate was 0.729 for tamarack, lower than estimates reported for most other conifers.

By using haploid megagametophytic tissue from mature trees, Cheliak and Pitel (1985) investigated inheritance and linkage of 29 allozymes in tamarack. In general, segregation ratios conformed to those expected for traits under single gene Mendelian control. It was also confirmed that the tight linkage between pairs of loci reported in many conifer species (e.g. Conkle 1981), also existed in tamarack.

Allozyme Variation in Forest Trees

Estimation of the relative amount and geographic distribution of genetically controlled variation is a central topic of tree resource management (Conkle 1979). General geographic variation of allozymes has been studied in many coniferous species, e.g. bristlecone pine (Hiebert and Hamrick 1983), jack pine (Ross and Hawkins 1986), Jeffrey pine (Furnier and Adams 1986), lodgepole pine (Wheeler and Guries 1982; Yeh et al. 1985), pitch pine (Guries and Ledig 1982), ponderosa pine (O'Malley et al. 1979), western white pine (Steinhoff et al. 1983), Douglas-fir (Yeh and O'Malley 1980), black spruce (O'Reilly et al. 1985; Yeh et al. 1986), Sitka spruce (Yeh and El-Kassaby 1980), and western larch (Fins and Seeb 1986).

Bergmann (1975, 1978) found that some allozymes showed environmentally dependent variation in Norway spruce (Picea abies). The frequency of the allele group Aph-B1/B2 markedly increased with latitude, whereas the allele group Aph-B3/B4 was more frequent in the southern parts of its range. It was therefore concluded that natural selection caused the geographic variation pattern and that one or several temperature variables might function as a predominant selective force.

Mitton et al. (1980) indicated that in ponderosa pine (Pinus ponderosa) heterozygote excesses in a locus were associated with slope aspect, and in another locus clinal differentiation was increased with decreasing elevation.

Steinhoff et al. (1983) reported that the southern populations of Pinus monticola were similar to each other, but differed from the northern populations in allelic frequency patterns.

Only few studies concerning the relationship between allozyme variation and edaphic and climatic effects have been reported. Grant and Mitton (1977) detected substantial differentiation of peroxidase enzymes in Engelmann spruce and subalpine fir along an elevational gradient. There were significant differences in gene frequences among Krummholz, flag, and spire growth forms for each species. An example of an edaphic effect was shown by Furnier and Adams (1986), who found that in Jeffrey pine, the populations on ultramafic soil were similar in allele frequencies and genetically different from those populations on a broader range of soils. Thiebaut et al. (1982) investigated the polymorphism of peroxidases 1 and 2 in beech (Fagus sylvatica L.). The variation of allelic frequencies showed the influence of climate. The climatic parameters involved were moisture and temperature regimes.

Not only the variation of allelic frequency, but also some biochemical properties of enzymes have been related to environmental factors. Vidgren and Hagman (1982) studied variation in the activity of the catalase enzyme on young seedlings of Scots pine (Pinus sylvestris L.). The activity of the enzyme expressed per milligram fresh weight of seedlings decreased with decreasing latitude of origin.

Although published studies show that forest trees are

among the most variable plants (Hamrick 1979), some exceptions have been reported. Copes (1981) stated that isozymes of western red cedar seedlings (Thuja plicata Donn) lacked variation in band patterns of 9 enzymes. Torrey pine (Pinus torreyana Parry ex Carr.), occurring in only two populations, was composed of identical homozygous genotypes at 59 isozyme loci (Ledig and Conkle 1983). In red pine (Pinus resinosa Ait.), Fowler and Morris (1977), as well as Simon et al. (1986) found no differences among individuals in the number and mobility of some isozymes. The authors attributed the low level of genetic variation to "evolutionary bottleneck". Genetic variation in allozymes of western larch (Larix occidentalis Nutt.) was studied by Fins and Seeb (1986). The authors concluded that for all measures of variation, this species scored lower than most, but within the range observed for western conifers. Furthermore, most of the variation was found within rather than between the population groups.

In contrast with the great body of data about allozyme variation in other North American forest trees, little information on tamarack is available. Knowles and Perry (1987) investigated allozyme variation in 10 tamarack populations from northwestern Ontario. The results showed that tamarack exhibited overall homogeneity in isozyme structure across the geographic area studied, with a small amount of patchy differentiation existing.

According to the literature reviewed above, allozyme variation appears to be distributed nonrandomly within and

among populations of forest trees. Such distribution is possibly attributed to the type of selection in temporally and spatially variable environments.

MATERIALS AND METHODS

Allozyme variation was studied in rooted cuttings from 1157 trees taken from 44 populations of tamarack sampled from northern Ontario between 46° 16′ N. and 50° 06′ N. latitude (Fig. 1). The location, elevation, and sample size of each population are listed in Table 1. These populations were part of the samples that D. Joyce (1987) selected and collected to assess genetic variation in cold hardiness of tamarack. The populations were selected from varied climatic areas within two forest regions -- the Boreal Forest Region and the Great Lakes-St. Lawrence Forest Region. The populations also represented the geographic distribution of the species in the potentially commercial range of tamarack in northern Ontario (Joyce 1987).

These populations were represented by wild seedlings dug up in the spring of 1986. The ages of individual seedlings were not estimated, but most seedlings were under 45 cm in height. After removal, the wild seedlings were transferred to a greenhouse and potted in 3.8-Liter containers. In July 1986, branch ends were removed from each sample seedling. They were vegetatively propagated in Spencer-Lemaire `Tinus' roottrainers (350 mL volume) filled with peat and vermiculite (1:1 ratio by volume). Temperature in the greenhouse was 18 - 28°C, with natural lighting. The new ramets were approximately 25 cm in height by January 1987.

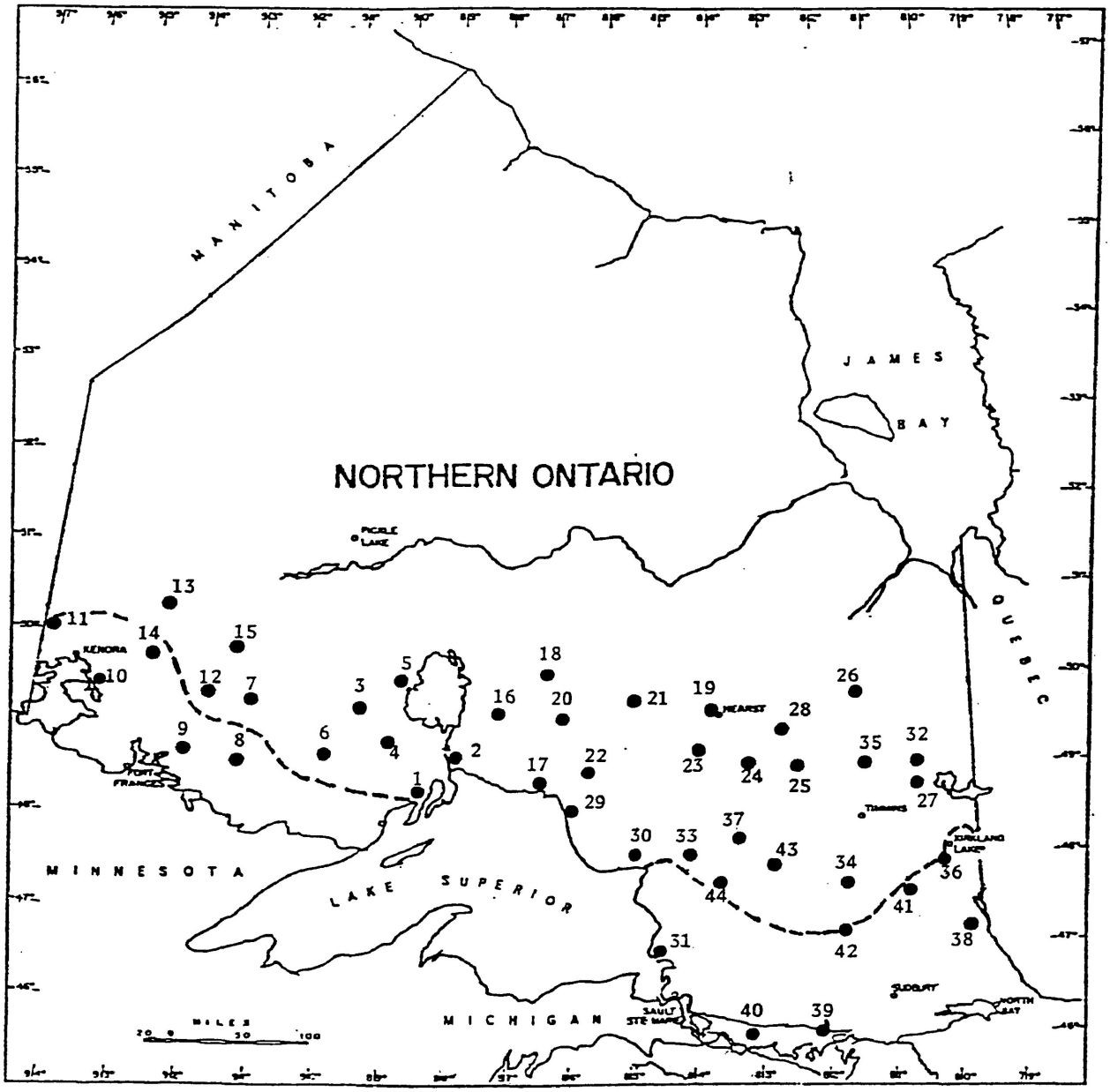

Fig. 1. Location of the 44 tamarack populations sampled in this study. The dashed line represents the junction of the Boreal Forest Region to the north and the Great Lakes - St. Lawrence Forest Region to the south.

Table 1. Details of the location and sample sizes of the 44 tamarack populations in this study.

