ISOZYME VARIATION WITHIN THE FRASER FIR POPULATION ON MT. ROGERS, VIRGINIA

by

Kenneth Edward Diebel

Dissertation submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Forestry

APPROVED:

P. P. Feret, Chairman

S. M. Zedaker

R. G. Oderwald

B. Wallace

E. Nilsen

August, 1989

Blacksburg, Virgnia

ISOZYME VARIATION WITHIN THE FRASER FIR POPULATION ON MT. ROGERS, VIRGINIA

by

Kenneth Edward Diebel

Committee Chairman: Peter P. Feret
Forest Biology

(ABSTRACT)

The Fraser fir (Abies fraseri (Pursh) Poir.) on Mt. Rogers is an isolated relic population and part of the southern Appalachian spruce-fir ecosystem. The population has, so far, been able to withstand the impacts of insect infestation and the possible influence of atmospheric deposition factors which may be causing mortality in other regions of the southern Appalachians. It was hypothesized that population vigor may be due to a unique genetic structure. The objective of this study was to determine the amount of genetic diversity within this population and to relate observed diversity to environmental variables.

To quantify the genetic structure 304 trees from 35 plots were genotyped for 13 isozyme loci. Four loci were polymorphic using the 95% criterion. At a fifth locus there were two rare alleles with a combined frequency of approximately 3%. Range wide studies of eastern fir species have shown that other populations are more diverse.

There were no significant differences in gene frequencies among three arbitrarily defined subpopulations or among the 35 plots. There were no significant correlations between any environmental characters and isozyme frequencies.

There was a significant difference among subpopulations for seed weight and germination value as well as a slight, yet significant, correlation between seed weight and elevation, germination value and elevation, and germination value and aspect. Spatial autocorrelation analysis, Wright's F-statistics, Nei's genetic distances and Gregorius' "δ" index all indicated little or no substructuring of the population. It is suggested that a population bottleneck (a drastic reduction of population numbers), which may have occurred following the last glaciation, is the cause for the relatively low genetic diversity found in the population. The lack of substructure is likely due to extensive gene flow.

ACKNOWLEDGMENTS

I would like to thank all of my committee members for their help and advise throughout the course of this project. I particularly want to thank Dr. Feret for the financial, moral, and intellectual support he provided. Even though ate all my food and drank all my water during our trips to Mt. Rogers I would not have been able to collect field data without his work ethic and sense of humor. Dr. Zedaker and were very gracious in allowing me to use their data. It helped to make this study more complete. , , , and were tremendous lab assistants. Finally, I would like to thank my wife . She put up with my foul moods, early morning work habits, and was a great editor all while pursuing her own education. All I can say is that I'm very lucky to have such a great person supporting and helping me.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

The population of Fraser fir growing on Mt. Rogers is unique because of its apparent resistance to environmental disturbance. The population remains vigorous despite increased amounts of air pollutants and the spread of the balsam wooly aphid (Adelges piceae Ratz.) (White 1984). This is in direct contrast to the fir growing in the Great Smoky Mountains National Park which are in a state of decline (Personal communication; Dr. S. Zedaker). Dull et al. (1988) estimated that 91% of the fir in the park are dead or dying. They also classified 30% of the hectares of the spruce-fir type in the southern Appalachians as having heavy to severe fir mortality. It is obvious that the wooly aphid is a severe threat to the fir populations in the south east.

A unique genetic makeup could be one of the underlying causes for the remarkable vigor of the Mt. Rogers fir population. If true, then an important question becomes one of determining how much genetic diversity is present the population.

The question of genetic diversity is also important for the long term continuation of this population. The population is vigorous at the present time. However, the environment is in a constant state of flux. A genotype

that is advantageous now may not be in the future. One of the central theories in population genetics holds that for long term success a population must have a large amount of genetic diversity so that some genotypes may survive as the environment changes.

The Mt. Rogers population of fir provides a unique opportunity to study the effects of site and stand type on genetic diversity because of the great variety of sites and stands the trees grow in, even though they occupy a relatively small area of roughly 400 hectares. Few studies of forest tree species have quantified these effects. Site and stand characteristics having a potentially large impact on genetic variation of populations include: elevation, aspect, age class distribution, species composition, spatial distribution, flowering phenology and the mating system.

The specific objectives of this project were to:

1. determine the relationships, if any, between site characters and genetic variation of the Fraser fir forests on Mt. Rogers, Va.

2. quantify the amount of within and between stand genetic variation.

3. quantify variability in flowering phenology and determine if variation patterns are related to patterns of genetic variation.

LITERATURE REVIEW

Introduction

This literature review is focused on the genetic variability of conifers so as to provide the reader with a foundation to compare the genetic diversity of the Fraser fir population on Mt. Rogers with other conifer species. This study focuses on microgeographic genetic variation therefore a discussion of the pattern of genetic diversity of other conifer species over both large and microgeographic areas was included. Next there is an evaluation of Hamrick and his coworker's "life history" hypothesis. This hypothesis attempts to explain the causes for the patterns of variability and may provide the basis for explaining the patterns of variability observed in this study of Fraser fir. A comparison of isozyme and morphological variability patterns observed in previous studies is included because two morphological variables were measured in this study and were compared to isozyme variables.

The pattern of genetic variability in marginal populations is reviewed because the Mt. Rogers fir population grows at the margin of the species distribution and presumably at the margin of its ecological tolerance.

This section reviews information from studies of woody and non woody plants as well as studies of Drosophila. A discussion of four hypotheses which attempt to explain the causes of observed genetic patterns concludes the section.

Finally, the paleohistory of the spruce-fir ecosystem in the southeast United States is summarized. The hypothesized changes to the vegetation on Mt. Rogers and vicinity will receive special attention because of the potential impacts on the genetic variability of the population these changes may have had. There is also a discussion of anthropogenic disturbances and the future of the spruce-fir forests.

Genetic Variability of Conifers

Forest trees, particularly conifers, are among the most genetically variable of organisms. Hamrick (1979) compared isozyme variabilities of 11 conifer species to over 100 species of monocots and dicots. Conifers had a mean heterozygosity (i.e. the number of heterozygous loci in the average individual) of 27% while monocots had 16.5% and dicots had 11.3%. A loci is heterozygous if the alleles at that loci are different.

These results may be misleading. In many of the early studies of forest trees only the most variable and easily extracted enzymes were sampled. This meant that

heterozygosity estimates were made from only a few variable loci causing upwardly biased estimates.

In a review of studies which sampled 20 or more loci, Guries and Ledig (1982) showed that observed heterozygosities were much lower than Hamrick (1979) had reported. For example the mean heterozygosity of pitch pine (Pinus rigida Mill.) was 13.8% (Guries and Ledig 1982), lodgepole pine (Pinus contorta ssp. latifolia) 16.1% (Yeh and Layton 1979), and Douglas-fir (Pseudotsuga menziesii Mirb.) 15.5% (Yeh and O'Malley 1980). Recent studies have provided similar estimates. Mean heterozygosity for black spruce (Picea marianna Mill.) was 12% (Yeh et al. 1986) and 17% for Douglas-fir in southwestern Oregon (Merkle and Adams 1987). Guries and Ledig (1982) concluded that conifers are not much more diverse than many herbaceous monocots. Even though tree species are not as diverse as previously thought the revised estimate still places conifers well ahead of vertebrate and invertebrate animals and ahead of most dicot plants (Hamrick 1979).

When discussing mean heterozygosities it is incorrect to interpret observed heterozygosities literally. Falkenhagen (1985), in his review of isozyme studies in provenance research, criticizes Yeh and O'Malley's (1980) statement that a mean heterozygosity of 15% represents a

large amount of variation in Douglas-fir. Falkenhagen rejects this statement because he incorrectly interprets the value of 15% as meaning that 85% of the genes in an average Douglas-fir are homozygous.

Careful reading of Yeh and O'Malley's paper and a knowledge of electrophoretic techniques shows how incorrect this interpretation is. Electrophoresis separates isozymes mostly by charge differences. Most, 16 out of 20, of the common amino acids have a neutral charge at physiological pH. A change in charge occurs only if a positively charged amino acid is substituted for a negative one, or vice versa or if a charged amino acid is substituted for a neutral amino acid. Thus only about 36% of all amino acid substitutions are detected (Wallace 1981). Starch gel electrophoresis, the commonly used technique in studies of forest trees, can detect even fewer variants. Yeh and O'Malley state that a heterozygosity of 15% means, assuming that the loci sampled are representative of the genome, that at least 45% of the structural genes in the average Douglas-fir are heterozygous. The percentage may even be higher, up to 60% because of the limitations of starch gel electrophoresis.

Mean heterozygosity is not the only measure of diversity. The percentage of polymorphic loci, the number of loci for which there is more than one kind of allele, is

usually high in conifers. A loci, by convention, is considered polymorphic if the rare allele occurs at a frequency greater than or equal to 5%. Hamrick et al. (1981) reported an average of 67% polymorphism in a review of 28 studies. Dropping studies sampling less than 20 loci only reduced the percentage to 66%. Recent studies provide comparable results. For example, the estimated percentage of polymorphic loci for lodgepole pine is 51.4% (Dancik and Yeh 1983), jack pine (Pinus banksiana Lamb.) 46% (Dancik and Yeh 1983), white pine (Pinus monticola Dougl. ex D. Don) 65% (Steinhoff et al. 1983), black spruce 38% (Yeh et al. 1986), Douglas-fir 71.7% (Merkle and Adams 1987) and western larch (Larix occidentalis Nutt.) 30.4% (Fins and Seef 1986).

The average number of alleles per locus is another common measure of genetic diversity. A normal diploid individual has two alleles per locus. These alleles may be identical (homozygous) or different (heterozygous). All the individuals in a population may be homozygous for a particular locus. Such a population has only one allele for the locus. A diverse population may have individuals homozygous for one kind of allele, homozygous for a second or third or more kind of allele and there may be heterozygous combinations of all the kinds of alleles.

Diverse populations often have more than two kinds of alleles per locus.

Hamrick et al. (1981) reported that the average number of alleles per locus in conifer species ranges from 1.00 for red pine (Pinus resinosa Ait.; Fowler and Morris 1977) to 3.54 for Norway spruce (Picea abies (L.) Karst.; Lundvist 1979). The average of 20 different conifer species was 2.29. Hamrick et al. (1979) reviewed reports documenting allelic diversity of 113 taxa which were mostly herbaceous plants. The mean average number of alleles per locus was 1.69. Hamrick and his coauthors (1981) concluded that most conifers have more alleles than most other plant species.

It is difficult to compare the average number of alleles per locus among studies which have vastly different sample sizes. Ewens (1972) demonstrated that the average number of alleles per locus is highly dependent on sample size. A better measure of allelic diversity is the effective number of alleles index developed by Crow and Kimura (1970). Some examples of estimates of the effective number of alleles in conifer species are: black spruce 1.24 to 1.38 alleles per locus (Boyle and Morgenstern 1987), Norway spruce 1.65 to 1.75 (Lundvist 1979) and bristle cone pine (Pinus aristata Engelm.) with a gene pool average of 1.49 (Hiebert and Hamrick 1983). An example of the

effective number of alleles for an angiosperm species is European beech (Fagus sylvatica L.) with a gene pool average of 1.55 (Gregorius et al. 1986).

Isozyme variation patterns can differ greatly among conifer species, although most conifers have a relatively high degree of variability. The number of polymorphic loci varies from 100% in Douglas-fir (Hamrick 1979) to 0% for red pine (Fowler and Morris 1977). The causes for these large differences among species has been the subject of many speculative articles. The hypothesis presented by Hamrick (1979), Hamrick et al. (1979), Hamrick et al. (1981), Mitton (1983) is that plant species differ in their genetic structure depending on several ecological and life history characteristics. These characteristics include population size, geographical range, mating system (i.e. selfing vs. outcrossing), means of pollen/seed dispersal, longevity, successional status, fecundity and population history. The relatively high degree of variability found in most conifer species is thought to be related to their large populations, continuous geographic ranges, primarily outcrossing mating system, wind dissemination of pollen and seed, high fecundity and longevity combined with a long generation interval.

According to the "life history" hypothesis, outcrossing should maintain a high degree of genetic

heterogeneity. In spite of this Brown (1979) noted that many outcrossing plant species have an excess of homozygotes in comparison to Hardy-Weinberg equilibrium expectations. Brown called this phenomenon the "heterozygosity paradox" and suggested three possible causes. The first is negative heterosis. This term means that heterozygotes are at a selective disadvantage for some reason. The second cause he suggested is the Wahlund effect. This effect is the result of unrecognized population subdivison. Treating two or more populations as one population reduces the estimated heterozygosity because of the mathematical properties of averages. The final cause could be partial selfing or mating between closely related individuals.

Forest trees occasionally have a deficiency of heterozygotes at some loci. Some examples include: pitch pine (Guries and Ledig 1982), loblolly pine (Roberds and Conkle 1984), western larch (Fins and Seef 1986), black spruce (Boyle and Morgenstern 1987), lodgepole pine and jack pine (Dancik and Yeh 1983). There are also reports of an excess of heterozygotes (Desparts and Simon 1987; Ross and Hawkins 1986 and others) but most loci are in frequencies matching those predicted by Hardy-Weinberg equilibrium.

Hamrick's hypothesis predicts that species with small population size and/or limited geographic distributions will be less genetically diverse. An example of a species with a small population and an extremely limited geographic range is Torrey pine (Pinus torreyana Parry ex Carr.). It is possible that only 9,000 individuals exist in two small populations (Ledig and Conkle 1983). Ledig and Conkle (1983) investigated the isozyme variability of this species. Every sampled tree was homozygous for each of the 59 loci tested. The remarkable uniformity of Torrey pine was attributed to a severe reduction in population numbers (i.e. a population bottleneck). The authors speculate that population numbers may have at one time been as low as 50 individuals.

Population histories may be the overriding factor causing the low variabilities found in two other conifer species, red pine and western red cedar (Thuja plicata Donn ex D. Donn). Unlike Torrey pine the populations of these two species are fairly large and both grow on a variety of sites. Copes (1981) found the western red cedar trees he sampled were homozygous for every locus tested. He did not speculate on a cause but Mitton (1983) speculates that at least part of the cause is the low frequency of occurrence of this species in a typical stand. Fowler and Morris (1977) and Simon et al. (1986) found no variability at any

of the loci they sampled in red pine. Fowler and Morris (1977) suggested that the extreme homozygosity in this species is due to a population bottleneck that might have occurred during the Wisconsinan glaciation period.

Red pine may not be as devoid of variability as previously reported. Allendorf and others (1982) found null alleles at three loci in red pine. The average frequency of the null allele was low (.0028) as was mean heterozygosity (.1%). The authors speculate that the null alleles may be the first mutations to enter the population since the supposed population crash.

According to the "life history" hypothesis species differ genetically because of differences in successional status. There are several problems with this aspect of the "life history" hypothesis. Classifying species according to successional status is difficult especially when there continues to be considerable debate about successional processes and patterns (Oliver 1981). Another problem is the lack of empirical data addressing the relationship between genetic diversity and succession. Beckman and Mitton (1984) conducted one of the few studies of forest trees designed to investigate this relationship. The study sampled four stands of ponderosa pine (Pinus ponderosa Dougl. ex Laws.) which the authors assumed to be in different stages of succession. Their hypothesis was that

stands in the early stages of succession would be similar to stands on xeric sites and that older stands would be similar to stands on mesic sites. This was based on the Clemensian ecological theory that plants modify the environment as succession progresses. They chose to investigate the peroxidase enzyme because in previous studies the frequency of this enzyme differed significantly among xeric and mesic sites.

Study results indicated that the oldest stand (late in succession) had a significantly greater frequency of the "common" allele than the other stands. The genetic structure of this stand compared favorably with mesic stands sampled in the previous studies. The second youngest stand had a significant excess of heterozygotes and a much lower frequency of the "common" allele. This again was a pattern found for xeric sites in the previous studies. The authors concluded that genetic variability patterns changed in response to changes presumably brought about by succession.

While Hamrick's hypothesis may partially explain the causes of the genetic diversity patterns found in conifer species, studies comparing the genetic diversity of short-lived highly fecund plants with long-lived plants of low fecundity are lacking (Mitton 1983). Until such studies

are completed all the factors involved in Hamrick's life history hypothesis are difficult to assess.

Given that the genetic composition of a population may differ because of successional status, population history, and differences in geographic range, the questions now are what is the pattern of isozyme variability within and between populations of typical conifer species and what are the causes for that pattern? For most tree species 80 to 90% of isozyme variation is within populations (Mitton 1983). Therefore the amount of inter-population variation is very small. Human populations also have this pattern of within population variance overwhelming between population variance (Hartl 1980).

A series of studies of ponderosa pine exemplify isozyme variation. Significant genetic differentiation occurred among stands within 100 m east to west transects (Mitton et al. 1977; Mitton et al. 1980). Comparison of stands 10 m apart revealed as much variation as stands 100 m apart (Linhart et al. 1981a; Linhart et al. 1981b). The variation was presumably due to limited seed dispersal and limited effective pollen flow (Turner et al. 1982).

Recent studies confirm this pattern of variation. White pine populations typically contain 85% of the total variation in the species (Steinhoff et al. 1983), black spruce 94% (Yeh et al. 1986), Douglas-fir 99% (Merkle and

Adams 1987), Jeffery pine (Pinus jeffreyi Grev. and Balf.) 86% (Furnier and Adams 1986) and western larch 91% (Fins and Seef 1986). These percentages are derived from Wrights' F statistics (Wright 1978) or Nei's genetic distance (Nei 1978) which is the equivalent of Wright's F statistics.

Morphological characters tend to have a different pattern of variation than isozymes. Falkenhagen (1985) compared the results of his provenance (area from which seeds were obtained) investigations into the morphological characters of Sitka spruce (Picea sitchensis (Bong.) Carr.) and the isozyme study of Yeh and El Kassaby (1980). Morphological characters are apparently strongly adapted to the local environment. Simple univariate and multivariate canonical correlations between such variables as height growth of seedlings grown in a common environment and latitude of origin were highly significant and large. The variation attributable to inter-provenance variation ranged from 3% to 39%.

The isozyme study revealed very little inter-provenance variation and no correlations to the environment of origin. Nei's genetic differences calculated from the isozyme allele frequencies range from .004 to .03 with an average of .014. These are relatively low values indicating little population differentiation (Yeh and El

Kassaby 1980). Genetic distances were not correlated with geographical distances and did not conform to the known paleohistory of Sitka spruce (Falkenhagen 1985).