Population	Location	Latitude (°N)	Longitude (°W)	Elevation (m)	No. of trees
1	Sibley	48.36	88.48	274	23
2	Helen Lake	49.04	88.13	244	25
3	McMullen Lake	49.35	89.47	457	28
4	Max Creek	49.11	89.22	457	32
5	Gull River	49.47	89.07	305	25
6	Upsala	49.00	90.25	488	29
7	Ignace	49.24	91.36	457	23
8	Atikokan	48.44	91.38	396	23
9	Mine Center	48.45	92.34	360	15
10	Bunny Lake	49.30	94.03	335	27
11	Sand Lake	50.03	94.48	320	22
12	Dyment	49.35	92.19	396	27
13	Ear Falls	50.30	93.09	366	26
14	Vermilion Bay	49.49	93.14	381	27
15	Sioux Lookout	50.06	91.47	366	29
16	Jellicoe	49.42	87.28	335	34
17	Steel River	48.49	86.42	229	19
18	Nakina	50.05	86.46	335	34
19	Hearst	49.44	83.54	244	36
20	Caramat	49.42	86.19	335	35
21	Mistake River	49.47	85.10	244	32
22	Manitouwadge	49.04	85.53	366	29
23	Hale	49.22	84.01	305	26
24	Rufus Lake	49.13	83.09	290	31
25	Saganash Lake	49.06	82.25	260	30
26	Frazerdale	49.49	81.34	213	21
27	Tunis	48.49	80.50	290	37
28	Harty	49.28	82.40	244	25
29	Pic River	48.42	86.15	260	31
30	Catfish Creek	48.13	84.51	427	27
31	Hibbard Bay	47.01	84.46	198	21
32	Wade Lake	49.04	80.36	305	22
33	Flame Lake	48.12	84.05	457	9
34	Gogama	47.42	81.45	350	26
35	N.Driftwood River	49.07	81.22	274	30
36	Engelhart River	48.01	80.15	305	32
37	Missinaib Lake	48.22	83.26	366	19
38	Latchford	47.16	79.46	381	29
39	Massey	46.16	82.08	260	24
40	Iron Bridge	46.17	83.14	183	28
41	Gowganda	47.40	80.41	381	20
42	Edinburg Lake	47.22	81.48	396	18
43	Shawmere River	48.00	82.55	427	32
44	Hoey Lake	47.50	83.39	488	21

Root Preparation

Root tips of tamarack were used for electrophoretic assay in this study. From January to June 1987, about 15 mg of actively growing root tips were excised from each ramet and placed in a 0.5 mL conical, polystyrene sample cup (Monoject Scientific) with 30 uL of extraction buffer (Appendix I). The cups were kept in a refrigerator for about 20 hours, then homogenized with a motorized teflon grinding head. Homogenization of the tissues released the enzyme into the buffered extract solution.

Starch Gel Preparation

Molds for the gels were formed by four plexiglass strips (10 x 200 mm) in a rectangular arrangement. Starch gels, 12.5% w/v, were then prepared (starch from Connaught Laboratories Limited) as follows: Approximately 1/3 of the gel buffer was added to the dry starch in a 1000 mL Erlenmeyer flask to make a suspended solution free of lumps. The remaining buffer was heated in a microwave oven to boiling, and then added to the starch suspension and swirled vigorously. This starch solution was then returned to the heat until it boiled throughout. A vaccuum was then applied to the solution until only large bubbles were left. The solution was poured into the gel molds and allowed to set. After about 15 minutes the gel molds were placed into plastic bags to prevent dehydration, and left at room temperature overnight.

Electrophoretic Procedure

The crude homogenate in the cups was absorbed onto 2 x 12 mm filter paper wicks. These wicks were inserted along a cut 20 mm from the edge of the gel. The gel would accommodate 40 samples along the longitudinal axis. Marker dyes (dilute red food colouring) were also loaded on the gel for tracking purpose. After loading the gel, 2/3 of the total running voltages was applied until the tracking dye had migrated 5 - 10 mm. The sample wicks were then removed and full running voltages were applied. To ensure that the gels were kept cool, electrophoresis was carried out in a refrigerator. Electrophoresis was continued until the buffer front had migrated approximately 8 cm.

The starch gel was sliced horizontally into 7-9 slices 1 mm thick using plexiglass guides and nylon thread. Each gel slice was placed in a tray and stained for the following enzyme systems: aspartate aminotransferase (AAT), diaphorase (DIA), esterase (fluorescent)(FLE), fumarase (FUM), glutamate dehydrogenase (GDH), glucose-6-phosphate dehydrogenase (G6P), hexokinase (HK), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), mannose-6-phosphate isomerase (MPI), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6PG), and shikimic acid dehydrogenase (SKDH). Inheritance of AAT, GDH, G6P, IDH, MDH, PGI, PGM, and 6PG has been previously described by Cheliak and Pitel (1985). Details of enzyme assays,

including the extraction, gel and electrode buffers, and composition of each of the enzyme staining solutions, are listed in Appendices I and II.

After the staining solutions had been added, the gels were incubated at 37°C until bands appeared, and then the gel phenotypes were interpreted.

Gel Interpretation

When an enzyme system was apparently controlled by multiple loci, those isozymes and the corresponding loci were identified by the symbol of the enzyme and a hyphenated numeral. The locus specifying the most anodally migrating isozyme was designated as 1, the next as 2, and so on (e.g. Aat-1, Aat-2). Within each locus, the most common allele was designated as 1. The other alleles were numbered according to their descending frequencies: the allele with highest frequency was assigned as 2, and the next highest one as 3, and so on. The exceptions are a few alleles for Mdh-4 and Pgi-2 whose frequencies were lower at the beginning of the laboratory analyses, but higher up to the final days, than their preceding alleles. Homozygotes for a particular allele were scored as 11, 22, 33, etc. Heterozygotes were scored as 12, 13, 23, etc.

Data Analysis

For each population, three measures of genetic variation were calculated: the average number of alleles per locus, percentage of polymorphic loci per population (two

criteria -- the frequencies of the most common alleles are less than and equal to 0.99 and 0.95) , and the average heterozygosity. Two measures of heterozygosity were used: observed heterozygosity (H_o) based on a direct count, and expected heterozygosity (H_e) based on the unbiased estimate of Nei (1978):

$$H_e = 1 - \sum p_i^2,$$

where p_i is the frequency of the ith allele.

Genotypic distributions were tested for agreement with Hardy-Weinberg expectations. Allelic and genotypic distributions were also tested for heterogeneity among populations. In both instances, the log-likelihood G-Statistic (Sokal and Rohlf 1981) was used to compare row by column observed frequencies. Alleles with low frequencies of occurrence (less than 1) in a population were bulked with the allele with the next lowest frequency (Snedecor and Cochran 1967).

The organization of genetic variation was examined using the F-statistics developed by Wright (1969; Nei 1977). This method was originally devised to examine hierarchical structuring in populations utilizing the correlation between uniting gametes within and among subpopulations and for the population as a whole. The estimate of F_{IS}, a measure of the deviation from Hardy-Weinberg proportion within subpopulations, was calculated as

$$F_{IS} = (H_S - H_O) / H_S,$$

where H_O and H_S are observed and expected heterozygosity, respectively. The calculational formula for H_S is identical to H_e above. Positive F_{IS} values indicate deficiencies of heterozygotes while negative values indicate an excess. The estimate of F_{ST}, a measure of genetic differentiation among populations, was calculated as

$$F_{ST} = (H_T - \bar{H}_S) / H_T,$$

where $\bar{H}_S$ is the average of expected heterozygosities among subpopulations, or subpopulation heterozygosity, and H_T is the total heterozygosity, namely $1 - \sum \bar{p}_i^2$.

The genetic distance statistic (D) developed by Nei (1972) was used to estimate the amount and pattern of geographic differentiation between all pairs of populations. Genetic distance is defined as

$$D = -\log_e I,$$

where I represents the normalized identity of genes between pairs of populations. If two populations have the same alelles in the same frequency, I = 1; when two populations have no alleles in common, I = 0. Cluster analysis of the resulting matrix of genetic distances was made using the unweighted pair group means analysis (UPGMA) procedure (Sneath and Sokal 1973). The relationship between genetic and geographic distances among the populations was examined using correlation analysis. The relationship was further investigated by the non-parametric Mantel Test of Matrix Correspondence (Mantel 1967; Smouse et al. 1986).

Sproule (1988) described the detailed procedures of the analysis. The test statistic is the sum of cross products:

$$Z_{YX} = \sum_{ij} (X_{ij}Y_{ij})$$

where X and Y are the distance matrices, Z_{YX} is the summation of the observed values over all ij pairs. Then the genetic distance matrix is randomly permuted by Monte Carlo randomization while the geographic matrix is held rigid. The resulting values comprise the permutation statistic, Z'_{YX}. The two tests (Z_{YX} and Z'_{YX}) are compared. Since larger Z'_{YX} values than Z_{YX} suggest positive association between the X_{ij}'s and the Y_{ij}'s, the proportion of the n! permutations for which Z'_{YX} is greater than or equal to Z_{YX} (the observed value) is the probability of obtaining the observed value by chance alone.

The pattern of multilocus variation in tamarack was examined by submitting the data to a discriminant functions analysis. This procedure functions to statistically maximize variation in an attempt to distinguish among the populations within a species. It has proven useful to the understanding of factors affecting genetic structure in forest trees (Knowles 1985; Yeh et al. 1985; Yeh et al. 1986). Yeh et al. (1985, 1986) described the detailed procedures of discriminant analysis. Prior to the analysis allozyme profiles of individual trees were coded according to their genotypes, with each allele being assigned the value of 0.5 if present, and 0 if absent, using an algorithm developed by Smouse and Neel (1977). According to

this method, each polymorphic locus is represented by a vector with n - 1 independent dimensions, where n is the number of alleles at the locus. Thus, at a triallelic locus with alleles $\underline{A}_1$, $\underline{A}_2$, and $\underline{A}_3$, the vector Y = (1, 0; 0.5, 0.5; 0.5, 0; 0, 1; 0, 0.5; 0, 0) represents the two dimensions for the six possible genotypes $\underline{A}_1\underline{A}_1$, $\underline{A}_1\underline{A}_2$, $\underline{A}_1\underline{A}_3$, $\underline{A}_2\underline{A}_2$, $\underline{A}_2\underline{A}_3$, $\underline{A}_3\underline{A}_3$, respectively.

In order to examine the relationship between genetic variation and environmental factors, the discriminant scores for each significant ($p < 0.05$) discriminant function were regressed against the environmental variables. Ten environmental variables were used: latitude (LAT), longitude (LONG), elevation (ELEV), minimum January temperature (MJAN), average January temperature (AJAN), maximum July temperature (MJUL), average July temperature (AJUL), annual snowfall (ASNOW), annual days with rain (ARAIN), and annual total precipitation (APREC). The multiple regression model for each discriminant function had the above-mentioned ten independent terms and was modified by backward elimination to a final model retaining only terms significant at $p < 0.05$. The climatic data in connection with the 44 populations were obtained from "Canadian Climate Normals, Temperature and Precipitation, 1951 -- 1980, Ontario" (Environment Canada 1982). The weather station nearest to the stand sampled, within a range of 60 kilometers, was chosen for the environmental information.

RESULTS

Allele Frequency and Genetic Variability

A total of 20 loci was inferred from the 14 enzyme systems surveyed. Twelve of these loci (Aat-1, Aat-2, Fum, Gdh, G6p, Hk, Mdh-1, Mdh-2, Mdh-3, Mpi, Pgi-1, and Skdh) were monomorphic. The remaining 8 loci (Aat-3, Dia, Fle-2, Idh, Mdh-4, Pgi-2, Pgm, and 6pg) were polymorphic in at least one population. In total, 40 alleles were detected in this study. Loci were classified as polymorphic whenever two or more alleles were detected in a population. Allelic frequency data at the eight polymorphic loci are presented for each population (Appendix III).

Table 2 gives the values of H_e per locus per population. Averaging H_e across the 44 populations yielded the following values listed in descending order: Pgi-2 (0.560), Mdh-4 (0.553), Aat-3 (0.369), 6pg (0.150), Dia (0.142), Pgm (0.034), Fle-2 (0.016), Idh (0.003).

Polymorphic loci fell into three classes according to their average heterozygosities. The loci Dia, Fle-2, Idh, Pgm, and 6pg had low levels of heterozygosity and were monomorphic in some populations surveyed. One allele (allele 1) predominated in all populations.

Table 2. Expected heterozygosity of 44 tamarack populations at eight polymorphic loci.

Population	Aat-3	Dia	Fle-2	Idh	Mdh-4	Pgi-2	Pgm	6pg
1	.440	.083	.000	.000	.623	.575	.043	.122
2	.480	.039	.000	.000	.578	.576	.077	.180
3	.469	.069	.069	.000	.664	.617	.000	.069
4	.390	.031	.031	.000	.495	.642	.061	.219
5	.365	.218	.000	.039	.486	.423	.000	.113
6	.428	.185	.000	.000	.632	.485	.034	.285
7	.364	.043	.000	.000	.468	.647	.000	.194
8	.227	.000	.000	.000	.552	.555	.043	.194
9	.124	.000	.000	.000	.620	.620	.000	.000
10	.444	.226	.000	.000	.578	.658	.072	.000
11	.325	.000	.044	.000	.666	.681	.000	.044
12	.384	.198	.000	.000	.594	.444	.036	.168
13	.334	.204	.000	.038	.662	.652	.000	.074
14	.324	.172	.036	.000	.619	.580	.036	.071
15	.262	.212	.034	.000	.619	.524	.000	.067
16	.457	.057	.000	.000	.663	.656	.029	.208
17	.444	.100	.000	.000	.632	.615	.000	.100
18	.309	.000	.084	.000	.626	.567	.084	.084
19	.461	.219	.027	.000	.534	.646	.000	.239
20	.493	.082	.000	.000	.628	.542	.000	.157
21	.390	.219	.000	.000	.474	.482	.031	.144
22	.158	.128	.034	.000	.548	.521	.000	.098
23	.375	.074	.074	.038	.425	.646	.038	.204
24	.425	.271	.000	.000	.511	.629	.000	.121
25	.375	.153	.000	.000	.424	.391	.000	.124
26	.495	.046	.000	.000	.452	.644	.000	.046
27	.253	.193	.000	.000	.579	.514	.000	.149
28	.444	.113	.039	.000	.463	.669	.000	.147
29	.412	.121	.032	.000	.549	.610	.032	.148
30	.475	.105	.105	.000	.630	.527	.000	.226
31	.472	.172	.000	.000	.595	.472	.000	.133
32	.375	.201	.000	.000	.528	.536	.000	.268
33	.475	.105	.000	.000	.512	.568	.000	.346
34	.375	.204	.000	.000	.615	.382	.038	.109
35	.406	.153	.000	.000	.492	.521	.033	.124
36	.305	.195	.000	.000	.404	.512	.031	.061
37	.361	.361	.000	.000	.422	.586	.051	.188
38	.307	.349	.000	.000	.595	.430	.034	.262
39	.249	.117	.000	.000	.477	.500	.080	.117
40	.357	.191	.101	.000	.600	.528	.035	.293
41	.139	.420	.000	.000	.420	.579	.335	.219
42	.346	.105	.000	.000	.494	.606	.105	.153
43	.375	.031	.000	.000	.614	.509	.000	.219
44	.387	.091	.000	.000	.550	.577	.133	.133
Mean	.369	.142	.016	.003	.553	.560	.034	.150

The locus Aat-3 had a moderate level of heterozygosity. This locus had one major allele (allele 1), predominating in all populations. No monomorphic population was found.

The loci Mdh-4 and Pgi-2 were highly polymorphic and the frequency of the most common allele varied considerably among the populations. These two loci had no single allele predominating in all populations. At Mdh-4, allele 2 was the most common allele in populations 10, 11, and 15. At Pgi-2, allele 3 occurred with the highest frequencies in populations 7 and 11.

Three measures of genetic variability that summarize data from all 20 loci are presented in Table 3: (1) the average number of alleles per locus (A), (2) percentage of polymorphic loci per population (P), and (3) the expected and observed heterozygosity (H_e and H_o). The polymorphic loci Aat-3, Idh, and 6pg segregated for two alleles, Dia and Fle-2 segregated for three alleles, Pgm segregated for four alleles, and Mdh-4 as well as Pgi-2 for six alleles.

The values of A ranged from 1.25 in population 9 to 1.65 in populations 14 and 23. An average population of tamarack had 1.46 alleles per locus and 2.60 alleles per polymorphic locus. Since two criteria for polymorphic loci are often found in the literature, two percentages (99% and 95%) of loci polymorphic per population are given in Table 3. An average population of tamarack was polymorphic for 22.73 - 28.75 percent of its loci depending upon the criterion of polymorphism used. The average expected and observed heterozygosity were 0.091 and 0.087, respectively.

Table 3. Genetic variability in 44 tamarack populations[a]

Pop	A	P_1	P_2	H_e	H_o
1	1.45	30.0	20.0	.094	.098
2	1.45	30.0	20.0	.097	.092
3	1.55	30.0	15.0	.098	.089
4	1.55	35.0	20.0	.093	.075
5	1.50	30.0	25.0	.082	.070
6	1.45	30.0	25.0	.102	.084
7	1.35	25.0	20.0	.086	.061
8	1.40	25.0	20.0	.078	.078
9	1.25	15.0	15.0	.068	.063
10	1.55	25.0	20.0	.099	.104
11	1.45	25.0	15.0	.088	.082
12	1.55	30.0	25.0	.091	.080
13	1.45	30.0	20.0	.098	.110
14	1.65	35.0	20.0	.092	.080
15	1.40	30.0	20.0	.086	.083
16	1.55	30.0	20.0	.103	.078
17	1.40	25.0	25.0	.095	.088
18	1.40	30.0	15.0	.088	.085
19	1.50	30.0	25.0	.106	.103
20	1.45	25.0	20.0	.095	.104
21	1.45	30.0	25.0	.087	.083
22	1.45	30.0	25.0	.074	.071
23	1.65	40.0	20.0	.094	.100
24	1.45	25.0	25.0	.098	.084
25	1.45	25.0	25.0	.073	.067
26	1.45	25.0	15.0	.084	.079
27	1.40	25.0	25.0	.084	.073
28	1.45	30.0	25.0	.094	.081
29	1.50	35.0	25.0	.095	.097
30	1.50	30.0	30.0	.103	.098
31	1.40	25.0	25.0	.092	.086
32	1.40	25.0	25.0	.095	.095
33	1.40	25.0	25.0	.100	.117
34	1.40	30.0	25.0	.086	.077
35	1.50	30.0	25.0	.086	.082
36	1.45	30.0	20.0	.075	.073
37	1.40	30.0	25.0	.099	.113
38	1.50	30.0	25.0	.099	.097
39	1.45	30.0	25.0	.077	.065
40	1.60	35.0	30.0	.105	.095
41	1.40	30.0	30.0	.106	.112
42	1.45	30.0	30.0	.090	.103
43	1.40	25.0	20.0	.087	.078
44	1.40	30.0	25.0	.093	.081
Mean	1.46	28.6	22.7	.091	.087

[a]A: average no. of alleles per locus; P_1 and P_2: Percentage of loci polymorphic, where the frequency of the most common allele is $\leq .99$ (.95); H_e and H_o: expected and observed heterozygosity.