Falkenhagen concluded from these data and other evidence that isozymes are not useful tools for provenance research. His objections are that isozyme variation patterns do not match morphological variation patterns, isozymes are not traits subjected to intense selection pressures and are not traits of interest to plant breeders. They are not of interest because they have little or no effect on traits such as shape, size, or metabolic rate.

Not all studies have shown that isozymes are unrelated to traits of economic importance (see Mitton and Grant 1984 for a review). Some isozyme genotypes are apparently subject to heavy selection pressures. Koehn et al. (1980) and (1983) have shown selective advantages for certain isozyme genotypes in Mytillus edulis a sea mollusk. There are several examples of correlation of isozyme genotypes to environmental variables in forest trees (Millar 1983; Fryer 1987; Furnier and Adams 1986; Cheliak et al. 1988; Yeh et al. 1985). There are also examples of isozyme variation patterns being similar to morphological variation (Fryer 1987; Grant and Mitton 1977; Yeh and Arnott 1986). However for every example of a significant correlation to the environment or morphology one can find another showing a

complete lack of a relationship (Wheeler 1982; Neale and Adams 1985; Merkle and Adams 1987; Feret 1974 etc.).

Perhaps an explanation for the lack of a consistent relationship between morphological and isozyme variation is that selection pressures are completely different for the two types of characters. It also seems likely that certain enzymes, those critical to metabolic pathways, are subject to greater selection pressures than others. The choice of enzymes studied could explain the presence or absence of correlation.

A few recent studies have demonstrated variability patterns of a few key enzymes to be correlated with soil moisture conditions (O'Reilly et al. 1985; Stutz and Mitton 1988). Stutz and Mitton's study is perhaps the most interesting. They investigated the relationship of soil moisture to the variability of two enzyme systems, phosphoglucomutase (PGM) and uridine diphosphate (UDP) in Englemann spruce. These two enzymes are important in the glycolytic pathway and the glycogen synthesis pathway respectively. The authors selected two locations in Rocky Mountain National Park each of which contained two stands located side by side. One stand was on a dry upland site and the other on a wet lowland site.

The results of the study indicated that allele frequencies within locations were significantly different for UDP but not for PGM. The "fast" UDP allele was more common on the wet sites. There was also a significant difference within locations for PGM genotypes. Trees heterozygous at the PGM locus were more common on the dry sites. The authors concluded that the best explanation of these results, although not the only one, was selection favoring the UDP fast allele on wet sites and selection favoring PGM heterozygotes on dry sites. It is important to realize that these types of studies can not in themselves provide proof of selection (Endler 1986). They do however provide direction for future studies. An additional problem with this particular study is the lack of replication. Only two replicates is not enough to verify that the observed correlations are not due to some factor other than soil moisture on the site.

Genetic Variation within Stands

Since most isozyme variation is within populations, there has been considerable interest in quantifying the pattern of diversity on a microgeographic scale. As is the case for most isozyme studies of forest trees, studies differ. Some authors conclude that stands have distinct spatial patterns of gene or genotype frequencies (Linhart

et al. 1981a; Knowles 1984; Knowles and Grant 1985; Gregorius et al. 1986). These authors assume that these spatial patterns were caused by limited seed dispersal and mating of related individuals. Other studies did not detect any within stand spatial pattern (Guries and Ledig 1977; Roberds and Conkle 1984; Epperson and Allard 1989). Some suggested causes of this randomness are random mating and random seed dispersal.

Guries and Ledig's (1977) study of pitch pine is an example of random genetic variation within stands. They sampled and mapped every individual within a 50 m diameter circle in four stands. The calculated inbreeding coefficient was nearly zero for each stand and there was no clustering of genotypes within any of the 2000 m^2 circular areas. The authors concluded there was essentially random mating within the circular area.

Roberds and Conkle's (1984) study of loblolly pine is another example of within stand randomness. They sampled two natural stands located directly adjacent to each other. Both stands were arbitrarily divided into subsections so that 15 trees were sampled from each subsection. There were no significant differences among the subsections. The subsections were redrawn in several different ways but there were still no significant differences. The authors concluded there was no spatial structure of genotypes

within either stand and there was little differentiation between the two stands because of random mating and random seed dispersal.

There are several examples of studies which detected spatial patterns of genetic variation within stands. The study conducted by Linhart et al. (1981a) was one of the first reports of within stand spatial structure. They determined the seven locus genotype for all ponderosa pine trees growing in a two hectare stand. They grouped the trees into six clusters based on observed spatial patterns. Allele frequencies for two loci were significantly different among the clusters. They concluded that each cluster was a clump of trees highly related to each other. These clumps were formed, according to these authors, by limited seed dispersal. Outcrossing apparently did not disrupt the family structure of this population.

Knowles (1984) reported finding spatial structuring in lodgepole pine stands. She established five evenly spaced belt transects within four separate stands. The results from genotyping each tree encountered on each transect indicated a limited amount of within stand substructuring. Allele frequencies for one locus in one stand were significantly different among the five transects. What is more striking is the pattern of genotype frequencies. Based on visual inspection of the spatial pattern of

genotypes within a stand, clumps of similar genotypes are obvious.

It is difficult to draw conclusions about within stand genetic variance when the evidence is so conflicting. Perhaps more studies of different species in various stand conditions would provide a definitive conclusion. Until then the assumption must be that each stand has a different pattern of genetic structure.

Genetic Variation in Marginal Populations

Populations at the ecological or geographic margins of a species' distribution are often quite different from populations in central locations. In many species of Drosophila the frequency of chromosomal inversion polymorphisms decrease from the center to the margin of the range (Carson 1955). A chromosomal inversion occurs when a segment of the chromosome is inverted with respect to the "normal" chromosome. Characteristic loops in the chromosome serve to identify the various types of inversions. Several types of inversions can occur in the same population which are therefore defined as having polymorphic chromosome inversions (Wallace 1981). Frequency of inversions in plant species usually have the opposite pattern. Inversions are more or as common at the margins as they are at the center (Levin 1978). Allozyme

heterozygosities, on the other hand, do not decline or increase in marginal populations for almost all the Drosophila species (Brussard 1984). There is no published comprehensive review of plant allozyme heterozygosity in marginal populations. Articles reviewed here indicate that for some species isozyme heterozygosity decreases in marginal populations (Tigerstedt 1973; Wheeler and Guries 1982; Bergmann and Gregorius 1979; Rick et al. 1974; Keeler 1978; Yeh and Layton 1979; Schumaker and Babbel 1980; Guries and Ledig 1982). For other species heterozygosity does not change (Levin 1977; Yeh and O'Malley 1980).

Why are inversion polymorphisms less frequent at the margin of most of the Drosophila species distributions but more or as common in marginal populations of most plants? Why should these polymorphisms increase or decline at all? Why is there no change in allozyme heterozygosity for Drosophila? There are many hypotheses which attempt to answer these questions.

Carson (1955) proposed the homoselection-heteroselection hypothesis to explain the decrease from the center to the margin of inversion polymorphisms in Drosophila. One assumption in this hypothesis is that inversion polymorphisms impart heterotic buffering. Selection in central populations favors heterozygotes

because the optimum conditions for growth and survival in central populations place a premium on heterotic buffering.

Directional selection is the major force operating in marginal populations, according to Carson's hypothesis, because of a reduction of niches. Structural homozygotes will impart more fitness than heterozygotes under these conditions because structural homozygosity allows for free recombination. Free recombination allows the population to produce novel combinations of alleles which could be beneficial in the harsh environmental conditions at the margin.

Da Cunha and Dobzhansky (1954) proposed an alternative hypothesis. The frequency of inversion polymorphisms are directly proportional to the number of niches available to the species at a particular location. They, of course, assumed there are more niches available at the center of a species distribution. This hypothesis is distinctly different from Carson's because he claimed heterozygosity is not a specific adaptation, as did Da Cunha and Dobzhansky, but is only a buffer to the environment.

Soule' (1973) proposed a third hypothesis specifically intended to explain the contrast between chromosome inversions and allozyme diversity patterns in Drosophila. This hypothesis emphasized epistasis rather than heterosis and therefore is called the epistasis cycle hypothesis.

Epistasis is the interaction among genes which are not allelic partners.

Soule'(1973) claimed that heterosis is a minor component of fitness. Regulatory genes and epistasis are the key parts of fitness because these two factors are important in controlling developmental and physiological processes. Inversion polymorphisms combine the effects of these two factors. Heterosis is a general effect contributing relatively the same amount to fitness regardless of the character state. Heterosis, Soule' stated, is usually associated with increased vigor, rate of development, and biomass.

Soule' suggested that selection pressures are completely different for chromosome polymorphisms and structural gene heterozygosity. This is the key point of his argument. He assumed directional selection to be operating in marginal populations. This type of selection reduces the number of chromosomal polymorphisms. However selection still favors allozyme heterozygosity, which he claimed is responsible for a great deal of heterosis, since heterosis increases vigor in both marginal and central populations.

Wallace (1984) proposed a fourth hypothesis. This hypothesis attempts to explain the differing patterns of inversion polymorphism distributions of plant and

Drosophila species. Wallace suggested there are two possible causes for the differences. The first is that rank order selection has a greater impact on plant species. Rank order selection is the elimination, starting with the least fit individual and progressing towards the most fit, of excess numbers of organisms until the population is at the carrying capacity of the environment. The second possible cause is that flies are highly mobile and can make choices while plants can not.

Wallace assumed that plant populations in centrally located areas are typically dense. There is a great need for self thinning to reduce population numbers to the carrying capacity. Rank order selection proceeds, according to Wallace, most efficiently if there is a large amount of variation in fitness within the population. Fitness variation can be maintained in populations if selection favors heterozygotes and matings occur mostly among the surviving heterozygotes. Chromosomal inversion polymorphisms prevent free recombination of genes which decreases the amount of fitness variation in future generations. Thus rank order selection can operate more efficiently in the absence of inversion polymorphisms.

Wallace further assumed that marginal populations are usually much less dense than central populations and intraspecific rank order selection is much less important.

Maximum individual fitness, supposedly imparted by the chromosomal inversion, increases the probability of success in the harsher marginal environment. This is because interspecific competition becomes more important at the margin. Therefore high individual fitness is at a premium.

This may adequately explain the situation with plants but what about Drosophila? Wallace contends that the mobility of flies and their ability to decide when and where to lay eggs allows them to avoid the rigorous self thinning that central plant populations must undergo. If populations are too dense the females may not lay as many eggs or the flies may move to less crowded areas. Drosophila species do not pay the penalty of having blocks of genes incapable of recombining and the resulting lack of variation in relative fitness.

The situation at the margin is different in two important ways. The determinants for success in these populations where habitats suitable for offspring survival are rare are the probability of survival to adulthood of an embryo and the probability of the female finding a place to lay her eggs. As conditions become more difficult those females who produce great numbers of reasonably fit individuals will have an advantage over females who produce few but super viable offspring (i.e. those with chromosomal polymorphisms). The probability of survival of the

monomorphic type will increase because of their greater production.

Brussard (1984) evaluated all four of these hypotheses. He discounted Soule's explanation of the maintenance of allozyme heterozygosity as a result of heterosis. Brussard pointed out there is only weak and contradictory evidence in favor of single locus allozyme heterosis but there is strong theoretical evidence which discounts Soule's assumption of multilocus allozyme heterosis. Brussard maintained that migration is the key to maintenance of high levels of allozyme heterozygosity in Drosophila.

Brussard discounted Da Cunha and Dobzhansky's hypothesis of levels of inversion polymorphisms being directly related to ecological amplitudes because of lack of evidence demonstrating that certain genotypes have better fitness in certain niches. Carson's hypothesis of heterotic buffering was also criticized because of weak and contradictory evidence of the heterotic effect of inversion polymorphisms.

However Brussard didn't totally discount Carson's ideas. He agreed that at the margin directional selection puts a premium on genetic flexibility, thus eliminating inversion polymorphisms. Stabilizing selection, according to Brussard, is the predominant force in central

populations. This type of selection eliminates extreme phenotypes and favors the average. Thus the number of inversion polymorphisms increase because the lack of recombination reduces the number of extreme phenotypes. Finally, Brussard did not find any incompatibilities between his modified version of Carson's hypothesis and Wallace's hypothesis.

These models do not specifically include historical factors to explain genetic diversity patterns. Whether a marginal population is a relic of a once larger one or the result of a species expanding its range, founder effects and bottleneck effects play an important role in determining the genetic structure of the population.

Founder effect is caused by the founding of a new population by a very small number of individuals which are not representative of the gene pool of the parent population. The new population may be quite different from the parent population because of sampling error. The bottleneck effect is the change in genetic makeup caused by a drastic reduction in population numbers either due to founding a new population or population retreat. The change may be caused by biased sampling, as is the case for founders effects, or because of increased inbreeding due to the limited numbers of individuals.

Nei et al. (1975) developed a model to simulate the effects of a drastic bottleneck on heterozygosity and the average number of alleles per locus. The critical factors were time since the bottleneck occurred and the rate of growth of the population following the bottleneck. In the generations immediately following the bottleneck heterozygosity and the number of alleles per locus declined rapidly. The model demonstrated that the decline in heterozygosity was not as precipitous if the rate of population increase was great. The decline soon leveled off to a minimum level and remained at this level for many generations. Then a rapid increase occurred until heterozygosity and the number of alleles reached or surpassed their original levels. The model showed that if population growth was rapid then the number of generations during which heterozygosity and number alleles were low was smaller than if population growth was slow. This model demonstrates that it is important when sampling a marginal population to know if a bottleneck event occurred, how long since the bottleneck, and what the growth rate of the population is.

Lodgepole pine is an example of how paleohistory may be important in determining the genetic makeup of marginal populations. Two separate studies (Wheeler and Guries 1982; Yeh and Layton 1979) document reduced allozyme

diversity in marginal populations of this species. MacDonald and Cwynar (1985) documented the quaternary history of lodgepole pine using fossil pollen. Most of the fossil pollen sample locations corresponded closely to the sample populations of Wheeler and Guries (1982) and Yeh and Layton (1979).

Fossil pollen samples indicate that the northern peripheral populations of this pine have only recently arrived. One population may be only 100 years old. Thus lodgepole must be expanding its range. Cwynar and MacDonald (1987) hypothesized that each peripheral population is the result of a long distance migration event and that only a few individuals founded each population. Bottleneck and founder effects may be responsible for the low diversity of the marginal populations since there has not been sufficient time for genetic diversity to develop once again. Evidence in support of this idea was the significant correlation between time of population establishment and allelic diversity.

The Spruce-fir Ecosystem of the Balsam Mountains

The following section is a discussion of the late quaternary history of the spruce-fir forests in the southeastern United States. It includes a summary of the recent disturbance history of the ecosystem with particular

emphasis on the events which occurred on Mt. Rogers, and a description of the topography and stand types on Mt. Rogers. The final topic in this section is a review of the current knowledge of the genetic variation patterns of eastern fir species.

Abies has been a part of the flora of the southeastern United States since the Eocene Epoch of the tertiary period (Delcourt and Delcourt 1984). About 70,000 years before present (YR BP) the earth's climate was similar to what we know today. Fir species ranged from New York to Illinois and from Ontario to South Carolina (Zavarin and Snajberk 1972). The climate gradually began to cool and the Wisconsin glaciation period started. Expansion of the ice sheet caused destroyed the vegetation immediately to the south of the glacier. However the cooler climate further south created conditions favorable for boreal forests which gradually expanded their range southward. Boreal forests extended as far as Georgia, Alabama and Louisiana. The western edge of the southern boreal forests extended to the Ohio and Mississippi river valleys (Delcourt and Delcourt 1984).

The range of fir species apparently was split in two, separated by the glacier which extended to the western plains. The fir west of the glacier probably survived in isolated drainage channels at the foot of the Rocky

Mountains. Fir species east and south of the glacier, although wide spread, apparently comprised a smaller percentage of the vegetation than the spruce species. Fossil pollen samples examined by Delcourt and Delcourt (1984) indicated that spruce comprised up to 60% of the vegetation in some areas. Fir usually comprised only 5 to 10% of the vegetation.

Between 18,000 and 14,000 YR BP the climate warmed and the ice sheet retreated to the present location of the Great lakes. The vegetation from the western refuge gradually migrated to the north and east. The boreal forests in the east expanded northward but still comprised a large portion of the vegetation in the Alabama, Georgia and Louisiana region.

A xerothermic period occurred between 10,000 and 4,000 YR BP. The boreal forests in the southeast became restricted to the high elevations in the Blue Ridge province. It was probably between these years that the northern populations of fir became separated from those in the south. This may have caused the formation of the separate species Abies fraseri in the south and Abies balsamea in the north (Robinson and Thor 1969).

This warm period probably caused changes in vegetation patterns at the local scale. Rheinhardt (1984) and others have noted the peculiar absence of fir on Whitetop

mountain. Whitetop (elevation 1682 m) is directly adjacent to Mt. Rogers (elevation 1746 m) in southwest Virginia and pure spruce stands cover the top of the mountain. Elevational differences are a key part of Rheinhardt's hypothesis for explaining the lack of fir on Whitetop.

Rheinhardt assumed that the forests on Whitetop and Mt. Rogers were similar before the xerothermic period, with both spruce and fir present. He further assumed that spruce is more tolerant than fir to hot dry conditions based on the observation that spruce is more common at lower elevations in present day forests. As the climate warmed the spruce and fir were gradually eliminated from the lower elevations on both of these mountains. At the height of the dry period fir may have been completely eliminated from White Top leaving only spruce at the very top. However, because of the 64 m difference in elevation fir survived at the summit of Mt. Rogers.

The xerothermic period ended approximately 4,000 YR BP. The climate once again began to cool and consequently the spruce-fir forests in the south began to expand. Approximately 2,000 YR BP fir was in upper South Carolina, western North Carolina, eastern Tennessee and southwestern Virginia. It constituted up to 15% of the vegetation in some areas (Delcourt and Delcourt 1984).

The spruce and fir which survived on Mt. Rogers and the spruce on White Top migrated back down the slopes of the mountains. Rheinhardt (1984) speculates that the fir did not recolonize White Top because it was incapable of crossing the low lying valley which separates the two mountains.

The spruce-fir forests continued to expand until recent times. This expansion stopped because of human activities. Dull et al. (1988) estimate that spruce-fir forests currently cover 26,610 hectares in the southern Appalachians. Korstian (1937) estimated that spruce-fir covered approximately 404,694 hectares before the logging which occurred at the turn of the century. Post logging slash fires, livestock grazing, alteration of soil characteristics by logging and competition from invading hardwoods have all contributed to the failure of spruce-fir regeneration (Pyle 1984).