Analysis of Population Genetic Structure

Allelic heterogeneity chi-square tests were performed on a per-locus basis among all populations (Table 4). Four of the eight polymorphic loci (Aat-3, Dia, Mdh-4, and Pgi-2) were found heterogeneous among the populations at $p < 0.001$. The among-population variability estimates showed that significant differentiation exists among populations at the above four loci. Hardy-Weinberg expectations were tested for all loci with two or more alleles within populations. Out of the 352 tests performed within populations only twelve (3%) showed a significant departure from the expected genotypic distribution, and eight of the twelve cases were due to an excess of homozygotes.

A summary of the F statistics is provided in Table 5. Values for F_{IS} are presented for each population at each locus. In those populations where alleles are fixed, the F_{IS} statistics have not been calculated. A small excess of heterozygotes was shown in the less variable loci Fle-2 and Idh, while no consistent patterns for the direction of deviation among the F_{IS} were found for the other loci. Population 7 showed the greatest value for mean F_{IS} (0.290). Average of F_{IS} over all loci was 0.050, indicating a 5% deficiency of heterozygotes relative to Hardy-Weinberg expectations. The extent of genetic differentiation among populations was measured by F_{ST} (Table 5). The values ranged from 0.018 for Idh to 0.054 for Pgm with a mean over all loci of 0.042. The result indicated that approximately 96% of

Table 4. Summary of allelic heterogeneity test for the eight polymorphic loci.

Locus	G	df	Probability
Aat-3	105.17	43	<0.001
Dia	83.14	39	<0.001
Fle-2	61.45	86	ns
Idh	16.33	43	ns
Mdh-4	185.05	84	<0.001
Pgi-2	172.07	86	<0.001
Pgm	29.99	22	ns
6pg	51.66	41	ns

Note: ns, not significant.

Table 5. Estimates of F_{IS} and F_{ST} for eight polymorphic loci

	Pop	Aat-3	Dia	Fle-2	Idh	Mdh-4	Pgi-2	Pgm	6pg	Mean
F_{IS}	1	.110	-.045	***[a]	***	-.117	-.059	-.022	-.070	-.038
	2	.167	-.020	***	***	-.107	-.111	-.042	.778	.047
	3	.314	-.037	-.037	***	.032	.017	***	-.037	.087
	4	.279	-.016	-.016	***	.052	.221	-.032	.429	.197
	5	.123	.265	***	-.020	.094	.244	***	-.064	.148
	6	.356	-.115	***	***	.018	.288	-.018	.275	.176
	7	.881	-.022	***	***	.349	-.074	***	.327	.290
	8	.233	***	***	***	-.103	-.097	-.022	.327	.003
	9	-.071	***	***	***	.032	.140	***	***	.072
	10	-.167	-.149	***	***	-.217	.212	-.029	***	-.048
	11	.302	***	-.023	***	.113	-.068	***	-.023	.071
	12	.229	-.125	***	***	.376	-.252	-.019	.339	.127
	13	-.268	-.130	***	-.020	-.220	.056	***	-.040	-.117
	14	.201	-.076	-.019	***	.341	-.022	-.019	-.038	.134
	15	.342	-.137	-.018	***	-.003	-.119	***	1.000	.036
	16	.356	-.030	***	***	.157	.328	-.015	.150	.247
	17	.250	-.056	***	***	.083	-.027	***	-.056	.072
	18	-.046	***	-.046	***	.154	-.037	-.046	-.046	.028
	19	-.324	.365	-.014	***	-.092	.011	***	.768	.034
	20	.132	-.045	***	***	-.092	-.319	***	-.094	-.097
	21	.279	-.143	***	***	-.055	.093	-.016	-.085	.048
	22	-.094	-.074	-.018	***	-.181	.008	***	-.055	.049
	23	-.128	-.040	-.040	-.020	-.176	-.072	-.020	.246	-.068
	24	.317	.285	***	***	-.072	.076	***	.466	.143
	25	.378	-.091	***	***	.058	-.023	***	-.071	.092
	26	.135	-.024	***	***	.053	.039	***	-.024	.067
	27	.039	-.121	***	***	.113	.369	***	-.088	.135
	28	.250	-.064	-.020	***	-.036	.282	***	-.087	.140
	29	.061	-.069	-.016	***	.061	-.217	-.016	.347	-.017
	30	.143	.647	-.059	***	-.116	.016	***	.179	.051
	31	.394	.447	***	***	-.120	-.111	***	-.077	.070
	32	-.091	-.128	***	***	-.119	-.017	***	.490	-.001
	33	-.169	-.059	***	***	-.084	-.565	***	.357	-.163
	34	.282	.246	***	***	.061	-.209	-.020	.646	.107
	35	.097	-.091	***	***	.051	-.023	-.017	.464	.055
	36	-.026	.198	***	***	-.159	.023	-.016	1.000	.025
	37	-.310	-.019	***	***	-.246	-.258	-.027	.441	-.148
	38	.215	-.186	***	***	-.102	.037	-.018	.342	.023
	39	.498	-.067	***	***	.038	.250	-.043	-.067	.161
	40	.499	-.120	-.057	***	.226	-.014	-.018	-.217	.101
	41	-.081	-.190	***	***	-.190	-.123	.403	-.143	-.066
	42	.036	-.059	***	***	-.125	-.282	-.059	-.091	-.137
	43	.000	-.016	***	***	.084	.080	***	.429	.106
	44	.138	-.050	***	***	.394	-.320	.641	.641	.134
	Mean	.149	-.009	-.037	-.020	.018	-.013	.127	.233	.050
F_{ST}		.045	.051	.028	.018	.044	.039	.054	.030	.042

[a] *** = insufficient variation for F_{IS} calculations.

the genetic variation in tamarack resided within populations, while only 4% of the variation was expressed among populations.

Geographic Differentiation in Tamarack

Multilocus genetic distances were calculated for all pairs of populations (Appendix IV). The population pair of 5 and 25 was most similar (D = 0.0003) and that of 20 and 41 was the most distinct (D = 0.016), with a very small average distance of 0.0045 for all pairs. Although associations between genetic and geographic distances were not evident from cluster analysis (Fig. 2) of the genetic distances, some trends were seen in the analysis: populations that were geographically close tended to cluster together. For instance, populations 1, 2, 3, 4, 17, 16, and 18, which were around Lake Nipigon, clustered together. So did the populations in the eastern part of the area sampled. Cluster analysis also indicated that populations 8, 10, 11, and especially population 41, were the most divergent of all populations and also distinctly separated from each other.

A correlation analysis performed to verify the relationship between genetic and geographic distances among populations yielded a value of $\underline{r} = 0.297$ ($\underline{r}^2 = 0.088$).

After a run of 10,000 permutations for the Mantel Test of Matrix Correspondence, no permuting values were found to exceed the observed value, indicating a significant ($p < 0.001$) correlation between genetic distance and geographic distance among populations.

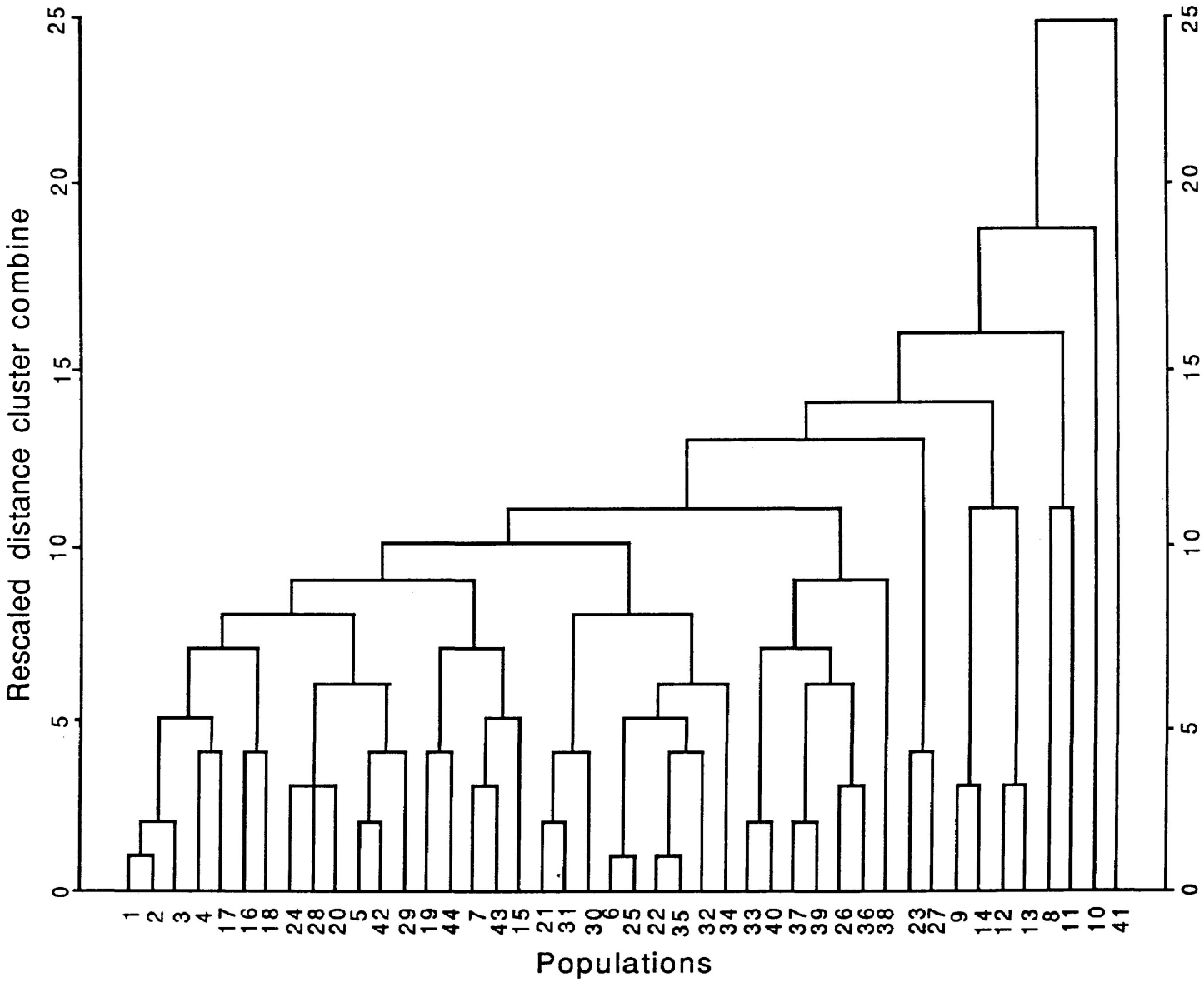

Fig.2. Dendrogram of genetic distance

Discriminant Analysis of Population Differentiation

The first seven of the 19 discriminant functions were significant and accounted for 67.4% of the total variation observed at the seven polymorphic loci (Table 6). Canonical R^2 values indicated that variation explained by the among-population differentiation in these scores ranged from 19% in the first discriminant function to 7% in the seventh discriminant function.

The first discriminant function was dominated by large loading factors for Pgm and Mdh-4. Scatter plot (Fig. 3) of populations showed population 41 to be very different from others, showing the same result as the cluster analysis (Fig. 2). Frequency of the most common allele for Pgm (0.775) of this population was very low compared to those for the other populations (see Appendix III). For locus Mdh-4, population 41 had only two alleles while each of the other populations possessed at least three alleles. The second function was dominated by large loading factors from loci Pgi-2, Mdh-4 and particularly Dia. This function was responsible for the separation of populations 5, 11, and 38 (Fig. 3). However, the first two functions were unable to adequately separate the other populations. Population 10, which was separated by cluster analysis, showed a difference from other populations in the sixth discriminant function. Discriminant analysis revealed similar results with that of cluster analysis of genetic distance, but failed to isolate population 8.

Table 6. Discriminant function coefficients generated from discriminant analysis for the first seven significant ($p < 0.05$) functions.[a]

	Discriminant function coefficient						
Locus	1	2	3	4	5	6	7
Aat-3	.3533	.0702	.4428	.0005	.1237	.4933	.0303
Dia	.4632	1.9273	-1.4787	2.2443	.7395	.4657	.6224
Fle-2	.1646	.5251	-.8354	.9148	.8602	.6375	.1404
Mdh-4	.9122	-1.5013	-.6008	1.0475	4.9878	-2.4359	.8113
Pgi-2	.6393	1.0951	2.6264	1.0240	-.9123	1.2272	1.0638
Pgm	.8178	-.4603	-.9730	-.3829	-.7782	.7466	-.4861
6pg	-.1511	.0833	.2416	.0784	.1775	-.0098	-.1500
Eigenvalue	.2338	.1555	.1216	.1180	.0958	.0826	.0732
Percent of variance	17.90	11.91	9.31	9.04	7.33	6.32	5.60
Canonical correlation (R)	.4353	.3669	.3292	.3249	.2956	.2762	.2611
Probability	<.0001	<.0001	<.0001	<.0001	<.0001	<.01	<.05

[a]Only the largest discriminant function coefficient at each locus is present. Locus Idh is omitted due to insufficient variation.

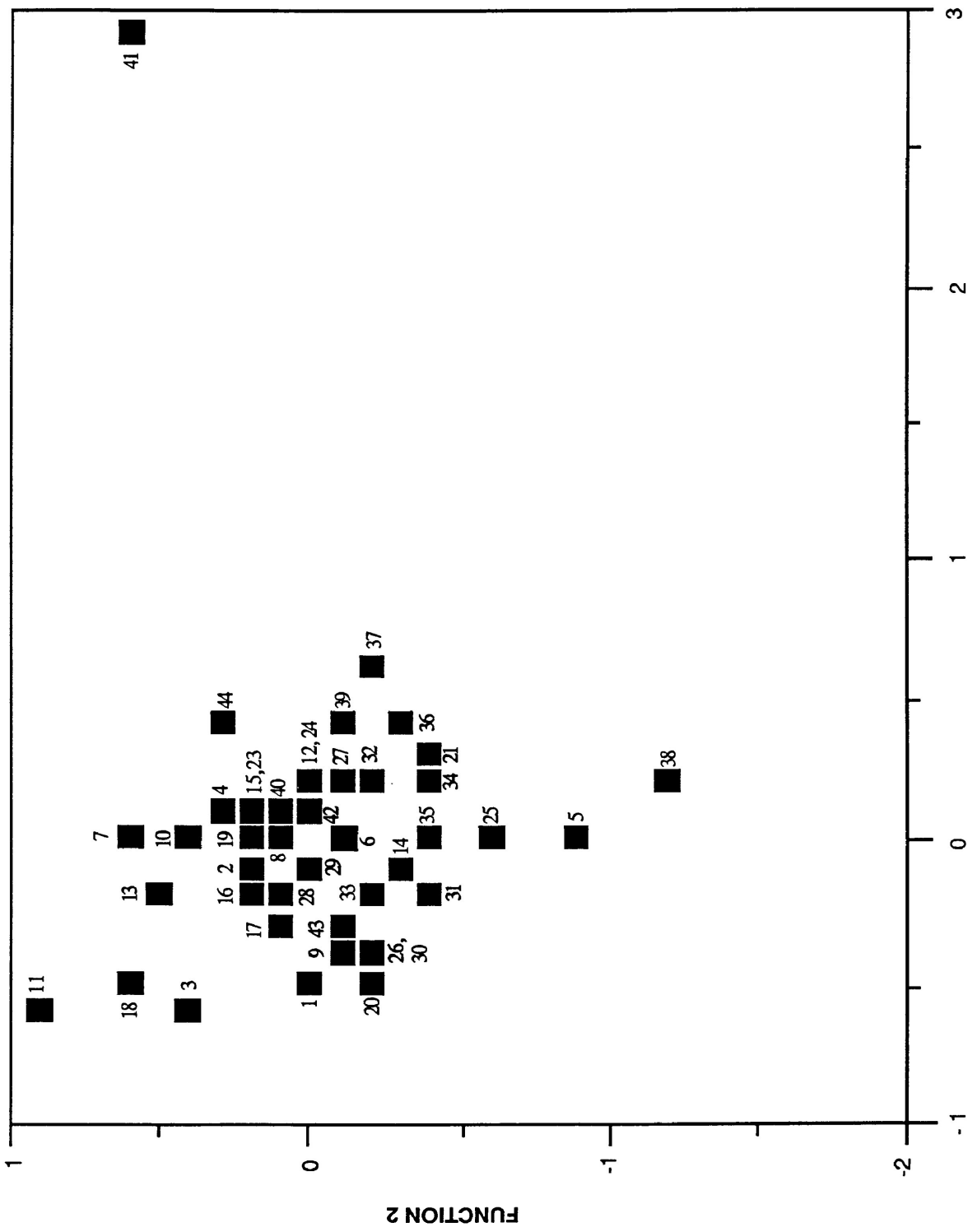

Fig. 3. Scatter plot of 44 tamarack populations on the axes of the first two functions of the discriminant analysis.

Correlation between Genetic Variation and Environmental Variables

The outcome of multiple regression analysis between the seven significant discriminant functions and the environmental factors are given in Table 7 (the multiple regression equations are listed in Appendix V). Both the geographic and climatic variables seemed to affect the genetic structure of tamarack, even though in no case did the regression models account for more than 5% of the variation in the discriminant functions. The general pattern of variation described by the first seven discriminant functions showed that latitude and winter temperature made the most important contribution to the genetic variation of tamarack. Longitude, water regime, and summer temperature contributed to a lesser extent. Further, since a larger absolute value of beta indicates a more important variable, the results suggested that latitude was a more important variable than winter temperature in the first discriminant function, longitude was the most important factor in the second discriminant function, and so forth. Note that elevation did not affect the distribution of genetic variation of tamarack.

Table 7. Result of regressing discriminant scores for the first seven discriminant functions against environmental variables. Only significant variables are listed.

Discriminant function	Location variable (Probability)	Beta	Canonical R^2
DIS1	LAT[a] (<.0001)	-.3931	0.043
	AJAN (<.0001)	-.2825	
DIS2	LONG (<.0001)	.1661	
	ASNOW (<.05)	.0778	0.036
	ARAIN (<.01)	-.1016	
DIS3	AJUL (<.001)	.1121	0.027
	ASNOW (<.01)	-.0876	
DIS4	LAT (<.05)	.1309	
	LONG (<.05)	-.0741	0.011
	MJAN (<.01)	.1474	
DIS5	ARAIN (<.0001)	.1873	0.030
	APREC (<.001)	-.1107	
DIS6	MJAN (<.01)	.1009	
	MJUL (<.0001)	.2336	0.014
	AJUL (<.001)	-.2143	
DIS7	AJUL (<.05)	-.0718	
	ASNOW (<.0001)	-.1491	0.015
	ARAIN (<.05)	.0856	

[a]Environmental variable code: LAT = latitude, LONG = longitude, MJAN = minimum January temperature, AJAN = average January temperature, MJUL = maximum July temperature, AJUL = average July temperature, ASNOW = annual snowfall, ARAIN = annual days with rain, and APREC = annual total precipitation.

DISCUSSION

The most important conclusions based on these analyses are: 1) tamarack contains a low level of genetic variability, 2) relatively little differentiation resides among populations, and 3) allelic heterogeneity does exist in tamarack and is at least partially associated with environmental variables.