Currently the spruce-fir forests are being reduced to even lower levels of abundance. Approximately 30% of the hectares of spruce-fir left have heavy to severe mortality of the fir (Dull et al. 1988). There is some speculation that atmospheric pollution may be responsible for some of the mortality (Eagar 1984; Bruck 1984 and 1985). However, while there is no definitive answer to the question of what is the primary cause of the tree mortality, the

overwhelming circumstantial evidence is in favor of insect infestation as the root cause (Dull et al. 1988). The insect responsible, the balsam wooly aphid, has been spreading through the southern Appalachians since the early 1960's (White 1984). The aphid is a sucking insect which in North America exclusively attacks fir trees (Eagar 1984). Girdling, due to morphological changes in the stem wood caused by chemicals in the saliva of the insect, kills the tree.

There is evidence suggesting that the spruce-fir stands may be able to recover from insect infestation. Since the aphid primarily attacks mature trees advance regeneration and seedlings produced immediately before the death of the mature tree may grow to replace the adult. Witter and Ragenovich (1986) estimated there were 14,026 fir seedlings per hectare in stands devastated by the aphid. The primary concern regarding the future of the fir in these stands is whether the insect will destroy these young trees before they are sexually mature.

Site Description and Disturbance History of Mt. Rogers

Mt. Rogers is located in Grayson and Smyth counties in southwestern Virginia (latitude 36°40'N, longitude 81°30'W). At 1746 m it is the highest elevation in Virginia. The spruce-fir forests cover approximately 400

hectares. Most of the stands are on Mt. Rogers itself, however there are many spruce and fir trees growing on the nearby spur ridges called Cabin Ridge and Pine Mt. Ridge.

Fraser fir grows at elevations as low as 1,600 m on the northeast side of the mountain while on the southwest side they extend to 1667 m (Rheinhardt and Ware 1984). Within these elevational ranges the stands are nearly pure spruce and fir with only an occasional yellow birch (Betula alleghaniensis Britton) or mountain ash (Sorbus americana Marsh.). At the lower elevations spruce is more common than the fir. The frequency of fir increases with elevation. At the summit is an almost pure stand of fir (Stephenson and Adams 1984).

The understory of the stands on Mt. Rogers at almost all elevations is thick and dense. Stephenson and Adams (1984) estimated there were over 300,000 stems per hectare for the higher elevations. Over two thirds of the stems were Fraser fir seedlings.

Stand composition is more variable on Cabin ridge. Yellow birch and mountain ash are more common as well as american beech (Fagus grandifolia Ehrh.), hawthorne (Cretageous spp.) and mountain maple (Acer spicatum Lam.). Fir grows at elevations as low as 1515 m, but only in small clumps. Spruce and fir are co-dominant on the ridge top itself. These stands are dense with little understory. At

1600 m , at the head waters of Cabin Creek which flows parallel to Cabin ridge, isolated fir trees grow in the open. These trees are short and almost have a Krumholtz form.

The stands on Pine Mountain ridge grow in a narrow band between 1600 m and 1636 m. These stands are similar to those growing on Mt. Rogers in that they have a dense understory and fir is more frequent at the upper elevations. However there is far more yellow birch and mountain ash growing here.

Railroad logging began in the area around 1905 (Pyle et al. 1985). Cutting occurred on most of Cabin Ridge, Pine Mountain Ridge, and the southeast flank of Mt. Rogers. Not all the trees were cut. Only those greater than eight inches in diameter somewhere near the top of the tree were cut (Pyle et al. 1985). The northeast side of Mt. Rogers was uncut because of the steep and rocky terrain. Cabin Ridge and Pine Mountain Ridge were also heavily disturbed by post logging slash fires and livestock grazing (Pyle et al. 1985).

In the 1950's and 60's Pine Mountain Ridge and Cabin Ridge were again cut over. These stands were thinned for Christmas trees and some small scale lumbering also occurred (Pyle et al. 1985). During these years approximately 50,000 seedlings were planted near Cabin

Creek to establish a Christmas tree farm. All cutting and Christmas tree farming ended in 1972 when the U.S. Forest Service purchased the land.

The balsam wooly aphid is present in the population of fir on Mt. Rogers. The indications are that the insect has been in the area since 1962 (Lambert et al. 1980). Trees attacked in the 1960's are still alive, although surveys in 1985 discovered the first incidence of fir mortality (Ghent et al. 1986). Grazing still occurs in the area and there is extensive use of the area for recreation.

Genetic Variation of Eastern Fir Species

There are two eastern fir species, balsam fir (Abies balsamea (L.) Mill.) and Fraser fir. The range of balsam fir is mainly in eastern Canada and the northeastern United States. There are a few disjunct populations in Pennsylvania, northern Virginia and West Virginia (Harlow et al. 1979). The high elevations in the Southern Appalachians are the only places were natural stands of Fraser fir exist. The most obvious morphological difference between these two species is the long and exerted bract of Fraser fir. The bract of the balsam fir is short and does not protrude beyond the cone scale. The disjunct populations of balsam fir have a bract which is intermediate between Fraser and balsam fir.

Fulling (1936) hypothesized that the disjunct populations were hybrids of the two major species. To test this hypothesis Robinson and Thor (1969) collected cones, foliage and branch samples from 15 populations of fir throughout the eastern United States. The disjunct populations of fir were intermediate in their morphological variability. Hybrid index values, hypodermal cell count, bract to cone scale length ratios and several other characters show a nearly linear north to south trend. According to Robinson and Thor (1969) hybrid populations should have greater morphological variability than either parent population because of the mingling of two different gene pools. Since they did not, they concluded that the disjunct populations of fir were not of hybrid origin.

Robinson and Thor (1969) hypothesized that north to south clinal variation patterns existed in the contiguous fir population before the xerothermic period. The differences among the main balsam fir population, the disjunct populations and the Fraser fir population are, according to Robinson and Thor, merely relics of the previous clinal variation.

Thor and Barnett (1974) sampled the same populations once again and this time they investigated the variability of chemical and wood properties. Again there was a fairly strong north to south clinal pattern of variation for all

characters studied. There were significant differences among populations, however there were no multiple comparison tests reported. Thor and Barnett (1974) concluded that their data supported Robinson and Thor's (1969) hypothesis of clinal variation in the once contiguous population.

Zavarin and Snajberk (1972) did not accept Robinson and Thor's (1969) hypothesis. They believed that the differences between Fraser fir and balsam fir are the result of the population separation caused by the xerothermic period. Chemical analysis of tissues collected throughout the range of both firs indicate that Fraser fir has considerably less diversity than balsam fir. Zavarin and Snajberk (1972) consider this to be the result of the population bottleneck which presumably occurred 8,000 to 4,000 YR BP. Thus the genetic differences between the two species could be due to genetic drift.

Isozyme studies conducted by Clarkson and Fairbrothers (1970) and Jacobs et al. (1984) indicate strong genetic similarity between the two eastern fir species. Nei's genetic distances fall within the range expected for one species (Jacobs et al. 1984). Jacobs et al. (1984) conclude that their results support Zavarin and Snajberk's (1972) hypothesis of genetic drift causing the genetic differences among the populations of eastern fir.

Further evidence of genetic similarity between Fraser fir and balsam fir are the successful artificial crosses made between the two species (Clarkson and Fairbrothers 1970; Hawley and DeHays 1985). Thus there are no barriers to sexual crossing. If such barriers existed, it would be significant evidence in favor of the distinctness of the two species. There are no reports concerning the fertility of the trees resulting from these crosses. It is possible that the hybrids are sterile which would be evidence of the distinctness of the two species.

Roller (1966) compared resin canal position, supposedly a powerful taxonomic tool, among balsam fir, Fraser fir and subalpine fir (Abies lasiocarpa (Hook.) Nutt.) a western species. All the trees sampled were grown in a common garden in Canada. The results indicate the close relations among all three species since there were no significant differences among them.

Witter and Ragenovich (1986) stated their belief that the fir growing on Mt. Rogers is not truly Fraser fir. Robinson and Thor's (1969) morphological data does seem to support this assertion. Close examination of their graphs reveals that the mean value of the hybrid index, hypodermal cell count and bract to cone scale length of the Mt. Rogers Fraser fir are less than for other Fraser fir populations. However there is considerable overlap of values among the

populations for each of these traits. The chemical and wood properties measured by Thor and Barnett (1974) do not show any large differences between Mt. Rogers and the other Fraser fir populations.

The chemical analysis Zavarin and Snajberk (1972) performed revealed that the population of fir growing on Mt. Rogers was distinctly different from the fir growing further south. In most statistical analysis the samples from Mt. Rogers were left out because they were so different from the rest of the populations.

Genetic distances, based on isozyme data, among three Fraser fir populations, Mt. Rogers, Mt. Mitchell and Clingmans Dome are relatively small (Jacobs et al. 1984). The values range from .01 to .02. However cluster analysis combined Mt. Mitchell and Clingmans Dome at the first iteration and excluded Mt. Rogers. This is an indication that Mt. Rogers differs genetically from other populations of Fraser fir.

The conclusion drawn from all of these investigations must be that, as Thor and Barnett (1974) state, there is only one species of fir in the eastern United States. However this species does have strong interpopulational differences in morphological and chemical traits. Isozyme variability does not have as distinct a pattern of interpopulational differences as the morphological and

chemical traits but there remains a suggestion of a similar pattern. The population of fir growing on Mt. Rogers may be distinct from the other "Fraser fir" populations. Thus a detailed study of the population is warranted.

MATERIALS AND METHODS

The primary objective of this study was to quantify the genetic diversity of the Fraser fir population on Mt. Rogers. To accomplish this it was necessary to sample a large number of trees, many more than the 20-30 trees per population sampled in other studies of the genetic variation of eastern fir species.

Another objective was to quantify the relationship, if any, between site/stand characteristics and genetic variability. Plots were located throughout the population to provide a sample from as many site/stand characteristics as possible. The statistical techniques used to evaluate this hypothesized relationship were multiple regression, and spatial autocorrelation analysis.

The next objective was to quantify the within and between stand genetic variability. Traditionally population geneticists have used Wright's F statistics and Nei's genetic distances to accomplish this type of an objective. These statistics were calculated in this study to provide a means of comparison with previous studies. Additional statistics, which may have better mathematical and statistical properties, were also calculated. These statistics are Gregorius' index, cluster analysis, and discriminate analysis.

Sampling

Seven transects were established to traverse nearly all elevations, aspects and stand characteristics of Mt. Rogers (Figure 1). Plots were located, with the aid of an altimeter and a topographic map, at every 30.5 m change in elevation. A total of 35 plots were established.

At each plot all trees greater than 7.62 cm in diameter at 1.37 m above the ground (dbh) and within a 20 by 20 m area were counted. It was assumed, based on observations, that all fir trees greater than 7.62 cm dbh were sexually mature. Tree heights were estimated to the nearest meter using a clinometer.

Simpson's index (Kimmons 1987), a measure of species diversity, was calculated using the following formula:

$$C = \Sigma(n_i/N)^2$$

where n_i is the importance value of the ith species, and N the total importance value for all species. Importance values were obtained by averaging the relative abundance (the numbers of individuals of a particular species relative to the total number of individuals), the relative dominance (the basal area of a particular species relative to the total basal area), and the relative frequency (the number of plots where a particular species was encountered relative to the total number of plots).

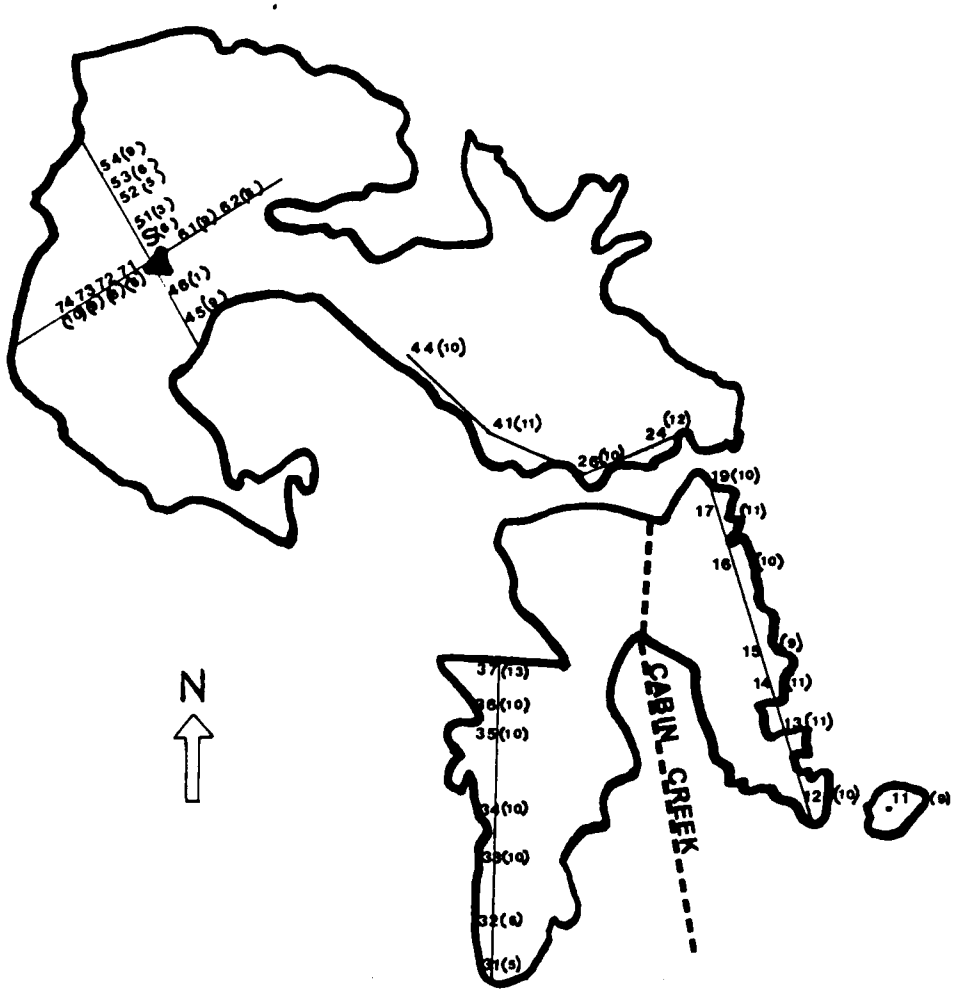


Figure 1. Distribution of spruce-fir stands on Mt. Rogers, Va. Lines indicate transects and numbers plot locations. Numbers in parenthesis are the number of trees genotyped per plot.

Age data for the Mt. Rogers fir population was collected by crews supervised by Dr. S. Zedaker and Niki Nicholas. Thirteen of their plots were adjacent to the plots established for this study. Plot configurations were exactly the same (20 by 20 meters). At each of these thirteen plots ten randomly chosen dominant or co-dominant spruce or fir trees were cored as close to the ground as possible. Cores were taken back to the lab for age estimation. Stand characteristics are summarized in Table 1.

The information from all plots was arbitrarily grouped into three subpopulations based on topography, disturbance history, and stand characteristics. The subpopulation hereinafter called Mt. Rogers encompasses stands growing on the mountain itself. These stands grow on all possible aspects from 1600 m to 1746 m in elevation. In the Mt. Rogers subpopulation the average tree age was 101 years (Table 1), there were 460 fir per hectare and Simpson's index was estimated to be .54. According to Pyle et al. (1985) Mt. Rogers stands have probably not been logged.

Stands on the Cabin Ridge and Pine Mt. Ridge areas were considerably younger than those on Mt. Rogers (Table 1) probably because they were extensively logged (Pyle et al. 1985). Both subpopulations had greater densities and greater species diversities than the Mt. Rogers

Table 1. A summary of the mean stand characteristics of each of the three subpopulation and the entire Mt. Rogers fir population. Numbers in brackets are one standard deviation.

Character	Mt. Rogers	Cabin Ridge	Pine Mt. Ridge	Total
N* =	13	18	4	35
Age (yr)	101 (24)	50.4 (30.2)	36.7**	68.8 (37)
Height (m)	10.9 (1.7)	9.4 (1.2)	8.8 (2.0)	7.2 (2.6)
Density (n/hec > 7.62 cm DBH)				
All trees	767 (299)	935 (803)	1094 (710)	891 (641)
Fraser fir	460 (252)	710 (593)	675 (471)	613 (481)
Simpson's Index	.54 (.18)	.69 (.20)	.60 (.24)	.63 (.22)

* N is the number of plots.

** Age data obtained from the SARRMAC project (Dr. S. Zedaker and Niki Nicholas personal communication, Dept. of Forestry, V.P.I. and S.U., Blacksburg, Va.). Ages were estimated from 5 plots on Mt.Rogers, 7 on Cabin Ridge, and 1 on Pine Mt. Ridge. No standard error can be calculated for the Pine Mt. Ridge subpopulation.

subpopulation (Table 1). Fir on Cabin Ridge grow at elevations ranging from 1515 m to 1600 m. The terrain is relatively flat and most fir grow on south facing slopes. The fir on Pine Mt. Ridge grow in a narrow band between 1600 m and 1636 m and only on a north facing slope.

Up to 10 fir trees per plot were chosen haphazardly for cone collection. At several plots there were less than 10 firs within the prescribed area. Thus fewer than 10 trees were sampled in these cases. Cones were taken from the top third of the crown, brought back to the lab, air dried and the seed separated from cone scales by hand. Empty seeds were removed using an air blower and a random sample of 50 seeds weighed. After soaking in distilled water at room temperature for 24 to 48 hours seeds were put in cold storage. After 45-60 days seeds were put on moist filter paper and placed in a growth chamber at 25^{o}C. A count of newly germinated seed was taken daily. A seed was considered germinated when the radicle extended 2 millimeters or more beyond the seed coat. Germinated seeds were removed from the growth chamber and put in cold storage until needed for enzyme extraction.

After counting newly germinated seeds for three weeks a germination value (GV) was calculated as recommended by Czabator (1962). The formula for GV is as follows;

GV = PV x MDG

MDG= total germination percent for the entire test, divided by the number of days in the test

PV = the maximum value of T

T = the total germination percent at each observation divided by the number of days since the beginning of the test.