Hamrick et al. (1981) reported that 20 conifer species had an average of 67% of their loci polymorphic per population, 2.29 alleles per locus, and mean individual heterozygosities of 0.207. Very similar results have also been obtained from more recent studies, e.g. in pitch pine (Guries and Ledig 1982), lodgepole pine (Yeh et al. 1985), Jeffrey pine (Furnier and Adams 1986), Douglas-fir (Merkle and Adams 1987). The data of this study (22.7 - 28.8% polymorphic loci, 1.46 alleles per locus, and average expected heterozygosity of 0.091) indicate a much lower level of variability than the mean values of some other conifers. The values of F statistics of tamarack fall within the range for other conifers (Table 8), but they are relatively low. Genetic distance analysis confirms that relatively little genetic differentiation has occurred among tamarack populations, even though the sampling area is large with a latitudinal range of 4.2^{o} and longitudinal range of 15^{o}.

Table 8. Population differentiation of some conifers

Species	F_{ST}	Reference
Larix laricina	0.04	This study
Larix occidentalis	0.09	Fins and Seeb (1986)
Picea mariana		
upland stands	0.07	
lowland stands	0.05	O'Reilly *et al.* (1985)
Picea sitchensis	0.08	Yeh and El-Kassaby (1980)
Pinus banksiana	0.02	Dancik and Yeh (1983)
Pinus contorta	0.06	Wheeler and Guries (1982)
Pinus jeffreyi	0.14	Furnier and Adams (1986)
Pinus monticola	0.15	Steinhoff *et al.* (1983)
Pinus ponderosa	0.12	O'Malley *et al.* (1979)
Pinus resinosa	0.00	Fowler and Morris (1977)
Pinus rigida	0.02	Guries and Ledig (1982)
Pinus sylvestris	0.02	Gullberg *et al.* (1985)
Pseudotsuga menziesii	0.03	Yeh and O'Malley (1980)
Sequoiadendron giganteum	0.10	Fins and Libby (1982)

There could be many reasons to account for the low level of variability and differentiation observed in tamarack. One plausible hypothesis for the low variability in tamarack involves an evolutionary bottleneck. Fins and Seeb (1986) proposed this hypothesis to explain low genetic variability in western larch. The concept of a "bottleneck" refers to a period during which only a few individuals survive to continue the existence of the population. Variation was once greater, but has been lost. Contemporary tamarack may be derived from small refugia during Pleistocene glaciation as is proposed for some other conifers (Critchfield 1984). These refugia could have played a role of genetic bottleneck and resulted in low genetic variability. In addition, many tamarack stands appear to have been established with seeds from a few individuals remaining after large destructive fires (Fins and Seeb 1986), and perhaps after disease and insect infections. Such stands would be expected to show low variability (Howe 1976). Tamarack is highly susceptible to fire damage because of its thin bark and shallow root system. Further, widespread damage through repeated defoliation may take place owing to its most serious parasite, the larch sawfly (Pauley 1965; MacGillivray 1969).

A well-known example of lack of variability is red pine, Pinus resinosa, (Fowler and Morris 1977; Allendorf

et al. 1982; Simon et al. 1986). It was attributed to a drastic reduction in size of red pine populations as a consequence of the Wisconsin glaciations. The same reason may be applicable to the low variability found in tamarack, although tamarack contains higher variability than red pine does.

Although the level of differentiation among populations found in tamarack is comparable to that observed for other conifers, it is still relatively low. A number of possibilities may account for the low differentiation.

Due to its continuous distribution, tamarack populations may be genetically linked by constant, or at least periodic, gene flow through a network of stands. Theoretically, as a wind-pollinated species, tamarack populations suffer no significant obstruction to gene flow. Such long distance pollen dispersal would theoretically lead to the relatively low differentiation among populations. However, the overall mean of F_{IS} is a positive value (0.050, Table 5), indicating a slight inbreeding in this species. Also, Knowles et al. (1987) reported relatively high levels of self-fertilization of tamarack in five populations from northern Ontario. Such evidence may suggest that gene flow does not seem to be a major or the only contributing factor for maintaining homogeneity among populations in this case.

A plausible hypothesis is the lack of time for differentiation since tamarack has occupied the area. Davis (1983) pointed out that tamarack migrated northward and eastward from the Great Plains and colonized the present-day

area of Ontario in the late Wisconsin approximately 8,000 -- 10,000 years ago. That is to say tamarack has occupied this area for only about 100 -- 200 generations since the last glaciation. Such a short period of time may not be enough for the species to display substantial differentiation (Gullberg et al. 1985).

Numerous studies on allozyme variation have shown that there is relatively limited genetic differentiation among forest tree populations. Results from the present study are consistent with this general conclusion. In their allozyme analyses of the same species and in the similar geographic region, Knowles and Perry (1987) found that little population differentiation had taken place. The present results, based on a larger sample of trees and more populations, as well as more loci assayed, are consistent with their findings. It should be noted that the F_{ST} value in this study is 0.042, higher than 0.032 in their data. This difference in estimates of F_{ST} is understandable considering the different numbers of sample trees and loci analyzed between the two studies.

In spite of the overall low genetic differentiation, the tamarack populations showed definite heterogeneity as seen in Table 4. This is what differs from the findings by Knowles and Perry (1987) in which no significant allelic heterogeneity was detected. Again, these differing results were anticipated due to the sampling strategy and the increased population numbers of the present study. A few

populations, particularly population No 41, did exhibit much different genetic structure from most other populations sampled. As illustrated in Fig. 3, Population 41 showed unusually great deviation. What has caused such deviation? If this population were physically isolated and predominantly selfing within the population for many generations, severe inbreeding may have caused a great difference in genetic structure from the other populations. However, no unique geographic or ecological circumstances for this stand were noticed. On the other hand, this population showed a slight excess of heterozygotes according to the F_{IS} statistic. So inbreeding does not seem to be the reason for the unusual deviation. It is possible that some kinds of selective pressures, which have not been detected, are underlying the genetic architecture of population 41. But it is more likely an example produced by stochastic processes such as genetic drift or sampling error.

Summarizing the results of the cluster analysis and discriminant function procedure, five populations, Nos 8, 10, 11, 38 and 41, are the most divergent ones from the other populations. A more detailed examination of this group of populations would help to elucidate the pattern of heterogeneity. All five populations happen to be in the Great Lakes-St. Lawrence Forest Region, thus their distinctive genetic structure may reflect the different selective pressures in this forest region. It should be noted that there are still some other populations in the Great Lakes-St. Lawrence region that do not significantly

differentiate from the populations in the Boreal region.

Results from the hardiness study by Joyce (1987) may help to further explain the peculiarity of the five populations. A relatively late occurrence of the first fall frost was found in the southwestern corner of northern Ontario as well as in eastern Ontario. Furthermore, populations with relatively low cold hardiness occurred in these two small areas. The five most divergent populations in the present study are also located in these two areas where the lower levels of cold hardiness were found. Such a parallel may suggest that like the physiological trait of cold hardiness, allozyme pattern in tamarack is, to a more or less extent, affected by specific environments. Various regimes of selection, either directly or via linkage, could produce these patterns (Endler 1977; Bergmann 1978). Linkage analyses and more refined environmental data would help to elucidate the importance of selection.

A question that may arise from the study is whether populations 8, 10, 11, 38, and 41, which were well separated by cluster analysis and discriminant functions analysis, were responsible for much of the genic diversity found among the populations. An analysis of gene diversity without the five populations showed the similar result with that of all the populations. The four loci, Aat-3, Dia, Mdh-4, and Pgi-2, were still found significantly heterogeneous in the X^2 test of homogeneity of allele frequencies across the residual populations.

The genetic distance statistic measures the amount and

pattern of geographic differentiation between populations. It is apparent that if population heterogeneity is largely the result of isolation by distance, then geographic distance and genetic distance are expected to be positively correlated. Cluster analysis showed that tamarack populations that were geographically close did tend to cluster together. An r^2 value of 0.088 was obtained from the correlation analysis, indicating that geographic distance accounted for approximately 9% of the variation in genetic distance among the populations. A correlation coefficient value of 0.297 means that as geographic distance between populations increases, genetic similarity decreases. This finding was further confirmed by the Mantel Matrix Correspondence procedure in which the genetic distance showed significant association with the geographic distance. These results suggest that geographic separation is a factor influencing the genetic variation in tamarack. In general, isolation by distance is an important aspect of differentiation in herbaceous plant species (Guries and Ledig 1981). For most tree species, however, an association between genetic distance and geographic separation has rarely been found among populations (Yeh and El-Kassaby 1980; Weber and Stettler 1981; Guries 1984; Gullberg et al. 1985; Merkle and Adams 1987). The difference between this result and those for many other forest trees may reflect the less effective gene flow in tamarack and the impact of varied climatic conditions for local stands.

The multiple regression analyses indicate that both

geographic and climatic variables appear to affect the genetic architecture of tamarack. Among all the environmental variables studied, latitude and winter temperature seem to be the most important factors influencing the genetic variation, with longitude and water index being next important factors. These findings parallel the results from Joyce (1987) in that cold hardiness of tamarack is related to latitude and longitude of population origins, while the effects of latitude dominate the determination of cold hardiness.

Allozyme variation observed in the present study has little to do with the elevation parameter. In contrast, population differentiation in cold hardiness was found related to elevation of population origins and could be detected over relatively small elevational distances. It is likely that the variation in elevation distances sampled in the current study is insufficient to detect significant variation in allozyme patterns.

CONCLUSIONS

1. Tamarack in northern Ontario has relatively low levels of genetic variability. Ninety-six percent of the variation is within populations, and four percent represents differentiation among populations. Although the latter figure is low, it is within the range reported for other conifers.

2. Significant allelic heterogeneity at some loci does exist among the populations. Five populations well differentiated from the other populations all occurred in two specific areas within the Great Lakes-St. Lawrence Forest Region. This finding partially parallels the results of the physiological study (Joyce 1987) in the same geographic areas.

3. Allozymic variation in this species appears to be associated with environmental variables. Latitude, longitude, temperature, and precipitation appear to have different levels of importance to the genetic variation. These results suggest that allozyme variation may be affected, directly or indirectly, by selective pressures.

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APPENDICES

APPENDIX I.

EXTRACTION, GEL AND ELECTRODE BUFFERS

1. Extraction buffer (Pitel and Cheliak, 1984):

0.1 M Phosphate buffer (pH 6.8)	100 mL
40M PVP (Polyvinylpyrrolidone)	4 g
360M PVP	1 g
Ascorbic acid	2.97 g
Diethyldithiocarbamic acid	0.45 g
B-mercaptoethanol	0.5 mL
Sodium metabisulfite	0.38 g
Sodium tetraborate	7.63 g

2. Gel buffers:

1) H gel buffer (Dilute stock, 4:1. Modified from Pitel and Cheliak, 1984)

L-Histidine HCl	20.96 g
EDTA	0.8 g
Distilled water	up to 2 L

pH 7.0 with 1 M Tris.

2) B gel buffer (Dilute stock, 9:1. Modified from Pitel and Cheliak, 1984)

Trizma base	76.2 g
RW electrode beffer	200 mL
Anhydrous citric acid	19.2 g
Distilled water	up to 2 L

pH 8.5.

3. Electrode buffers:

1) H electrode beffer

Trizma base	60.54 g
Distilled water	up to 4 L

pH 7.0 with 1 M anhydrous citric acid.

2) B electrode buffer

LiOH	10.07 g
Boric acid	74.16 g
Distilled water	up to 4 L

pH 8.1 with 1 M anhydrous citric acid.

APPENDIX II.

GELS AND STAINS USED FOR EACH ENZYME SYSTEM

Enzyme and EC #	Gel	Stain Recipe	
AAT -- Aspartate aminotransferase (E.C. 2.6.1.1)	2	Staining solution	50 mL
		5.3 g L-Aspartic acid and 0.7 g a-Ketoglutaric acid with 0.2 M Tris HCl (pH 8.0) up to 1 L.	
		Pyridoxal-5-phosphate	8 mg
		Fast Blue BB Salt	100 mg
DIA -- Diaphorase (E.C. 1.6.4.3)	2	0.2 M Tris HCl pH 8.0	45 mL
		MTT	10 mg
		2,6-Dichlorophenol indophenol	2 mg
		NADH	50 mg
FLE -- Fluorescent esterase (E.C. 3.1.1.1)	1	0.2 M Acetate buffer pH 5.0	24 mL
		4-Methylumbelliferyl acetate	10 mg
		Observe under longwave u.v. light.	
IDH -- Isocitric dehydrogenase (E.C. 1.1.1.42)	1	0.2 M Tris HCl pH 8.0	46 mL
		NADP	10 mg
		MTT	5 mg
		PMS	5 mg
		10% $MgCl_2$	1 mL
		DL-Isocitric acid	200 mg
MDH -- Malic dehydrogenase (E.C. 1.1.1.37)	1	0.2 M Tris HCl pH 8.0	22 mL
		0.5 M Malic acid	25 mL
		NAD	20 mg
		NBT	10 mg
		PMS	5 mg
		MTT	5 mg

APPENDIX II. (continued)

Enzyme and E. C. #	Gel	Stain Recipe	
PGI -- Phosphoglucose isomerase (E.C. 5.3.1.9)	2	0.2 M Tris HCl pH 8.0	45 mL
		NADP	10 mg
		10% $MgCl_2$	1 mL
		PMS	5 mg
		MTT	5 mg
		G6PDH	25 units
		Fructose-6-phosphate	25 mg
PGM -- Phosphoglucomutase (E.C. 2.7.5.1)	1	0.2 M Tris HCl pH 8.0	45 mL
		NADP	10 mg
		10% $MgCl_2$	1 mL
		PMS	5 mg
		Glucose-1,6-diphosphate	0.1 mg
		MTT	5 mg
		G6PDH	25 units
		Fructose-6-phosphate	300 mg
6PG -- 6-Phosphogluconate dehydrogenase (E.C. 1.1.1.44)	1	0.2 M Tris HCl pH 8.0	20 mL
		NADP	40 mg
		PMS	10 mg
		10% $MgCl_2$	4 mL
		MTT	20 mg
		6-Phosphogluconic acid (Na3 Salt)	20 mg

APPENDIX III.

ALLELIC FREQUENCY DATA AT EIGHT POLYMORPHIC LOCI FROM 44 TAMARACK POPULATIONS.

Locus/ Allele	Population 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Aat-3															
1	.674	.600	.625	.734	.760	.690	.761	.870	.933	.667	.796	.741	.789	.796	.845
2	.326	.400	.375	.266	.240	.310	.239	.130	.067	.333	.204	.259	.211	.204	.155
Dia															
1	.957	.980	.964	.984	.880	.897	.978	1.000	1.000	.870	1.000	.889	.885	.907	.879
2	.043	.020	.036	.016	.080	.103	.022	-	-	.130	-	.111	.115	.056	.121
3	-	-	-	-	.040	-	-	-	-	-	-	-	-	.037	-
Fle-2															
1	1.000	1.000	.964	.984	1.000	1.000	1.000	1.000	1.000	1.000	.977	1.000	1.000	.981	.983
2	-	-	.036	.016	-	-	-	-	-	-	.023	-	-	-	.017
3	-	-	-	-	-	-	-	-	-	-	-	-	-	.019	-
Idh															
1	1.000	1.000	1.000	1.000	.980	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.981	1.000	1.000
2	-	-	-	-	.020	-	-	-	-	-	-	-	.019	-	-
Mdh-4															
1	.500	.540	.446	.656	.680	.448	.652	.609	.467	.407	.364	.500	.385	.537	.414
2	.217	.340	.321	.266	.180	.362	.326	.217	.167	.500	.386	.389	.327	.241	.431
3	.283	.120	.179	.063	.140	.190	.022	.174	.366	.074	.227	.037	.288	.184	.155
4	-	-	-	.015	-	-	-	-	-	-	-	-	-	-	-
5	-	-	.018	-	-	-	-	-	-	.019	-	.056	-	.019	-
6	-	-	.036	-	-	-	-	-	-	-	.023	.018	-	.019	-
Pgi-2															
1	.587	.580	.536	.500	.740	.690	.326	.587	.500	.519	.341	.722	.404	.574	.638
2	.152	.140	.179	.188	.120	.120	.239	.087	.300	.166	.227	.111	.192	.111	.138
3	.239	.260	.250	.266	.120	.155	.435	.304	.200	.166	.386	.148	.385	.278	.224
4	.022	-	.035	.046	.020	.035	-	.022	-	.130	.046	.019	-	.037	-
5	-	-	-	-	-	-	-	-	-	.019	-	-	.019	-	-
6	-	.020	-	-	-	-	-	-	-	-	-	-	-	-	-
Pgm															
1	.978	.960	1.000	.969	1.000	.983	1.000	.978	1.000	.962	1.000	.981	1.000	.982	1.000
2	-	.040	-	.031	-	.017	-	.022	-	.019	-	.019	-	.019	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	.022	-	-	-	-	-	-	-	-	.019	-	-	-	-	-
6pg															
1	.935	.900	.964	.875	.940	.828	.891	.891	1.000	1.000	.977	.907	.962	.963	.966
2	.065	.100	.036	.125	.060	.172	.109	.109	-	-	.023	.093	.038	.037	.034

APPENDIX III. (continued)

Locus/ Allele	Population 16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Aat-3															
1	.647	.667	.809	.639	.557	.734	.914	.750	.693	.750	.548	.851	.667	.710	.611
2	.353	.333	.191	.361	.443	.256	.086	.250	.307	.250	.452	.149	.333	.290	.389
Dia															
1	.971	.947	1.000	.875	.957	.875	.931	.961	.839	.917	.976	.892	.940	.936	.944
2	.029	.053	-	.125	.043	.125	.069	.039	.161	.083	-	.108	.060	.064	.056
3	-	-	-	-	-	-	-	-	-	-	.024	-	-	-	-
Fle-2															
1	1.000	1.000	.956	.986	1.000	1.000	.983	.962	1.000	1.000	1.000	1.000	.980	.984	.944
2	-	-	.044	.014	-	-	.017	.038	-	-	-	-	.020	.016	.056
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Idh															
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.981	1.000	1.000	1.000	1.000	1.000	1.000	1.000
2	-	-	-	-	-	-	-	.019	-	-	-	-	-	-	-
Mdh-4															
1	.485	.473	.456	.625	.486	.688	.483	.731	.661	.733	.691	.541	.700	.613	.519
2	.250	.316	.368	.250	.300	.203	.466	.192	.194	.167	.262	.338	.180	.194	.240
3	.191	.211	.176	.111	.214	.109	.051	.058	.113	.100	.047	.121	.120	.194	.204
4	.029	-	-	.014	-	-	-	-	.032	-	-	-	-	-	-
5	.045	-	-	-	-	-	-	-	-	-	-	-	-	-	.037
6	-	-	-	-	-	-	-	.019	-	-	-	-	-	-	-
Pgi-2															
1	.441	.500	.588	.431	.629	.686	.655	.519	.516	.767	.500	.662	.460	.532	.648
2	.294	.341	.177	.194	.229	.188	.155	.212	.194	.117	.191	.189	.220	.274	.204
3	.250	.132	.235	.361	.100	.094	.155	.192	.258	.083	.262	.095	.260	.177	.093
4	.015	.027	-	-	.029	.032	.035	.039	.032	.017	.024	-	.060	.015	.055
5	-	-	-	-	-	-	-	.038	-	-	-	.054	-	-	-
6	-	-	-	.014	.016	-	-	-	-	.016	.023	-	-	-	-
Pgm															
1	.985	1.000	.956	1.000	1.000	.984	1.000	.981	1.000	1.000	1.000	1.000	1.000	.984	1.000
2	.015	-	-	-	-	.016	-	.019	-	-	-	-	-	.016	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	.044	-	-	-	-	-	-	-	-	-	-	-	-
6pg															
1	.882	.947	.956	.861	.914	.922	.948	.885	.936	.933	.976	.919	.920	.919	.870
2	.118	.053	.044	.139	.086	.078	.052	.115	.064	.067	.024	.081	.080	.081	.130