Phenological Observations

To determine the potential for gene flow by cross pollination the dates of female receptivity and pollen shed were recorded in the spring of 1987. Little or no flowering occurred in 1988 or 1989 so additional data could not be collected. Transect and plot locations were the same as previously described. Up to 10 trees per plot were scored using the following scale;

0 = no activity
1 = bud swell
2 = bud break, but no pollen shed or receptivity
3 = pollen shed, females receptive

Female strobili were considered receptive when the cone scales were open at 90^{o} angles. Male strobili were considered to be shedding pollen when upon manual movement of branches pollen was released. Weekly observations were made for a five week period.

Genotyping

The isozyme technique is particularly useful for studying conifers because of the ontogeny of conifer seed. The megasporocyte, the female mother cell, undergoes meiosis resulting in four haploid (1N) megaspores. Only one of these develops into a functional part of the ovule (Foster and Gifford 1974). As the functional megaspore matures several archegonia and megagametophyte tissue form. These organelles remain haploid (Liu 1971). A pollen grain fertilizes one or more archegonium and the megagametophyte serves as nutritive tissue for the developing embryo (Foster and Gifford 1974).

The inheritance of isozyme variants obtained from megagametophytes is easily determined because the megagametophyte can be regarded as a single gamete (Feret and Bergmann 1976). If, after sampling several megagametophytes from the same tree the alternative forms of an enzyme segregate in a 1:1 ratio, it can be concluded that the enzyme is the product of a single gene with two different alleles (Conkle and Adams 1977).

Assuming a one to one segregation of alleles in heterozygous parent trees, the probability of correctly classifying a heterozygote at a particular locus is equal to $1-[(0.5)]^{n-1}$ where n is the number of megagametophytes analyzed per parent tree (Cheliak and Pitel 1984). At

least five megagametophytes were sampled from each tree to determine its genotype. Sampling this number of megagametophytes ensured 93.75% confidence that the genotype is being correctly identified. While the stratification and germination procedures previously described worked well for most single tree seed lots, some seed lots failed to have adequate numbers of seeds germinate. Thus for a few plots the number of trees genotyped were low. The total number of trees genotyped was 304. Sample sizes per plot are in Figure 1.

Embryos' were separated from their megagametophytes and discarded. Megagametophytes were homogenized in three drops of a solution of 1% polyvinlypyroledone (PVP-40) and distilled water. The extract was absorbed onto filter paper wicks. A tracking dye was also absorbed onto separate wicks. The wicks were directly inserted into an 11% starch gel made with potato starch (Sigma Chemical Co.). Electrode and gel buffer systems were modified from Cheliak and Pitel (1984) and from Neale and Adams (1981). Complete recipes are in the appendix.

An electric current was applied until the dye had migrated 8-10 mm. The wicks were then removed and the current reapplied until the dye had migrated a total of 8 cm. Electrophoretic separation usually took about three hours. The gel was then cut into horizontal slices and

these were placed in a histochemical staining solution, in the dark at 37°C. Seven enzyme systems were assayed for each tree. These enzyme systems were:

phosphoglucomutase (PGM, EC 2.7.5.1)
glutamine oxaloacetate transaminase (GOT, EC 2.6.1.1)
6-phosphogluconate dehydrogenase (6-PGD,EC 1.1.1.44)
leucine aminopeptidase (LAP, EC 3.4.11.1)
malate dehydrogenase (MDH, EC 1.1.1.37)
glutamate dehydrogenase (GDH, EC 1.4.1.3)
isocitrate dehydrogenase (IDH, EC 1.1.1.42)

Enzyme staining solutions were modified from Cheliak and Pitel (1984). Complete recipes are in the appendix.

The enzyme banding patterns of each megagametophyte were recorded by measuring the migration distance of each band from the origin. To aid in interpretation of the staining patterns a known genotype was run alongside the unknown source.

The inheritance patterns for each of the enzyme systems used in this study were thoroughly investigated by Neale and Adams (1981) for balsam fir. Thus an in depth test of the inheritance of the enzyme phenotypes for the closely related Fraser fir was not necessary. To determine the reliability of laboratory procedures a small test of Mendelian inheritance was conducted. Ten to 15 presumed heterozygous trees for each of the variable enzymes were tested by running 13 to 20 (usually 20) seeds per tree. A chi square test determined the goodness of fit to the expected 1:1 ratio of alleles.

Data Analysis

If allelic frequencies do not conform to frequencies expected under Hardy-Weinberg equilibrium then the population is being subjected to one of the following forces: selection, random drift, mutation, or non-random mating. A chi square "goodness of fit" test determined if the observed genotype frequencies were in concordance with expectations under Hardy-Weinberg equilibrium. This was done for the entire population, for each of the three hypothesized subpopulations, transects, and for plots where sample numbers were sufficient.

Lewontin (1974) stated that observed linkage disequilibrium is a sensitive detector of selection. Linkage disequilibrium is non-random gametic association between alleles at different loci. Pairwise chi square tests for linkage disequilibrium were conducted for each possible combination of the variable loci. The testing procedure is outlined in Weir and Cockerham (1977), and Weir and Cockerham (1978). The calculation of Δ, the linkage parameter, gives a quantitative measure of the amount of disequilibrium. If $\Delta = 0$ then the population is in linkage equilibrium. A chi square test determined if the estimate of Δ was significantly different from zero.

The long term success of a population is dependent on its genetic diversity (Wallace 1981). Several measures

were used to quantify the diversity of the Mt. Rogers fir population. Crow and Kimura's (1970) "effective number of alleles" is considered to be the best among many available allelic diversity measures (Gregorius 1978; Routledge 1979). This index was calculated for the total population, and for each subpopulation. The formula used was

$$v = (\Sigma_i p_i^2)^{-1}$$

where v is the effective number of alleles, p_i is equal to the relative allelic frequencies. The harmonic mean of each of the v's for individual loci is the diversity of all the loci combined, i.e. the gene pool diversity (Gregorius 1978). The gene pool diversity was estimated by calculating the harmonic mean of the effective number of alleles of all the loci including the monomorphic loci.

Diversity was also quantified by calculating conditional heterozygosities for each of the variable loci. Gregorius (1978) stated that a proper evaluation of heterozygosity, the proportion of individuals which are heterozygous for the locus of interest, relates actual heterozygosity to the maximum possible given the observed allelic frequencies. Thus he recommended using conditional heterozygosity. Conditional heterozygosity is simply the actual heterozygosity divided by the maximum heterozygosity possible. For allelic frequencies (p) greater than 0.5 the maximum possible number of heterozygotes is equal to 2(1-

p). Conditional heterozygosities were calculated for the entire population and for each subpopulation, transect and plot.

The within and between stand genetic variation was evaluated and described using a variety of statistical tests. The first test was a contingency table chi square. Following the recommendations of Workman and Niswander (1970) this test determined if there was significant heterogeneity of allele frequencies among subpopulations, transects and plots.

Wright's F statistics (Wright 1978), as modified by Weir and Cockerham (1984), were also calculated to quantify within and between stand variability. These F statistics are the correlations between specified classes of gametes relative to a specified total population. FIT is the correlation (F) of genes within individuals (I) in the total (T) population; FST is the correlation of genes of different individuals in the same subpopulation (S) within the total population; FPT is the correlation of genes of different individuals from the same plot (P); FIS is the correlation of genes within individuals within subpopulations; FPS is the correlation of genes among plots within subpopulations; FIP is correlation of genes within individuals from the same plot. These values represent the percent of the total genetic variation explained by

population organization, i.e. variation among subpopulations, plots and plots within subpopulations.

Weir and Cockerham's (1984) modifications incorporate corrections for small and/or unequal sample sizes. They also devised a method for calculating the variances of each of the components. The variance estimate is obtained by jackknifing across loci. The jackknifing procedure consists of obtaining a series of estimates of a component by omitting each locus in turn. The variance of these estimates is then used to obtain the variance of the component.

A third procedure used was Nei's genetic distance (Nei 1973). The formula for this index is as follows;

$$D = -\text{Log}_e I$$

$$I = \frac{Jxy}{\sqrt{Jxx * Jyy}}$$

where;

Jxx= the probability that two alleles chosen at random from population x are identical

Jyy= the probability that two alleles chosen at random from population y are identical

Jxy= the probability that two alleles are identical when one is chosen from population x and the other from population y

To obtain an estimate of genetic distance for the gene pool arithmetic means of each of the J's were calculated and I was calculated as before.

A fourth technique for describing genetic variability is Gregorius' "δ" index (Gregorius and Roberds 1986). The formula for this index is as follows;

$$\delta = \Sigma_i c_j D_j$$

where;

$$D_j = .5\Sigma_i \left| pi(j) - \overline{pi(j)} \right|$$

pi(j) = the relative frequency of the ith genetic type in the jth subpopulation

pi(j) = the relative frequency of the ith genetic type in the complement of the jth subpopulation. The complement is the frequency of the ith genetic type in the rest of the population excluding the jth subpopulation.

c_j = the relative size of the jth subpopulation

The relative size of the jth subpopulation was derived from estimates of the number of mature Fraser fir trees per hectare combined with the sample size for each subpopulation. The G test (Zar 1984) determined if the D_j's represented a significant genetic difference between the plot and its compliment.

Additional techniques used to evaluate the genetic variation of this population were discriminant analysis and cluster analysis. The discriminant analysis used transformed data. The genotype of each individual tree was coded according to the scoring algorithm presented by Smouse and Neel (1977). For loci with two alleles coding took the following form: A tree homozygous for a fast

migrating allele was coded as a 1. If a tree was heterozygous it was coded as 0.5. Finally if it was homozygous for the slow migrating allele it was coded as 0.

The cluster analysis used Nei's genetic distances as the raw data for clustering. The two plots with the smallest genetic distance between them were combined into a cluster and all the genetic distances were recalculated with this new cluster treated as a new plot. Formation of new clusters followed the same criteria. This was repeated until all the plots were combined into one. The procedures for this clustering method are in Hartl (1980).

The statistical procedures described above only investigate the genetic relationships among the various population divisions without regard to the spatial patterns of the observed genetic diversity. If spatial patterns are shown to exist it could be an indication of selection. Two techniques, correlation between Nei's genetic distance and geographic distance, and Moran's I index, were used to investigate the relationship between geographic location and genetic variability. Kendall's rank correlation coefficient (Hollander and Wolfe 1973) was calculated to determine the relationship between geographic distance between each plot and the genetic distance between each plot.

Moran's I index calculates the correlation between a genetic variable and geographic distance or more precisely the extent to which the genetic variable of two plots diverge from the average of all others separated by a similar distance. The formulas and an in depth discussion of this index is in Sokal and Oden (1978a,1978b).

Moran's I was calculated for all possible pairs of plots within 13 arbitrarily determined distance classes. The genetic variables were allele frequencies, percent heterozygotes, and the frequency of doubly homozygous trees for each of the variable loci in pairwise combinations. A binary connection matrix was used following Sokal and Oden's (1978b) analysis of human blood groups. The resulting indices were plotted against geographic distance to produce correlograms as suggested by Sokal and Oden (1978a).

Obtaining an estimate of gene flow provides information to explain observed patterns of genetic variability. It is also a link between phenology patterns and the patterns of genetic variability. The formula suggested by Wright (1969) estimated the amount of gene flow occurring in this population. The formula used was;

$$Nm = \frac{1/FPS-1}{4}$$

where;

N = the local population size

m = the average rate of immigration in an "island" model of population structure

FPS= the correlation of genes among plots within subpopulations

Most isozymes are controlled by one or a few genes. Selection may act differently on these characters than on characters controlled by many genes. Two such characters, seed weight and germination value (GV) were investigated to determine if there is a correlation between morphological variation and isozyme variation. Seed weights and germination values were analyzed using nested analysis of variance. Procedures for estimating the appropriate F values for unbalanced data are in Steel and Torrie (1980). The Kolomogorov-Smirnoff test (Hollander and Wolfe 1973) as well as Box plots and stem and leaf diagrams were used to test the assumption of a normal distribution of data.

Kendall's Tau-b correlation coefficients (Hollander and Wolfe 1973) were calculated to determine the relationship between the number of polymorphic loci for each tree and its seed weight and GV. The relationships between seed weight and GV and environmental/stand characteristic variables such as elevation, aspect and stand density were also quantified using Tau-b and multiple

regression. Plots of residual and predicted values were examined, to verify assumptions, for the regression analysis.

RESULTS AND DISCUSSION

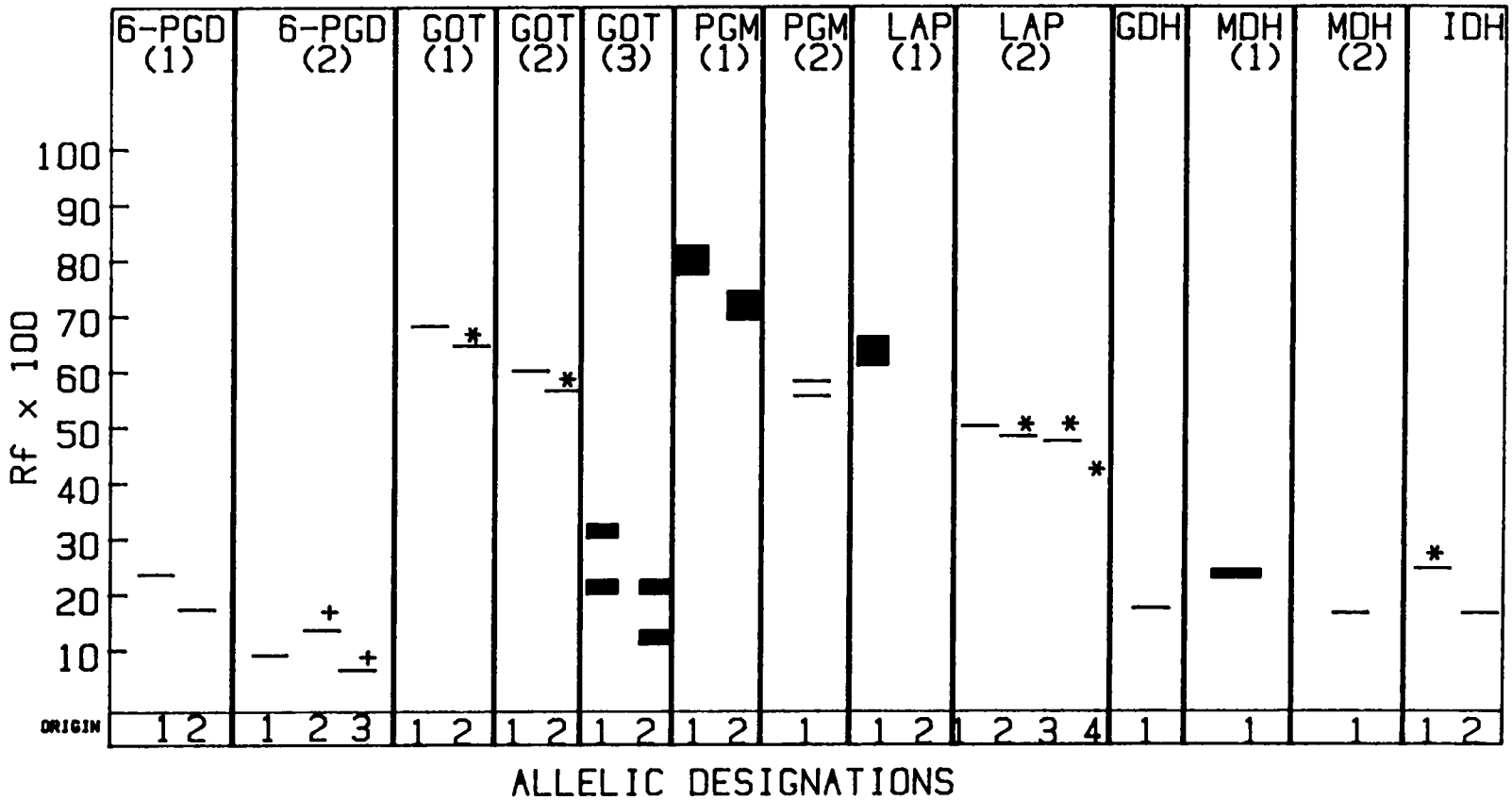
Allele and Genotype Frequencies

Enzyme staining patterns were similar to those Neale and Adams (1981) observed for balsam fir. Therefore enzyme nomenclature follows that used by Neale and Adams. Allelic designations are in Figure 2. The seven enzyme systems were coded by 13 loci, four of which were polymorphic using the 95% criterion. The percentage of polymorphic loci is therefore equal to 30.8%. The variable loci were:

PGM (1)
GOT (3)
6-PGD (1)
LAP (1)

where the number in parentheses is the locus designation (Figure 2).

In the Mt. Rogers population only two alleles were found for each of these loci. The second allele at the LAP (1) locus was a null (i.e. produces no visible band). The alleles at the other loci coded for either fast or slow (relative to each other) migrating enzymes. The inheritance of allelic variants was confirmed by chi square "goodness of fit" tests. All tests provided strong evidence that each of the trees tested was indeed heterozygous and the variants Mendelian (Table 2).



+ indicates alleles found in this study but not in Neale and Adams (1981).

* indicates alleles found in Neale and Adams (1981) but not in this study.

Rf = relative migration distance

Figure 2. Allelic designations for Mt. Rogers fir population. Nomenclature follows Neale and Adams (1981). Horizontal lines and blocks indicate allelic phenotypes.

Table 2. Tests for Mendelian inheritance of isozyme phenotypes in the Mt. Rogers fir population.

Loci	Tree I.D.	n*	Segregating phenotypes 1	2		Chi square	P
PGM(1)	747	20	8	12	OBS.		
			10	10	EXP.	0.8	.3-.5
	158	15	8	7	OBS.		
			7.5	7.5	EXP.	0.07	.7-.8
	546	15	5	10	OBS.		
			7.5	7.5	EXP.	1.7	.1-.2
	124	20	14	6	OBS.		
			10	10	EXP.	3.2	.05-.1
	528	20	12	8	OBS.		
			10	10	EXP.	0.8	.3-.5
	141	10	6	4	OBS.		
			5	5	EXP.	0.4	.5-.7
	222	20	14	6	OBS.		
			10	10	EXP.	3.2	.05-.1
	366	18	12	6	OBS.		
			9	9	EXP.	2.0	.1-.2
	723	20	10	10	OBS.		
			10	10	EXP.	0.0	>.9
	386	20	12	8	OBS.		
			10	10	EXP.	0.8	.3-.5
	256	15	7	8	OBS.		
			7.5	7.5	EXP.	0.07	.7-.8
	545	20	13	7	OBS.		
			10	10	EXP.	1.8	.1-.2
	182	20	13	7	OBS.		
			10	10	EXP.	1.8	.1-.2
	249	20	9	11	OBS.		
			10	10	EXP.	0.2	.5-.7
	413	20	9	11	OBS.		
			10	10	EXP.	0.2	.5-.7

* n= the number of megagametophytes sampled.

Table 2. (Continued)

Loci	Tree I.D.	n*	Segregating phenotypes 1	2		Chi square	P
6-PGD(1)	546	15	9	6	OBS.		
			7.5	7.5	EXP.	0.6	.25-.5
	115F	20	11	9	OBS.		
			10	10	EXP.	0.2	.5-.7
	429	20	8	12	OBS.		
			10	10	EXP.	0.8	.3-.5
	386	20	7	13	OBS.		
			10	10	EXP.	1.8	.1-.2
	249	20	8	12	OBS.		
			10	10	EXP.	0.8	.3-.5
	438	20	7	13	OBS.		
			10	10	EXP.	1.8	.1-.2
	624	20	10	10	OBS.		
			10	10	EXP.	0.0	>.9
	112c	20	11	9	OBS.		
			10	10	EXP.	0.2	.5-.7
	545	17	9	8	OBS.		
			8.5	8.5	EXP.	0.06	.7-.8
	182	20	11	9	OBS.		
			10	10	EXP.	0.2	.5-.7

* n= the number of megagametophytes sampled.

Table 2. (Continued)

Loci	Tree I.D.	n*	Segregating phenotypes 1	2		Chi square	P
GOT(3)	354	20	9	11	OBS.		
			10	10	EXP.	0.2	.5-.7
	429	20	7	13	OBS.		
			10	10	EXP.	1.8	.1-.2
	222	20	10	10	OBS.		
			10	10	EXP.	0.0	>.9
	196	20	7	13	OBS.		
			10	10	EXP.	1.8	.1-.2
	261	20	14	6	OBS.		
			10	10	EXP.	3.2	.05-.1
	551	20	9	11	OBS.		
			10	10	EXP.	0.2	.5-.7
	432	13	8	5	OBS.		
			6.5	6.5	EXP.	0.7	.3-.5
	182	20	11	9	OBS.		
			10	10	EXP.	0.2	.5-.7
	413	20	12	8	OBS.		
			10	10	EXP.	0.8	.3-.5
	126C	20	11	9	OBS.		
			10	10	EXP.	0.2	.5-.7

* n= the number of megagametophytes sampled.

Table 2. (Continued)

Loci	Tree I.D.	n*	Segregating phenotypes 1	2		Chi square	P
LAP(1)	354	15	8	7	OBS.		
			7.5	7.5	EXP.	0.07	.7-.8
	546	15	9	6	OBS.		
			7.5	7.5	EXP.	0.6	.25-.5
	115F	15	9	6	OBS.		
			7.5	7.5	EXP.	0.6	.24-.5
	366	18	11	7	OBS.		
			9	9	EXP.	0.9	.3-.5
	5310	20	11	9	OBS.		
			10	10	EXP.	0.2	.5-.7
	189	20	9	11	OBS.		
			10	10	EXP.	0.2	.5-.7
	459	17	10	7	OBS.		
			8.5	8.5	EXP.	0.5	.25-.5
	456	20	10	10	OBS.		
			10	10	EXP.	0.0	>.9
	545	20	10	10	OBS.		
			10	10	EXP.	0.0	>.9
	112C	20	10	10	OBS.		
			10	10	EXP.	0.0	>.9

* n= the number of megagametophytes sampled.

The allelic and genotypic frequencies for the three subpopulations and the entire population are in Table 3. Allele numbers and frequencies were similar to those reported by Jacobs et al. (1984) for their sample from Mt. Rogers. For example, they reported a frequency of 72% for the fast PGM (1) allele which is exactly the same as the estimate reported in this study. The largest differences between the present study and Jacobs et al. (1984) previous work were at the LAP (2) and MDH (2) loci. Jacobs et al.(1984) report three and two alleles respectively for these two loci. Trees sampled in the present study were monomorphic for both loci.

Out of a total of 20 sampled loci, Jacobs et al. (1984) report that 40% were polymorphic in the Mt. Rogers population. It was estimated, for the present study, that 30.8% of the loci were polymorphic. In comparison the balsam fir populations Jacobs et al. (1984) sampled had percentages of polymorphic loci that ranged from 50 to 60%. Sixty four percent of the 14 loci Neale and Adams (1981) sampled were polymorphic in the balsam fir population they studied.

At a fifth locus, 6-PGD (2), there were two rare alleles as well as a common third allele. This locus was not variable in the Neale and Adams (1981) study or in Jacobs et al. (1984). There were seven heterozygotes with

Table 3. Observed allelic and genotypic frequencies in fir populations on Mt. Rogers. Numbers in brackets are the allelic frequencies expressed as a percentage; plus or minus one standard error.

	Mt. Roger's		Cabin Ridge		Pine Mt. Ridge		Total	
N** =	87		174		43		304	
PGM(1)								
A1+	120	(69±13)	257	(74±12)	56	(65±8)	433	(72±12)*
A2	54	(31±13)	91	(26±12)	30	(35±8)	175	(28±12)
A1A1	42	(48±19)	98	(56±18)	19	(44±13)	159	(52±19)
A1A2	36	(41±21)	61	(35±17)	18	(42±11)	115	(38±18)
A2A2	9	(10±13)	15	(9±10)	6	(14±4)	30	(10±10)
GOT(3)								
A2	153	(88±9)	301	(87±9)	79	(92±3)	533	(88±9)
A1	25	(12±9)	47	(14±9)	7	(8±3	75	(12±9)
A2A2	71	(82±12)	136	(78±14)	36	(84±7)	243	(80±13)
A1A2	11	(13±11)	29	(17±13)	7	(16±7)	47	(16±13)
A1A1	5	(6±9)	9	(5±6)	0	(0)	14	(5±7)
6-PGD(1)								
A2	155	(89±5)	305	(88±9)	79	(87±6)	539	(89±8)
A1	19	(11±5)	43	(12±9)	7	(13±6	69	(11±8)
A2A2	68	(78±11)	134	(77±16)	37	(77±11)	239	(79±14)
A1A2	19	(22±11)	37	(21±14)	5	(21±11)	61	(20±13)
A1A1	0	(0)	3	(2±4)	1	(2±4)	4	(1±4)
LAP(1)								
A1	138	(79±10)	274	(79±11)	75	(87±6)	487	(80±11)*
A4	36	(21±10)	74	(21±11)	11	(13±6)	121	(20±11)
A1A1	56	(64±15)	109	(63±18)	33	(77±11)	198	(65±17)
A1A4	26	(30±11)	56	(32±16)	9	(21±11)	91	(30±15)
A4A4	5	(6±7)	9	(5±6)	1	(2±4)	15	(5±6)

* Significant heterogeneity among plots at a p-value of 0.15 using a chi square test as recommended by Workman and Niswander (1970).

** N equals sample size.

+ Allelic designations follow Neale and Adams (1981).

a slow migrating allele (A3) and the common allele (A1), five heterozygotes for a fast migrating allele (A2) and the common allele and four homozygotes for the fast migrating allele.

Heterozygosity and Diversity

Actual heterozygosities for most of the loci were not significantly different from the expected heterozygosities under Hardy-Weinberg equilibrium (Table 4). One exception was the GOT (3) locus. For this locus there were significant ($p = .05$) differences from Hardy-Weinberg expectations for the entire population, for Mt. Rogers, for Cabin Ridge and for most plots. There were fewer GOT (3) heterozygotes than expected except for Pine Mt. Ridge where there was no significant deviation from expectations. All actual heterozygosities for the other loci were slightly lower than expectations. There was little difference in actual heterozygosities among the subpopulations. However there was a slight trend for heterozygosity to be lower in the Pine Mt. Ridge subpopulation. Mean heterozygosity for the four polymorphic loci was 25.8%. In comparison to these results, Neale and Adams (1985) estimated a mean heterozygosity of eight polymorphic loci to be 26.6%. Jacobs et al. (1984) did not report an estimate of mean heterozygosity.

Table 4. The actual, predicted and conditional percentage of trees heterozygous for each of the variable loci in Fraser fir on Mt. Rogers.

		Subpopulation			
Loci		Mt. Rogers	Cabin Ridge	Pine Mt. Ridge	Total
PGM(1)	actual	41.4%	35.1%	41.9%	37.8%
	H-W	42.8	38.6	45.4	41.0
	condi.	66.6	67.0	60.0	65.7
GOT(3)	actual	12.6	16.7	16.3	15.5
	H-W	21.2*	23.3*	14.9	21.6*
	condi.	52.3	61.7	100.0	62.6
6-PGD(1)	actual	21.8	21.3	11.6	20.1
	H-W	19.4	21.5	14.9	20.1
	condi.	100.0	86.0	71.4	88.4
LAP(1)	actual	29.9	32.2	20.9	29.9
	H-W	32.8	33.4	22.3	31.8
	condi.	72.2	75.6	81.8	75.2

H-W = Hardy-Weinberg heterozygosity

condi. = conditional heterozygosity

* indicates significant deviations from H-W, p=.05

Conditional heterozygosities were highest for the 6-PGD (1) locus and lowest, on the average, for the GOT (3) locus. The one exception was the Pine Mt. Ridge subpopulation. For the Got (3) locus this subpopulation had the maximum number of heterozygotes possible given the observed gene frequencies. The conditional heterozygosities for the other loci did not vary greatly among the subpopulations. Average conditional heterozygosity was 73%.

Gregorius et al. (1986) reported conditional heterozygosities that ranged from 23% to 100% (mean = 51.8%) among three subpopulations within a single stand of European beech. Actual heterozygosities for beech generally were significantly lower than Hardy-Weinberg expectations. Whatever force caused this lack of heterozygosity in beech may also be responsible for the low conditional heterozygosity as well. The differences in conditional heterozygosities between the Mt. Rogers fir population and the European beech stand could be explained by the fact that the fir population is in Hardy-Weinberg equilibrium and the beech was not.

The effective number of alleles were low in this fir population (Table 5). Gregorius et al. (1986) reported values as high as 2.73 for a single stand of European beech. PGM (1), the most diverse locus in this population

Table 5. Crow and Kimura's (1970) effective number of alleles index, a measure of allelic diversity for Mt. Rogers Fraser fir.

	Subpopulation			
	Mt.Rogers	Cabin Ridge	Pine Mt. Ridge	Total
PGM(1)	1.75	1.63	1.83	1.69
GOT(3)	1.27	1.30	1.18	1.27
6-PGD(1)	1.24	1.27	1.18	1.25
LAP(1)	1.49	1.50	1.29	1.47
Gene Pool*	1.10	1.10	1.08	1.10

* includes monomorphic loci.

of fir, had a diversity index of 1.69. The Mt. Rogers fir population, which includes thousands of trees, had a lower diversity index (1.10) than a single stand of just a few hundred beech trees (1.55). Pine Mt. Ridge was the least diverse subpopulation (1.08) although the differences among subpopulations were small.

Reinhardt's (1984) hypothesized population bottleneck may be the cause of the low genetic diversity estimated for this population. The model developed by Nei et al. (1975) demonstrated that the number of generations since the bottleneck is a key influence on the genetic diversity of a population. Assuming that it takes 20 to 30 years for Fraser fir to reach sexual maturity (Johnson 1980) there could only have been, at the most, 200 generations in the 4,000 years since the bottleneck. Most likely the actual number of generations may be lower because heavy seed years only occur every four to five years (Schopmeyer 1974) and age data indicates that fir can live 100 years or more. Thus generation length may be as great as 35 to 100 years since the in situ trees may prevent the establishment of the next generation. The number of generations since the bottleneck may then be 40 or less. According to Nei's model, which simulated heterozygosity patterns after bottlenecks, the population could not have regained high levels of diversity, in the absence of

selection, in such a short time. The simulations showed that it would take several thousand generations to regain heterozygosity even in populations with high intrinsic growth rates.

The model developed by Nei et al. (1975) assumed that population numbers are extremely low during the bottleneck. There is no way to assess what the population size was on Mt. Rogers during the xerothermic period. Evidence suggests that it was extremely small. Delcourt and Delcourt (1984) found fir pollen that dated to this period in only one area and the amount of pollen found was extremely small. Reinhardt (1984) assumed that the fir was only able to survive in a small area at the summit. Assuming that fir survived only in areas above 1700 m in elevation and on the northeast side of the mountain, at most 5,700 trees survived on 12.4 hectares. The estimate of the number of survivors was obtained by using present day densities but it is also possible population densities were lower in this small area than those found under present conditions.

Organization of Genetic Variation

All indices and statistical tests indicated there is very little genetic differentiation among the population divisions (i.e. among subpopulations, transects, and

plots). Contingency table chi square tests indicated that allelic frequencies are not statistically heterogeneous among the three subpopulations (Mt. Rogers, Cabin Ridge, and Pine Mt. Ridge) nor among the transects within them. There was no significant heterogeneity among plots at the GOT (3) and 6-PGD (1) loci. There was weak evidence (p =.15) of differentiation among plots at the PGM (1) and LAP (1) loci (Table 3).

To determine the amount of within and between stand variation Wright's F statistics were calculated. FST, FPT and FPS values (Table 6) are all less than 1%. The total percent of the genetic variation present in the population explained by interplot or intersubpopulation differences is .35%. Thus more than 99% of the genetic variation, as measured by this index, is due to tree-to-tree variation.

Theoretically these three values (FST,FPT and FPS) can not be negative (Weir and Cockerham 1983). However because of the corrections for unequal/small sample size it is sometimes possible to have values less than zero. This is most likely the cause for the negative estimates for each of the individual loci (Table 6).

FIS represents the reduction in heterozygosity due to non random mating in subpopulations and FIP is representative of the non random mating occurring in plots. The Mt. Rogers fir population does not have a

great amount of inbreeding within subpopulations (FIS = .007; Table 6) but there is considerably more inbreeding occurring within plots (FIP = .101; Table 6). This could be the result of long distance pollen flow reducing the inbreeding within a subpopulation and short seed dispersal distances creating a situation were close relatives are likely to mate since they are close neighbors.

A second method of quantifying within and between stand variability is Gregorius' "δ" index. Analysis of this index leads to the same conclusions as the F statistics (Table 7). The estimated differentiation is 10 times higher than for the F statistics but the largest inter-subpopulation differentiation was only 8.2%. None of the subpopulation allelic frequencies were significantly different from their compliment.

The "δ" index indicated that interplot differences were larger than most of the intersubpopulation differences. Yet only in five cases were plot allelic frequencies significantly different from their compliments. These significant differences may be spurious since with an alpha level of .05 one would expect that seven of the 140 tests would be significant just due to random chance (type 1 error).

Nei's genetic distances (Table 8) also indicated very low differentiation among the plots within the total Mt.

Table 6. F statistics describing the hierarchical organization of genetic variation within the population of Fraser fir on Mt. Rogers. Numbers in brackets are plus or minus one standard errors.

Locus	FIT*	FPT	FST	FIS	FPS	FIP
PGM(1)	0.081	0.003	0.004	0.015	0.002	0.078
GOT(3)	0.272	-0.002	-0.003	-0.007	-0.001	0.274
6-PGD(1)	0.022	-0.002	-0.005	<0.001	-0.003	0.024
LAP(1)	0.065	0.003	0.005	0.01	0.002	0.062
ALL LOCI	0.102	0.001	0.002	0.007	0.0005	0.101
	(±.04)	(±.002)	(±.003)	(±.013)	(±.001)	(±.04)

FIT = the correlation (F) of genes within individuals (I) in the total (T) population.

FPT = the correlation of genes of different individuals from the same plot (P).

FST = the correlation of genes of different individuals in the same subpopulation (S).

FIS = the correlation of genes within individuals within subpopulations.

FPS = the correlation of genes among plots within populations.

FIP = the correlation of genes within individuals from the same plot.

Table 7. Subpopulation differentiation, expressed as the percent (Dj), difference in allele frequency between a plot and its compliment as determined by the "δ" index (Gregorius and Roberds 1986).

Plot	Locus	PGM(1) Dj**	GOT(3) Dj	6-PGD(1) Dj	LAP(1) Dj
11		17.7	3.7	29.0*	14.2
12		16.3	3.2	11.1	10.9
13		15.3	9.9	11.1	10.8
14		26.9*	9.3	1.6	1.4
15		23.5*	1.9	4.9	8.6
16		8.6	8.3	4.4	5.6
17		3.5	19.5*	7.8	16.2
19		1.6	3.1	9.5	10.7
21		0.7	3.7	5.0	8.6
22		1.6	7.2	4.4	15.1
23		3.5	3.1	9.5	5.6
24		18.3	5.9	6.3	3.0
26		3.5	8.2	10.9	0.5
31		18.9	13.2	10.8	19.7
32		4.9	13.2	6.0	2.9
33		11.8	2.0	0.7	15.8
34		1.6	2.1	4.6	0.5
35		8.9	12.6	6.1	16.4
36		19.4	7.3	0.8	16.3
37		1.6	13.9*	3.2	4.5
41		8.3	4.1	1.7	10.8
44		1.6	8.3	5.9	15.0
45		17.5	3.7	4.9	8.4
46		21.9	13.2	10.9	19.8
51		22.1	3.7	11.0	20.0
52		1.6	13.4	0.7	0.5
53		13.5	4.8	2.4	14.0
54		6.4	9.4	0.5	2.7
61		10.7	1.9	0.5	8.7
62		3.5	13.3	6.1	2.9
71		30.5	13.3	14.5	2.9
72		3.5	5.8	1.8	0.7
73		6.4	1.9	0.5	8.5
74		11.8	3.1	5.8	9.7
Sum.		3.5	20.9	2.4	22.9
Total	(δ)	9.5	7.6	5.5	9.8

* signifies a significant difference between the location's gene frequency and its compliment at p =.05 using the G-test.

** Dj = the proportion of alleles by which two plots effectively differ.

Table 7. (Continued)

Subpop. Locus	PGM(1)	GOT(3)	6-PGD(1)	LAP(1)
	Dj**	Dj	Dj	Dj
Mt. Rogers	2.5	0.001	0.2	1.6
Cabin Ridge	6.0	2.6	2.2	3.0
Pine Mt. Ridge	6.4	4.7	3.5	8.2
Total (δ)	4.7	2.0	1.6	3.4

** Dj = the proportion of alleles by which two subpopulations effectively differ.

Table 8. Nei's genetic distances (x 1000) among 35 plots of Fraser fir on Mt. Rogers.