APPENDIX III. (continued)

Locus/ Allele	Population 31	32	33	34	35	36	37	38	39	40	41	42	43	44
Aat-3														
1	.619	.750	.611	.750	.717	.813	.763	.810	.854	.768	.925	.778	.750	.738
2	.381	.250	.389	.250	.283	.187	.237	.190	.146	.232	.075	.222	.250	.262
Dia														
1	.905	.886	.944	.885	.917	.891	.763	.793	.938	.893	.700	.944	.984	.952
2	.095	.114	.056	.115	.083	.109	.237	.121	.062	.107	.300	.056	.016	.048
3	-	-	-	-	-	-	-	.086	-	-	-	-	-	-
Fle-2														
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.946	1.000	1.000	1.000	1.000
2	-	-	-	-	-	-	-	-	-	.054	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Idh														
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
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mdh-4														
1	.548	.614	.667	.519	.683	.750	.737	.552	.688	.571	.700	.667	.516	.548
2	.262	.296	.111	.250	.133	.156	.132	.241	.208	.214	.300	.222	.281	.381
3	.190	.090	.167	.231	.150	.094	.131	.207	.083	.161	-	.111	.203	.071
4	-	-	-	-	-	-	-	-	.021	.018	-	-	-	-
5	-	-	.055	-	.034	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	.036	-	-	-	-
Pgi-2														
1	.691	.636	.556	.769	.650	.656	.552	.724	.667	.643	.575	.556	.656	.571
2	.214	.182	.111	.116	.183	.219	.158	.052	.167	.214	.225	.139	.109	.167
3	.071	.159	.333	.115	.150	.094	.290	.207	.166	.107	.200	.250	.219	.262
4	.024	.023	-	-	.017	.031	-	.017	-	.036	-	.055	.016	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pgm														
1	1.000	1.000	1.000	.981	.983	.984	.974	.983	.958	.982	.775	.944	-	.929
2	-	-	-	.019	-	.016	-	-	-	.018	.175	.056	-	-
3	-	-	-	-	.017	-	.026	.017	.042	-	.050	-	-	.071
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6pg														
1	.929	.841	.778	.942	.933	.969	.895	.845	.938	.821	.875	.917	.875	.928
2	.071	.159	.222	.058	.067	.031	.105	.155	.062	.179	.125	.083	.125	.072

APPENDIX IV.

ESTIMATE OF GENETIC DISTANCE* (x 10^{-4}) BASED ON DATA FROM 20 LOCI BETWEEN 44 POPULATIONS OF TAMARACK.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	0																					
2	17	0																				
3	11	10	0																			
4	29	20	30	0																		
5	32	45	52	35	0																	
6	23	22	27	41	36	0																
7	66	50	56	20	94	91	0															
8	32	48	51	20	32	47	47	0														
9	51	102	75	73	77	92	95	42	0													
10	49	36	25	56	77	38	80	86	112	0												
11	49	61	37	55	116	79	42	57	50	55	0											
12	39	29	35	37	27	14	84	45	100	31	87	0										
13	34	57	35	55	89	59	50	49	44	53	12	72	0									
14	15	31	24	21	24	32	48	11	39	47	41	29	27	0								
15	40	50	39	51	48	28	78	38	57	32	46	19	33	20	0							
16	17	19	14	22	63	39	39	51	64	49	38	55	35	33	59	0						
17	20	27	15	35	54	34	62	62	56	33	45	45	41	36	45	11	0					
18	23	32	23	29	45	30	54	22	38	39	29	28	31	14	13	34	29	0				
19	32	23	30	18	59	49	23	50	103	61	61	58	46	36	69	20	40	55	0			
20	19	16	15	47	48	23	98	79	102	42	87	40	76	50	64	24	16	47	49	0		
21	33	40	47	28	4	33	82	39	81	65	112	25	87	28	50	51	41	46	47	39	0	
22	67	68	63	52	52	45	82	39	68	47	68	23	65	34	10	81	63	20	92	88	52	0
23	38	36	46	6	26	53	36	27	75	76	82	46	76	28	65	35	43	43	28	55	18	62
24	26	30	32	18	26	44	41	39	80	54	73	41	50	21	53	29	34	47	12	44	18	70
25	44	52	65	40	3	45	104	41	95	90	140	33	114	37	64	75	66	57	68	55	5	62
26	39	18	28	26	57	67	48	73	130	60	89	63	88	52	94	33	41	66	25	35	48	109
27	40	51	49	37	22	26	79	30	53	48	76	15	60	22	15	56	39	21	66	55	20	13
28	26	26	29	10	37	55	30	39	79	65	68	57	59	28	73	20	32	48	12	41	28	85
29	13	25	23	14	25	35	47	32	47	56	59	41	47	19	49	14	14	29	24	26	17	61
30	17	20	20	37	32	17	94	60	90	52	91	33	77	40	59	25	21	42	45	7	26	76
31	19	23	23	42	23	18	100	64	90	45	99	26	78	38	51	34	20	44	49	7	16	69
32	29	27	37	16	16	16	57	29	79	49	83	14	64	23	33	36	34	31	31	37	10	35
33	35	32	51	30	53	52	61	51	124	110	109	72	88	50	104	37	68	78	21	53	49	127
34	21	40	39	50	12	17	111	39	66	57	96	18	67	22	27	57	42	31	68	34	16	42
35	22	34	39	23	7	39	72	32	68	74	99	35	77	22	57	39	38	44	38	36	5	66
36	49	64	69	34	10	61	83	38	68	87	118	43	96	32	60	68	56	53	63	68	7	55
37	51	65	72	38	32	66	63	46	96	97	112	61	73	35	71	64	74	75	30	86	26	88
38	37	55	61	49	20	23	99	30	79	78	102	27	67	23	33	70	70	43	61	66	27	46
39	42	54	60	24	11	49	66	17	57	80	93	31	78	20	40	62	57	32	59	71	13	33
40	29	40	43	26	19	20	77	32	65	67	89	26	69	27	41	38	35	35	45	39	13	46
41	127	129	143	84	80	106	109	86	134	119	157	79	122	81	81	132	126	104	101	161	66	74
42	24	28	35	6	19	39	35	11	59	59	62	32	52	10	41	34	41	26	27	52	17	44
43	11	19	22	21	23	12	62	15	55	52	56	20	45	12	26	30	32	14	41	30	27	37
44	25	15	20	14	37	26	35	27	72	29	44	17	41	16	22	28	29	13	29	39	32	28

APPENDIX IV. (continued)

Population	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
23	0																					
24	17	0																				
25	27	33	0																			
26	33	28	59	0																		
27	36	40	29	83	0																	
28	10	8	42	16	55	0																
29	13	12	32	31	30	9	0															
30	39	36	38	41	43	34	19	0														
31	42	30	27	39	34	37	19	6	0													
32	17	19	20	49	13	27	17	24	21	0												
33	35	32	57	40	86	24	35	40	52	39	0											
34	50	39	20	74	17	56	30	25	16	21	67	0										
35	14	14	9	37	30	17	10	23	18	16	34	20	0									
36	18	25	10	63	25	33	23	51	38	23	70	31	10	0								
37	30	11	39	66	51	29	33	67	59	30	43	51	24	28	0							
38	51	41	32	95	24	62	43	47	43	20	58	16	32	41	36	0						
39	18	29	14	66	15	37	25	52	44	17	64	27	14	8	32	29	0					
40	22	28	24	68	17	35	16	19	23	7	45	20	17	25	37	21	22	0				
41	77	71	86	153	60	102	90	137	121	62	140	93	85	60	49	71	56	71	0			
42	8	14	25	38	26	14	14	39	38	14	36	32	13	19	25	31	11	22	64	0		
43	31	34	32	53	21	35	20	21	24	15	39	15	23	42	54	21	25	17	106	17	0	
44	27	26	45	36	24	29	24	37	34	17	54	34	32	44	50	40	26	32	79	16	16	0

*According to Nei (1972).

Note: Mean genetic distance = 0.0045.

APPENDIX V.

THE MULTIPLE REGRESSION EQUATIONS FOR THE FIRST SEVEN DISCRIMINANT FUNCTION SCORES WITH THE ENVIRONMENTAL VARIABLES.

$$Y_1 = 18.9241 - 0.0043\ Lat_i - 0.0131\ AJan_i.$$

$$Y_2 = -3.0146 + 0.0004\ Long_i - 0.0116\ ARain_i + 0.0002\ ASnow_i.$$

$$Y_3 = -1.3105 + 0.0105\ AJul_i - 0.0002\ ASnow_i.$$

$$Y_4 = -3.8573 + 0.0014\ Lat_i - 0.0002\ Long_i + 0.0056\ MJan_i.$$

$$Y_5 = -0.6488 + 0.0208\ ARain_i - 0.0001\ APrec_i.$$

$$Y_6 = 0.0749 + 0.0178\ MJul_i - 0.0197\ AJul_i + 0.0038\ MJan_i.$$

$$Y_7 = 1.1398 - 0.0003\ ASnow_i + 0.0094\ ARain_i - AJul_i.$$

where $Y_1, Y_2, \ldots, Y_7$ are the discriminant function scores 1, 2, ..., 7, respectively.