Plot	11	12	13	14	15	16	17	19	21	23	22	31	32	33	34	35	36	37	24	26	41	44	51	52	53	54	61	62	71	72	73	74	45	46
11																																		
12	20																																	
13	21	9																																
14	33	9	22																															
15	13	11	3	25																														
16	9	4	8	13	4																													
17	20	15	7	16	12	13																												
19	8	4	12	9	10	2	12																											
21	11	2	7	10	8	2	9	1																										
23	18	10	5	11	7	7	2	8	6																									
22	7	5	9	11	6	1	10	1	1	6																								
31	27	13	5	23	3	9	17	16	13	9	12																							
32	16	6	12	5	9	3	12	3	5	6	2	10																						
33	18	2	15	6	17	6	14	3	2	11	5	23	6																					
34	11	3	6	8	7	2	6	2	1	3	1	11	2	4																				
35	13	4	7	20	10	6	14	6	2	11	6	18	13	5	5																			
36	9	6	8	26	6	5	17	6	4	13	5	16	13	9	7	2																		
37	18	4	8	8	6	2	13	4	4	6	3	5	1	8	3	11	11																	
24	21	7	15	2	17	8	8	5	5	5	5	18	3	4	3	13	18	6																
26	20	1	6	9	6	3	13	5	3	7	4	6	4	6	3	7	8	1	8															
41	22	6	8	4	9	6	6	6	5	2	5	8	2	7	2	12	15	2	2	3														
44	24	7	6	8	7	6	9	8	7	3	6	4	3	11	4	14	15	1	5	3	1													
51	42	13	14	6	22	19	9	17	14	6	17	18	11	14	10	22	30	11	6	11	4	6												
52	17	3	10	6	8	2	13	3	3	6	2	8	1	5	2	10	10	0	5	1	2	3	12											
53	20	2	16	4	18	6	17	3	3	11	5	21	5	1	4	8	12	6	4	5	6	9	13	4										
54	11	4	3	14	5	3	6	4	1	4	2	11	7	5	1	2	3	6	7	4	5	7	14	6	7									
61	19	4	10	4	12	5	9	3	3	5	4	13	3	3	2	9	13	3	2	4	2	4	7	3	3	5								
62	12	6	10	9	5	1	12	3	4	6	2	7	1	8	3	11	10	1	6	3	3	3	14	1	7	6	4							
71	31	17	32	3	31	17	20	11	16	15	13	31	7	12	12	29	34	13	4	18	9	13	12	10	9	21	8	12						
72	11	3	5	10	4	1	7	2	1	3	1	8	2	5	0	5	6	2	5	2	2	3	11	2	5	1	3	2	15					
73	14	5	3	12	3	2	6	5	3	2	3	4	3	9	2	8	8	2	6	2	2	2	10	3	9	2	4	2	17	1				
74	26	5	9	3	12	8	8	7	5	3	7	10	3	7	3	13	17	3	2	4	0	1	3	3	6	6	2	5	9	4	4			
45	8	5	5	22	3	2	13	5	3	9	3	10	9	9	4	2	1	7	14	5	11	10	24	7	11	2	9	6	29	3	4	12		
46	44	13	19	4	23	17	16	17	16	10	17	15	8	16	12	27	33	8	7	10	4	5	3	9	12	18	7	11	9	12	11	3	26	
Sum	15	8	13	23	18	12	16	9	5	16	10	29	19	6	9	1	5	18	16	13	18	22	27	16	10	5	13	18	33	10	14	18	7	35

Rogers fir population. The largest value indicated a 4% difference between plot 11 which is in the Cabin Ridge subpopulation, and plot 46 in the Mt. Rogers subpopulation. Some of the smallest differences were less than .1% (e.g. plots 41 and 44; Table 8). Plots 11 and 36, both in the Cabin Ridge subpopulation, were the most genetically different plots based on Nei's index.

A cluster analysis (Figure 3), using Nei's genetic distance, indicated a random association among all the plots. There is no indication that plots near each other or in the same subpopulation or the same transect form clusters. What is most striking about the cluster analysis is that plot 11 did not "join" a cluster until the last step in the clustering algorithm. This indicates that plot 11 may be genetically distinct relative to the other plots. However, this is inconsistent with the results of Gregorius' "δ" index which did not indicate differentiation of plot 11. A discriminant analysis did not detect any pattern of genotypic variation (Table 9). The p values for the first two discriminant functions were large and the adjusted canonical correlations were relatively low. Previous studies (Yeh et al. 1985; Cheliak et al. 1988) which used discriminant analysis techniques did detect significant patterns even when traditional statistics (multiple regression, F statistics, etc.) did not. Since

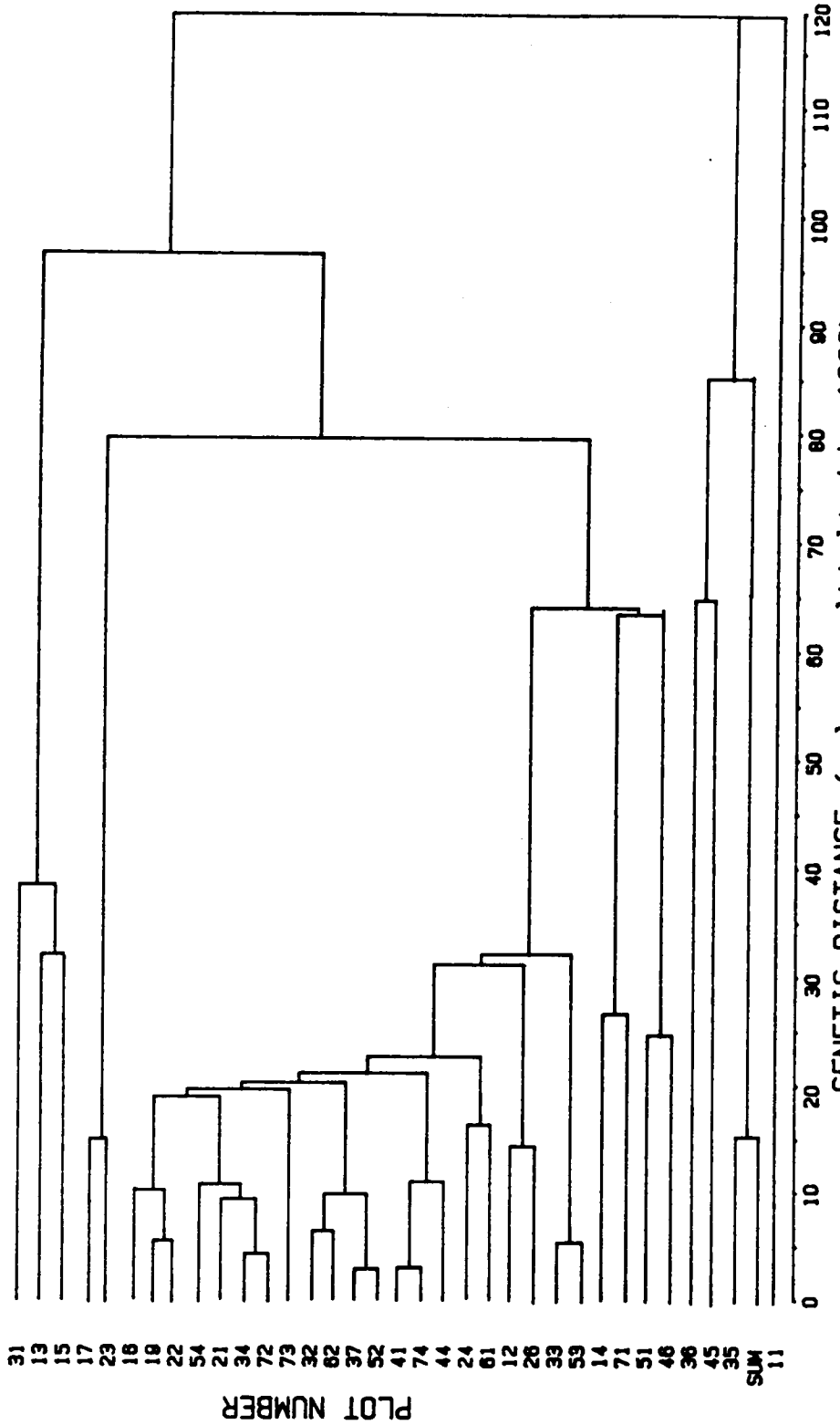


Figure 3. A dendrogram depicting the results of a cluster analysis of Fraser fir on Mt. Rogers.

Table 9. The results of a discriminant analysis of Mt. Rogers Fraser fir genotypes.

	Standardized discriminant function coefficients	
Locus	1	2
PGM(1)	-0.6832	0.5399
GOT(3)	0.4675	-0.0185
6-PGD(1)	0.2221	0.7394
LAP(1)	0.6535	0.3181
Eigen value	0.2253	0.1442
p-value	0.238	0.7330
Adj. canonical corr.	0.2957	0.1981

sensitive statistical techniques as well as the traditional techniques do not detect a pattern the conclusion must be that the Mt. Rogers fir population is a unified population with no subdivisions.

Moran's I index and correlations between Nei's genetic distance and geographic distance support the conclusion there is no pattern in the genetic variation observed in this population of fir. While some of the Kendall coefficients (Table 10) and the I indices (Figures 4 and 5) are statistically significant, the situation is, again, one in which these could be spurious results.

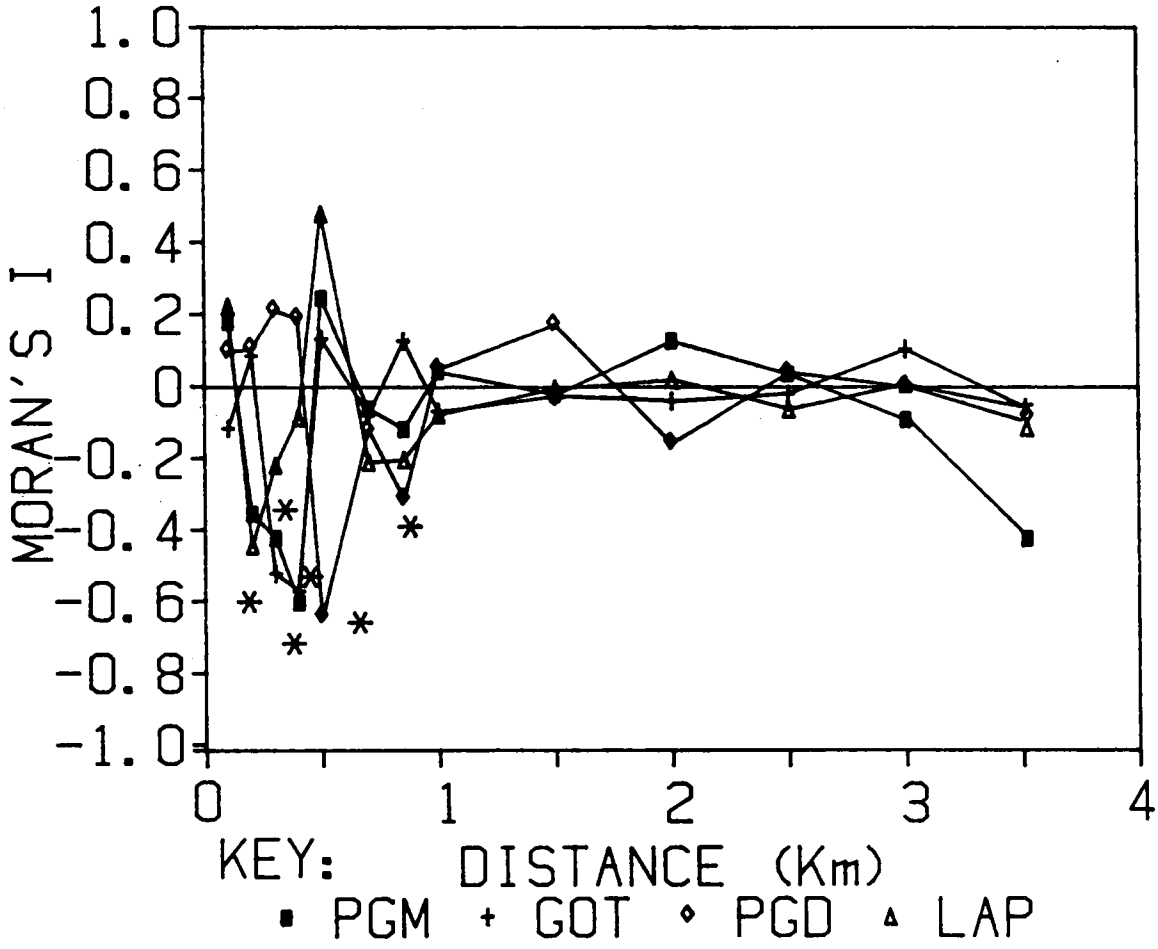
The correlograms (Figures 4 and 5) conform closely to the shape Sokal and Oden (1978b) found using an artificially created data set that was in random spatial order. The indices fluctuate from positive to negative and usually are quite close to zero. No indices were significantly different from zero at the .05 alpha level and only a few were significant at the .10 alpha level.

The results of a weighted regression analysis (weights were the sample size per plot) indicated there was no relationship between any of the genetic variables and any of the stand characteristics (i.e. elevation, aspect, and density). There is a weak ($p = .06$, $R^2 = .09$) trend for the PGM (1) A1 allele to decrease with increasing elevation.

Table 10. Correlations between Nei's genetic distances and geographic distances of Fraser fir on Mt. Rogers.

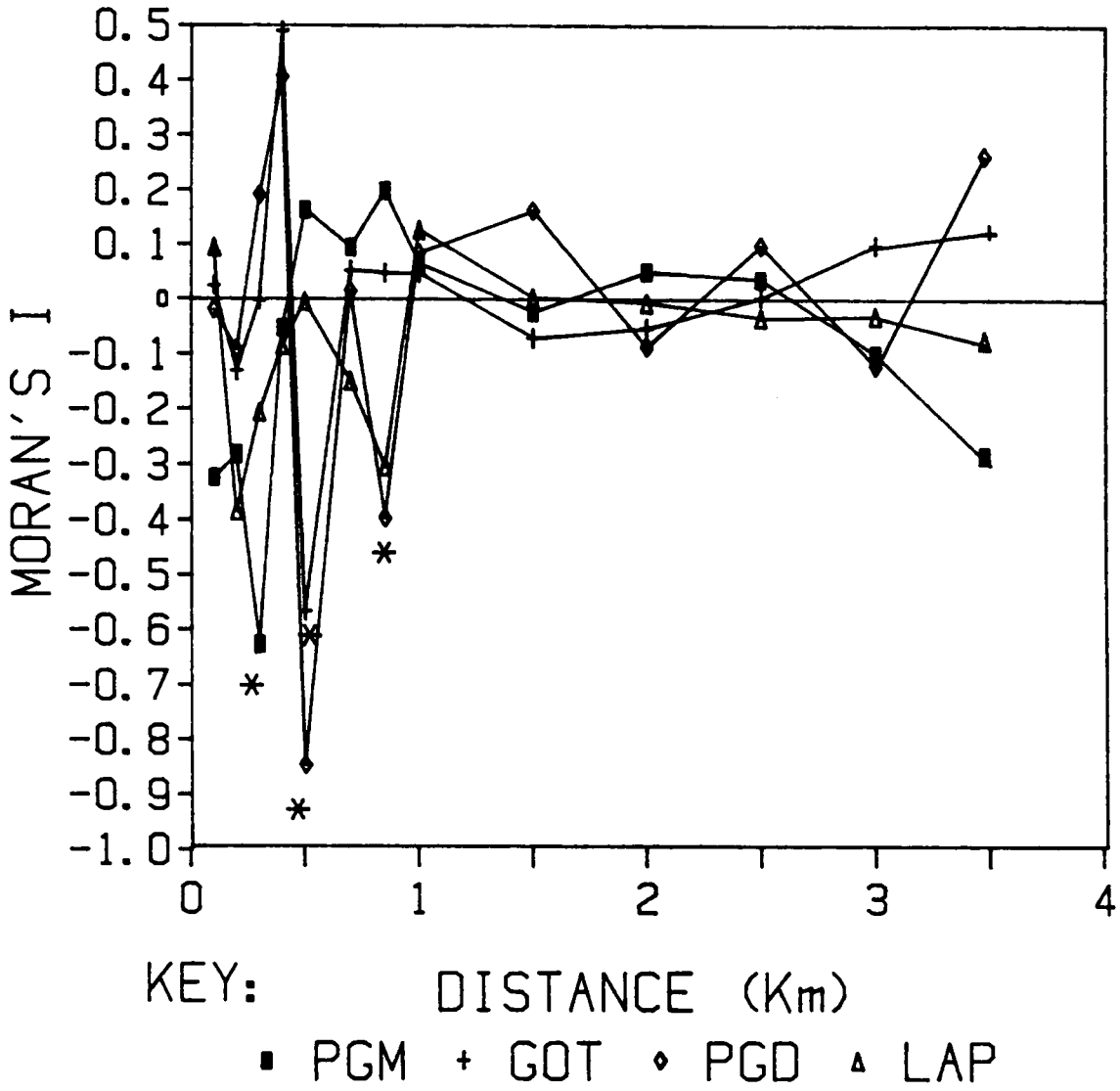
Plot	Mean genetic distance	Mean geographic distance (m x 100)	Correlation coefficient (Kendall Tau B)
11	0.018	21.79	0.132
12	0.006	17.83	-0.105
13	0.010	15.46	0.022
14	0.011	14.47	-0.336****
15	0.010	13.59	0.026
16	0.006	13.58	-0.091
17	0.011	13.66	-0.024
19	0.006	14.08	-0.111
21	0.005	12.20	0.039
22	0.007	12.10	-0.092
23	0.005	12.51	-0.104
31	0.013	18.39	-0.034
32	0.006	17.68	-0.225*
33	0.008	16.02	-0.015
34	0.004	15.31	-0.062
35	0.010	13.69	0.183
36	0.011	13.22	0.200*
37	0.005	12.54	-0.233*
24	0.006	13.66	-0.213*
26	0.008	12.26	0.048
41	0.014	12.24	-0.140
44	0.015	12.83	0.044
45	0.007	14.00	0.174
46	0.057	14.89	0.019
51	0.056	17.01	0.291**
52	0.007	17.21	0.187
53	0.013	17.48	0.255**
54	0.005	17.90	0.093
61	0.008	15.00	0.062
62	0.006	16.15	-0.141
71	0.005	17.27	0.193
72	0.006	18.74	0.099
73	0.016	19.24	0.212*
74	0.004	19.83	0.113
summit	0.005	20.05	0.030

* indicates significance at the .10 level; ** .05 level; **** .001 level



* indicates significance at the .10 level.

Figure 4. A correlogram depicting the correlation of allele frequency among Mt. Rogers Fraser fir plots separated by a similar distance.



* indicates significance at the .10 level

Figure 5. A correlogram depicting the correlation of heterozygosity among Mt. Rogers Fraser fir plots separated by a similar distance.

Gene Flow And Phenological Observations

The lack of population substructuring and the randomness of the variability observed could be the result of a combination of high levels of gene flow and low selection intensities. The most likely form of gene flow is through pollen dispersal. Phenological observations demonstrated there is the potential for mating throughout the population.

The timing of pollen shed and female receptivity were closely correlated in this population. On the same tree, if pollen was being shed, its female strobili were receptive. Therefore only female receptivity is presented (Figure 6). The first trees to be receptive were those at the lowest elevations and southwest aspects. The last trees to be receptive were those growing on the summit of Mt. Rogers. This negative relationship between flowering and elevation is a pattern typical of many different plant species (Stern and Roche 1974).

Once swollen, female strobili developed to mature strobili rapidly. Once-a-week observations were not sufficiently frequent to observe all of the stages of development. Therefore the results of the phenological observations must be considered approximate estimates of the actual dates of receptivity.

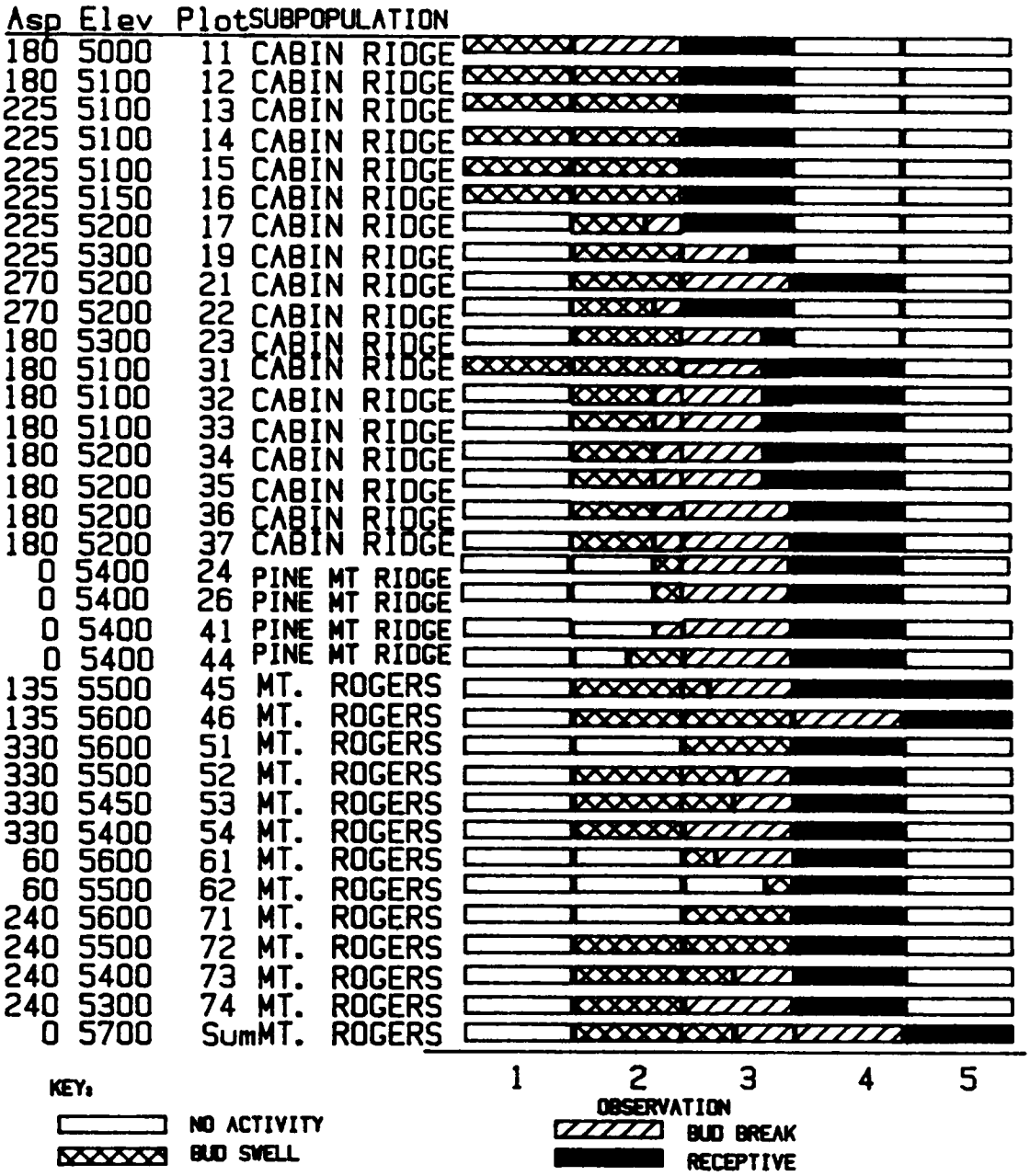


Figure 6. Observations of female receptivity of Fraser fir on Mt. Rogers. Each plot was visited once a week for five weeks. Observation 1 was during the week of April 23, 1987. Changes in hatching pattern within an observation indicates the percentage of trees which were in that stage of development at that time.

It appears as though there is a distinct difference in the timing of receptivity between Cabin Ridge and the other two subpopulations (Figure 6). The trees on Cabin Ridge were receptive during the third week of observation and showed no activity by the next observation. In contrast the Mt. Rogers and Pine Mt. Ridge areas were not receptive until the fourth observation. It is possible that these trees could have become receptive in a very few days after the third observation and therefore there is potential for pollen exchange throughout the population. Certainly year to year variation in phenology would likely cause variation in mating patterns. This variation would be expected to create years with greater or less overlap between Cabin Ridge populations and the other populations sampled.

Effective Population Size

Nm, the effective population size, is estimated to be 525. This value is quite large, well above 1, the critical value Wright (1969) estimated for gene flow to overcome subpopulation differentiation caused by genetic drift. The estimate of Nm in the present study may be conservative. Slatkin (1985) states that Wrights' F statistics are adequate if migration in the population conforms to an "island" model. If migration is more similar to a "stepping stone" model then Wrights' F statistics will

underestimate gene flow. Given the slight elevational trend in flowering phenology observed in this study, the Mt. Rogers populations may more closely represent the stepping stone model.

Crawford (1984) devised a method to estimate N, the average effective population size. His method estimates the neighborhood area based on the variance of the parent-offspring dispersal distribution. The parent-offspring dispersal distribution is composed of two factors. One is the standard deviation of pollen dispersal and the second is the standard deviation of seed dispersal.

The standard deviation of pollen dispersal distances (σ_p) of conifers range from 15.2 to 61 m (Wright 1953 and 1962). The large standard deviations of 61 m may be the result of studying open grown trees. In dense stands the standard deviation may be closer to 15.2 m (Epperson and Allard 1989). Most seed falls within 61 m of the mother tree (Stern and Roche 1974). In dense stands the standard deviation of seed dispersal (σ_s) should be about 15.2 m (Epperson and Allard 1989). The estimated neighborhood area [$A = 4\pi(\sigma_p^2/2 + \sigma_s^2)$], letting σ_p and σ_s equal 15.2 m, is .45 hectares. All of these values are gross estimates and are only intended to provide an indication of the possible neighborhood size.

There are approximately 613 firs greater than 1.2 cm in diameter per hectare on Mt. Rogers and the surrounding area. If the assumption that every fir tree greater than 1.2 cm in diameter is a potential contributor to the next generation is correct, then N is estimated at 276. Wright (1943) stated that if N is approximately 200 then a moderate amount of differentiation is possible.

There is no indication of subpopulation differentiation in this population. Perhaps the estimate of 15.2 m for σ_p and σ_s, while appropriate for the dense stands found in this population, may be too conservative for the open stands that are also present. If the estimate of σ_p and σ_s is increased to 30.5 m then the neighborhood area is 1.75 hectares and N is equal to 1,075. With effective population sizes as great as 1,000 there should be universal panmixa (Wright 1943). With such crude estimates of parent-offspring dispersal distributions it is difficult to arrive at a precise estimate of N. Based on the available information it is likely that N is large, on the order of 1,000.

If the estimate of N is accurate then m is equal to .525. However, this estimate may be meaningless. Slatkin (1985) believes that using census data to estimate N, which is then used to estimate m, is not valid. Census data only provides information about the current population size.

There is no way to account for past fluctuations in population numbers.

The lack of subpopulation differentiation may also be a result of uniform selection pressures or the lack of intense selection pressures altogether. Uniformity of selection pressures could be the result of a uniform environment. This seems unlikely. Despite the fact that the fir population covers only a small area, there is a 221 m change in elevation, stands grow on all aspects of the mountain, and there are considerable differences in the disturbance histories of each of the hypothesized subpopulations. To further quantify environmental differences among plots an estimate of the intensity of solar radiation was calculated. The amount of solar radiation arriving at a particular location is dependent on several factors some of which are; time of year, aspect, angle of slope, and elevation. Estimates of solar radiation used in this study included all of these factors. Aspect and angle of slope were determined in the field for each plot. Daily solar radiation (MJ m^{-2} day^{-1}) at the two equinoxes was graphically estimated from a chart presented in Monteith (1973). Each estimate was corrected for elevation by regression equations developed by Becker and Boyd (1957). Values ranged from 10.32 MJ m^{-2} day^{-1} at plot 74 (the lowest plot on the north west side of Mt. Rogers)

to 23 MJ m^{-2} day^{-1} at plot 23 (located on a south facing slope in the Cabin Ridge subpopulation). The solar radiation values were ranked from high to low and compared to the cluster patterns presented in Figure 3. There was no apparent relationship between the amount of solar radiation and any of the cluster patterns. Selection pressures on the isozyme loci studied must be weak or entirely absent.

Gregorius et al. (1986) cautioned that the frequent reports (Roberds and Conkle 1984; Epperson and Allard 1989 and others) of no subpopulation differentiation may be the result of low genetic diversity at the loci studied rather than the absence of differentiating forces. It follows then that the most diverse loci should be the most differentiated. This appears to be the case for the fir populations used in this study. The locus with the greatest allelic diversity, PGM (1), also had the greatest differentiation among subpopulations. LAP (1) was the second most diverse locus as well as approximately equivalent to PGM (1) (Tables 4,5,6, and 7).

Linkage Disequilibrium

Chi square tests indicate that most of the variable loci are in random association. There is one exception. It appears that PGM (1) and 6-PGD (1) are not randomly

associated (Table 11). The linkage disequilibrium value (Δ) for these two loci is extremely small but significantly different from zero.

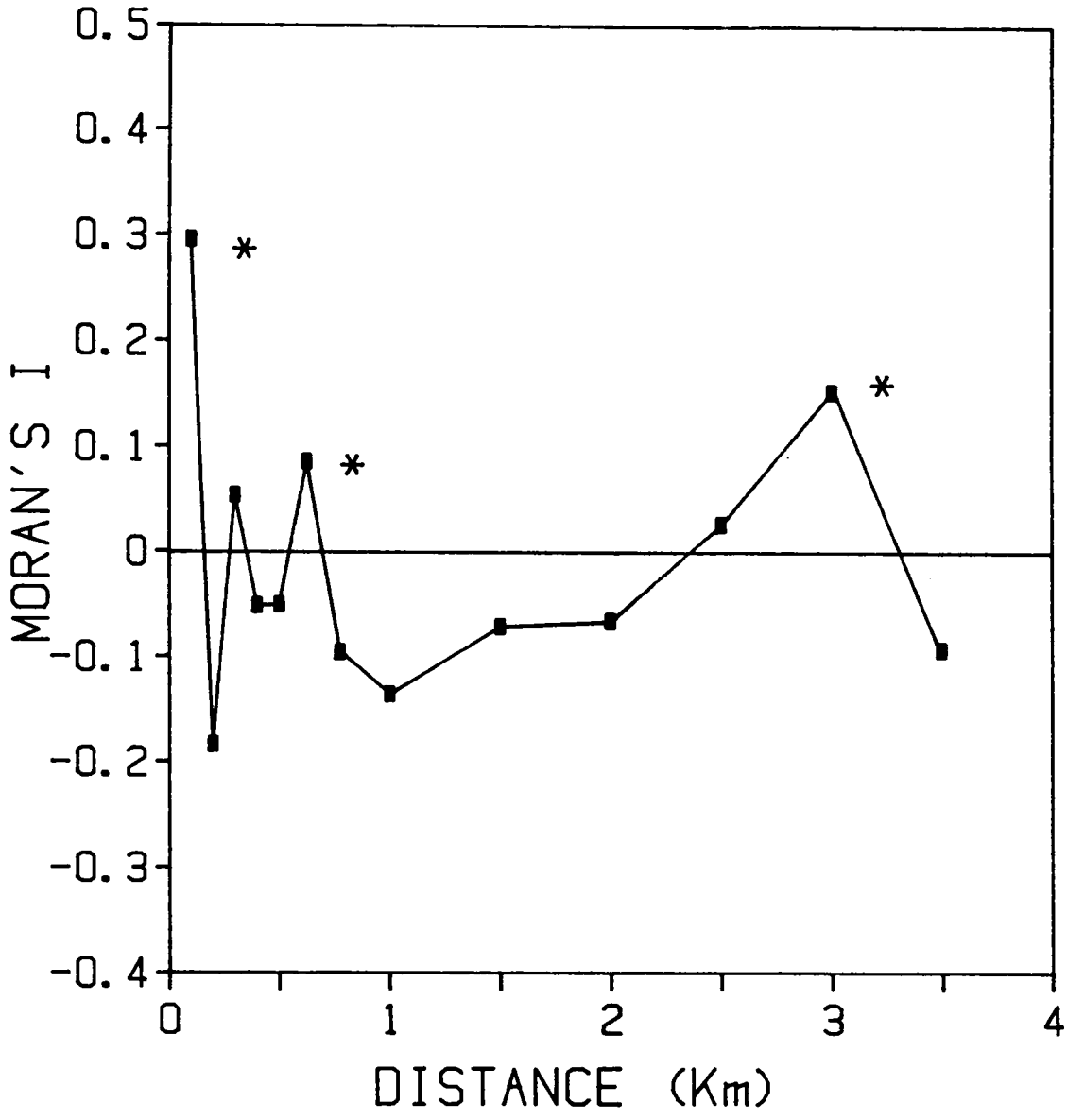
There are several forces which can cause linkage disequilibrium. One force is selection favoring a particular multi-locus genotype (Weir and Cockerham 1977). Lewontin (1974) emphatically stated that observed linkage disequilibrium can be a sensitive detector of natural selection. While it is possible for selection to be acting on the combination of PGM (1) and 6-PGD (1) the selective force is not immediately obvious. There is no correlation between spatial location and the frequency of double homozygotes for the common alleles for these two loci. Only three of 13 Moran's I indices were significantly different from zero ($p=.10$, Figure 7). It is likely that these significant differences are due only to random chance.

A second force capable of causing linkage disequilibrium is random drift caused by small population size (Hill and Robertson 1968; Ohta and Kimura 1969). Linkage disequilibrium can thus be established in a population because of a population bottleneck or a founding event. Brown and Allard's (1971) study of an artificial population of Zea mays is an example of linkage disequilibrium resulting from founders effects.

Table 11. Observed frequency of PGM(1)-6-PGD(1) genotypes in the Mt. Rogers fir population.

Alleles		6-PGD(1) 22	21	11	Δ*	Chi sq.	P
		# of Trees					
PGM(1)	11	127	31	1	.021	6.5	.05-.01
	12	88	24	3			
	22	24	6	0			

* the linkage disequilibrium parameter (Δ) of Weir and Cockerham (1977) with a chi square test of the null hypothesis that Δ equals zero.



* indicates significance at the .10 level.

Figure 7. A correlogram depicting the correlation of double homozygotes for the common alleles of PGM (1) and 6-PGD (1) among plots separated by a similar distance.

As the population begins to increase in size after a bottleneck or a founding event the amount of disequilibrium declines (Hartl 1980). The rate of decline is dependent on, along with other factors, the frequency of recombination (r) among the loci. The frequency of r is determined by how closely linked the loci are. If the loci are on different chromosomes (i.e. they are completely unlinked) then by definition r is equal to .5. With an r of .5 linkage disequilibrium will disappear in 10 generations if no other factors affecting disequilibrium are considered (Hartl 1980).

Neale and Adams (1981) demonstrated that PGM (1) and 6-PGD (1) were unlinked in balsam fir. It must be assumed, since it is likely that these are actually the same species, that these loci are unlinked in Fraser fir as well. It is likely that there has been at least 20 generations since the hypothesized bottleneck. The observed linkage disequilibrium is not entirely due to bottleneck effects. Perhaps the linkage disequilibrium was caused by the bottleneck and some other force has slowed the decay of the disequilibrium.

Non random mating, in particular self fertilization, slows the decay of linkage disequilibrium (Weir and Cockerham 1973). There is the potential for mating between close relatives in the Mt. Rogers Fraser fir population.

Low seed dispersal distances increases the likelihood of relatives growing near each other. Most pollen falls within a few meters of the parent tree. Self fertilization is also possible. Phenological observations indicate that male and female strobili on the same parent tree mature at the same rate. There are no known barriers to self fertilization in fir species (Liu 1971). Thus, inbreeding may have slowed the decay of linkage disequilibrium, originally caused by the hypothesized bottleneck, for it can still be detected in the current generation.

Seed Weight and Germination Value

Since the Kolomogorov-Smirnoff test, Box plots and stem and leaf diagrams indicated that germination values were not normally distributed the data was transformed using a log base 10 transformation. All parametric tests were performed on the transformed data. Seed weight values appeared to be normally distributed.

The results of the nested analysis of variance indicated that mean seed weight and mean $\log_{10}$ GV differed significantly among subpopulations (Table 12). Mean seed weights for plots within subpopulations were not significantly different but mean GV was significantly different ($p= .005$) among plots within subpopulations. Duncan's multiple range test indicated that on the average

seeds from Mt. Rogers were significantly smaller than those from trees growing in the other two subpopulations. Germination values for each of the subpopulations were each significantly different from the rest of the population. The average GV from trees growing on Mt. Rogers were less than the other two subpopulations.

Regression analysis revealed a significant yet relatively weak relationship between average seed weight per plot and elevation ($p = .045$, $R^2 = .09$). Seeds from higher elevations tended to be lighter than those from lower elevations. All other environmental variables were not significant either singly or when included in a multiple regression model. Average GV per plot was also related to elevation and to aspect ($p= .001$, $R^2 = .49$). Trees from northeastern aspects at high elevations had lower germination values than trees on southwestern aspects at low elevations. This may be due to variation in stratification requirements.

There was no correlation between seed weight or GV and the number of heterozygous loci per tree. Kendall's correlation coefficient was much less than .01 and the p value was greater than .95 for seed weight. The correlation coefficient for GV and number of heterozygous loci was less than .001 and the p value equal to .835.

Table 12. Mean seed weight and germination value by subpopulation. Numbers in brackets are plus or minus one standard errors.

Subpopulation	Seed weight (gms)	GV ($\times 10^5$)
Mt. Rogers	0.46 (±.102) A*	14 (±26) A
Cabin Ridge	0.51 (±.05) B	30 (±33) B
Pine Mt. Ridge	0.52 (±.05) B	46 (±4) C
PR > F**	0.0005	0.0001

* Means followed by the same letter within a column do not differ significantly (p = .05) based on Duncan's multiple range test.

** This is the probability (PR) of obtaining an F ratio larger than that observed for the subpopulation main effect.

The significant differences among the subpopulations in seed weight and GV are an indication of a variable environment and possible genetic substructuring of the population. Seed weight is a character that is greatly influenced by the environment, but there can also be a strong genetic element involved (Harper 1977).

The differences in GV among subpopulations must be, in large part, due to genetic differences. GV is only weakly related to seed weight (R^2 = .13; p = .05). Treatment of the seeds was uniform throughout storage and germination. Thus maternal effect (seed weight) and environmental variance have been eliminated or accounted for. One source of non genetic variation not accounted for was the environmental effect during seed development. Perhaps the seeds from trees growing at high elevations and on northeastern aspects were subjected to colder or wetter conditions relative to seeds from low elevations and southwest aspects which could have influenced their subsequent germination.

SUMMARY AND CONCLUSIONS

Heterozygosity and Diversity

The genetic diversity of the Fraser fir population on Mt. Rogers is low. This study found that only four of the 13 loci sampled were polymorphic using the 95% criterion. Neale and Adams (1981) sampled the same 13 loci in a population of balsam fir and found that eight loci were polymorphic. Jacobs and others (1984) sampled other Fraser fir populations and found an average of 50 to 60% of the loci they sampled were polymorphic. They estimated that 40% of the loci they sampled from the Mt. Rogers population were polymorphic. This is comparable to the results of this study.

The xerothermic period probably caused a population bottleneck 8000 to 4000 years ago. That population bottleneck may be the principle cause of the low allozyme heterozygosity and diversity of the Mt. Rogers Fraser fir population.

Population Structure and Selection

The isozyme data provided no evidence of subpopulation differentiation on Mt. Rogers. Analyses of the population using several different statistical techniques all support the conclusion that Fraser fir is a panmictic population.

Phenological observations indicate the potential for pollen flow throughout the population. Migration is likely the predominant force (i.e. selection and drift) acting to create subpopulations.

There is no evidence of strong selective forces acting on the isozyme loci studied. Correlations between genetic variability and site characteristics do not exist. Linkage disequilibrium, which can be a sensitive detector of selection, was found. However, the disequilibrium observed between PGM (1) and 6-PGD (1) is probably the result of the hypothesized bottleneck and nonrandom mating within the Mt. Rogers fir population.

The variability patterns of the two non-molecular characters studied (seed weight and GV) tend to contradict the conclusions drawn from the isozyme data. The isozyme data indicates no substructuring of the population. The two non-molecular characters both indicate strong substructuring. This may be evidence of disconnected molecular and morphological evolution. Future studies should include detailed studies of both the morphology of in situ trees and seedlings grown in a common garden. This may help to determine if there are subpopulations. If morphological analysis led to the conclusion that subpopulations existed, it would be an indication of genetic diversity and perhaps evidence of selection.

The Mt. Rogers fir population has proven to be resilient. It has survived and is surviving both natural and anthropogenic disturbances. There are likely to be more disturbances in the future. If the isozyme data is representative of the entire gene pool then this population may not survive new environmental challenges due to the lack of genetic diversity.

LITERATURE CITED

Allendorf, F.W., K.L. Knudsen and G.M. Blake. 1982. Frequency of null alleles at enzyme loci in natural populations of ponderosa and red pine. Genetics. 100:497-504.

Becker, C.F. and J.S. Boyd. 1957. Solar radiation availability on surfaces in the US as affected by season, orientation, latitude, altitude, and cludiness. J. Solar Energy Sc. and Eng. 1:13-21.

Beckman, J.S. and J.B. Mitton. 1984. Peroxidase allozyme differentiation among successional stands of ponderosa pine. Am. Mid. Nat. 112:43-49.

Bergmann, F. and H.R. Gregorius. 1979. Comparison of the genetic diversities of various populations of Norway spruce, Picea abies. Proc. Conf. Biochem. Genet. Forest Trees, Umea,Sweden. pp. 99-107.

Boyle, T.J.B. and E.K. Morgenstern. 1987. Some aspects of the population structure of black spruce in central New Brunswick. Silvae Genetica. 36:53-60.

Brown, A.H.D. 1979. Enzyme polymorphism in plant populations. Theor. Popul. Biol. 15:1-42.

Brown, A.H.D. and R.W. Allard. 1971. Effects of reciprical recurrent selection for yield on isozyme polymorphisms in maize (Zea mays L.). Crop Sci. 11:888-893.

Brown, A.H.D. and G.F. Moran. 1979. Isozymes and the genetic resources of forest trees. IN: Isozymes of North American Forest Trees and Forest Insects. M.T. Conkle (ed.) U.S.D.A. Forest Serv. Gen. Tech. Rep. PSW-48. pp. 1-10.

Bruck, R.I. 1984. Decline of montane boreal ecosystems in central Europe and the Southern Appalachian Mountains, TAPPI Proc 1984:159-163.

Bruck, R.I. 1985. Boreal montane decline in the Southern Appalachians : potential role of anthropogenic pollution. In: Air pollution effects on forest ecosystems. Proc. Symp. St. Paul, MN May 8-9, 1985. pp. 137-155.

Brussard, P.F. 1984. Geographic patterns and environmental gradients: the central-marginal model in Drosophila revisited. Annu. Rev. Ecol. Syst. 15:25-64.

Carson, H.L. 1955. The genetic characteristics of marginal populations of Drosophila. C.S.H. Symp. Quant. Biol. 20:276-287.

Cheliak, W.M., and J.A. Pitel. 1984. Techniques for starch gel electrophoresis of enzymes from forest tree species. Information Report PI-X-42. Petawawa National Forestry Institute. Canadian Forestry Service.

Cheliak, W.M., J. Wang and J.A. Pitel. 1988. Population structure and genic diversity in tamarack, Larix laricina (Du Roi) K. Koch. Can J. For. Res. 18:1318-1324.

Clarkson, R.B. and D.E. Fairbrothers. 1970. A serological and electrophoretic study of eastern North American Abies (Pinaceae). Taxon. 19:721-727.

Conkle, M.T. and W.T. Adams. 1977. Use of isozyme techniques in forest genetics research. In: Proc. 14th Southern Forest Tree Improvement Conf. Gainsville, Fla. pp.219-226.

Copes, D.L. 1981. Isozyme uniformity in western red cedar seedlings from Oregon and Washington. Can. J. For. Res. 11:451-453.

Crawford, T.J. 1984. The estimation of neighborhood parameters for plant populations. Heredity 52:273-283.

Crow, J.F. and M. Kimura. 1970. Introduction to Population Genetics Theory. Harper and Row, New York.

Cwynar, L.C. and G.M. MacDonald. 1987. Geographical variation of lodgepole pine in relation to population history. Am Nat. 129:463-469.

Czabator, F.J. 1962. Germination value: An index combining speed and completness of pine seed germination. For. Sci. 8:386-396.

Da Cunha, A.B. and T. Dobzhansky. 1954. A further study of chromosomal polymorphism in Drosophila willistoni in relation to environment. Evolution 8:119-134.

Dancik, B.P. and F.C. Yeh. 1983. Allozyme variation and evolution of lodgepole pine (Pinus contorta var. latifolia) and jack pine (Pinus banksiana) in Alberta. Can J. Genet. and Cytol. 25:57-64.

Delcourt, H.R. and P.A. Delcourt. 1984. Late quaternary history of the spruce-fir ecosystem in the southern Appalachian mountains. In: P.S. White ed. The southern Appalachian spruce-fir ecosystem, its biology and threats. NPS, Southeast Regional Office, Research/Resource Management Rpt. No.71. pp. 22-35.

Desparts, M. and J.P. Simon. 1987. Genetic structure and variability in populations of black spruce in the subartic region of northern Quebec. Can. J. For. Res. 17:1006-1012.

Dull, C.W., J.D. Ward, H.D. Brown, G.W. Ryan, W.H. Clerke and R.J. Uhler. 1988. Evaluation of Spruce and Fir Mortality in the Southern Appalachian Mountains. U.S.D.A. Forest Service Southern Region, Protection Report R8-PR 13.

Eagar, C. 1984. Review of the biology and ecology of the balsam woolly aphid in Southern Appalachian spruce-fir forests. In: P.S. White ed. The southern Appalachian spruce-fir ecosystem, its biology and threats. NPS, Southeast Regional Office, Research/Resource Management Rpt. No.71. pp. 36-50.

Endler, J.A. 1986. Natural Selection in the Wild. Princeton Univ. Press, New Jersey.

Epperson, B.K. and R.W. Allard. 1989. Spatial autocorrelation analysis of the distribution of genotypes within populations of lodgepole pine. Genetics. 121:369-377.

Ewens, W.J. 1972. The sampling theory of selectively neutral alleles. Theor. Popul. Biol. 3:87-112.

Falkenhagen, E.R. 1985. Isozyme studies in provenance research of forest trees. Theor. Appl. Genet. 69:335-347.

Feret, P.P. 1974. Genetic differences among three small stands of Pinus pungens. Theor. Appl. Genet. 44:173-177.

Feret, P.P. and F. Bergmann. 1976. Gel electrophoresis of proteins and enzymes. In: Modern Methods in Forest Genetics. Miksche (ed.) Springer-Verlag, Berlin. pp. 49-77.

Fins, L. and L.W., Seef. 1986. Genetic variation in allozymes of western larch. Can. J. For. Res. 16:1013-1018.

Foster, A.S. and E.M. Gifford. 1974. Comparative Morphology of Vascular Plants. W.H. Freeman and Company. San Francisco.

Fowler, D.P. and R.W. Morris. 1977. Genetic diversity in red pine: evidence for low heterozygosity. Can. J. For. Res. 7:341-347.

Fryer, J.H. 1987. Agreement between patterns of morphological variability and isozyme band phenotypes in pitch pine. Silvae Genetica. 36:199-206.

Fulling, E.H. 1936. Abies intermedia, the Blue Ridge fir, a new species. J. So. Appl. Bot. Club. 1:91-94.

Furnier, G.R. and W.T. Adams. 1986 Geographic patterns of allozyme variation in Jeffery pine. Am. J. Bot. 73:1009-1015.

Ghent, J., S. Covington, S. Jennings, and D. Tolman. 1986. Evaluation of balsam woolly adelgid on Mt. Rogers National Recreational Area. Rep. No. 86-1-8. U.S.D.A. Forest Serv. R-8, Forest Pest Management.

Grant, M.C. and J.B. Mitton. 1977. Genetic differentiation among growth forms of Engelmann spruce and sub-alpine fir at tree line. Arct. Alp. Res. 9:259-263.

Gregorius, H.R. 1978. The concept of genetic diversity and its formal relationship to heterozygosity and genetic distance. Math. Biosci. 41:253-271.

Gregorius, H.R., J. Krauhausen and G. Muller-Starck. 1986. Spatial and temporal genetic differentiation among the seed in a stand of Fagus slvatica L. Heredity. 57:255-262.

Gregorius, H.R. and J.H. Roberds. 1986. Measurement of genetical differentiation among subpopulations. Theor. Appl. Genet. 71:826-834.

Guries, R.P. and F.T. Ledig. 1977. Analysis of population structure from allozyme frequencies. In: Proc. 14th Southern Forest Tree Improvement Conf. Gainsville, Fla. pp. 246-253.

Guries, R.P. and F.T. Ledig. 1982. Genetic diversity and population structure in pitch pine. Evolution. 36:387-402.

Harlow, W.M., E.S. Harrar, and F.M. White. 1979. Textbook of Dendrology.McGraw-Hill Book Company. New York.

Hamrick, J.L. 1979. Genetic variation and longevity. In: Topics in Plant Population Biology. O. Solbrig, S. Jain, G. Johnson and P. Raven (eds.). Columbia Univ. Press. pp. 84-113.

Hamrick, J.L., Y.B. Linhart, and J.B. Mitton. 1979. Relationships between life history characteristics and electrophoretically-detectable genetic variation in plants. Ann. Rev. Ecol. Syst. 10:173-200.

Hamrick, J.L., J.B. Mitton, and Y.B. Linhart. 1981. Levels of genetic variation in trees: Influence of life history characteristics. In: Isozymes of North American Forest Trees and Forest Insects. M.T. Conkle (ed.). U.S.D.A. Forest Serv. Gen. Tech. Rep. PSW-48. pp. 35-41.

Harper, J.L. 1977. Population Biology of Plants. New York. Academic Press.

Hartl, D.L. 1980. Principles of Population Genetics. Sunderland, Mass. Sinauer.

Hawley, G.J. and D.H. DeHays. 1985. Hybridization among several North american firs. I. Crossability. Can. J. For. Res. 15:42-49.

Heibert, R.D. and J.L. Hamrick. 1983. Patterns and levels of genetic variation in Great Basin bristlecone pine. Evolution. 37:302-310.

Hill, W.G. and A. Robertson. 1968. Linkage disequilibrium in finite populations. Theor. Appl. Genet. 38:226-231.

Hollander, M. and D.A. Wolfe. 1973. Nonparametric Statistical Methods. John Wiley and Sons. New York.

Jacobs, B.F., C.R. Werth and S.J. Guttman. 1984. Genetic relationships in abies (fir) of eastern United States: An electrophoretic study. Can. J. Bot. 62:609-619.

Johnson,K. 1980. Fraser fir and Balsam woolly aphid: Summary of information. S. Appalchian Res. Resour. Mange. Coop. Rep.

Keeler, K.H. 1978. Intrapopulation differentiation in annual plants: Electrophoretic variation in Veronica peregrina. Evolution 32:638-645.

Kimmons, J.P. 1987. Forest Ecology. MacMillan Publishing Company. New York.

Knowles, P. 1984. Genetic variability among and within closely spaced populations of lodgepole pine. Can. J. Genet. and Cytol. 26:177-184.

Knowles, P. and M.C. Grant. 1985. Genetic variation of lodgepole pine over time and microgeographical space. Can. J. Bot. 63:722-727.

Koehn, R.K., B.L. Bayne, N.W. Moore and J. F. Siebenal. 1980. Salinity related physiological and genetic differences between populations of Mytillus edulis. Biol. J. Lini. 14:319-334.

Koehn, R.K., A.J. Zera and J.G. Hall. 1983. Enzyme polymorphism and natural selection. In: Evolution of Genes and Proteins. M. Nei and R.K. Koehn (eds.). Suderland, Mass: Sinauer. pp. 115-136.

Korstian, C.F. 1937. Perpetuation of spruce on cut-over and burned lands in the southern Appalachian Mountains. Ecol. Monogr. 7:125-167.

Lambert, H.L., S.W. Morgan, and K.D. Johnson. 1980. Detection and evaluation of the balsam woolly adelgid infestations on Mt. Rogers-Virginia, 1979. U.S.D.A. Forest Serv. Southeastern area, State and Private Forestry. Forest Insect and Disease Management.

Ledig, F.T. and M.T. Conkle. 1983. Gene diversity and genetic structure in a narrow endemic, Torrey pine. Evolution. 37:79-85.

Lewontin, R.C. 1974. The Genetic Basis of the Evolutionary Process. Columbia Univ. Press, New York.

Levin, D.A. 1977. The organization of genetic variability in Phlox drummondii. Evolution. 31:477-494.

Levin, D.A. 1978. The origin of isolating mechanisms in flowering plants. Evol. Biol. 11:185-317.

Liu, T.S. 1971. A Monograph of the Genus Abies. National Taiwan Univ. Taipei, China.

Linhart, Y.B., J.B. Mitton, K.B. Sturgeon and M.L. Davis. 1981a. Genetic variation in space and time in a population of ponderosa pine. Heredity. 46:407-426.

Linhart, Y.B., J.B. Mitton, K.B. Sturgeon and M.L. Davis. 1981b. An analysis of genetic architecture in populations of ponderosa pine. In: Isozymes of North American Trees and Forest Insects. M.T. Conkle (ed.). Gen. Tech. Rep. PSW-48. pp. 53-59.

Lundvist, K. 1979. Allozyme frequency distribution in four Swedish populations of Norway spruce (Picea abies). I. Estimations of genetic variation within and among populations, genetic linkage and a mating system parameter. Hereditas. 90:127-143.

MacDonald, G.M. and L.C. Cwynar. 1985. A fossil pollen based reconstruction of the late Quaternary history of lodgepole pine (Pinus contorta ssp. latifolia) in the western interior of Canada. Can. J. For. Res. 15:1039-1044.

Merkle, S.A. and W.T. Adams. 1987. Patterns of allozyme variation within and among Douglas-fir breeding zones in southwest Oregon. Can. J. For. Res. 17:402-407.

Millar, C.I. 1983. A steep cline in Pinus muricata. Evolution. 37:311-319.

Mitton, J.B. 1983. Conifers. In: Isozymes in Plant Genetics and Breeding. Part B. S.D. Tanksley and T.J. Orton (eds.). Elsevier, Amsterdam. pp. 443-472.

Mitton, J.B. and M.C. Grant. 1984. Associations among protein heterozygosity, growth rate, and developmental homeostasis. Ann. Rev. Ecol. and Sys. 15:479-500.

Mitton, J.B., Y.B. Linhart, J.L. Hamrick and J.S. Beckmann. 1977.Observations on the genetic structure and mating system of ponderosa pine in the Colorado Front Range. Theor. Appl.Genet. 57:5-13.

Mitton, J.B., K.B. Sturgeon and M.L. Davis. 1980. Genetic differentiation in ponderosa pine along a steep elevational gradient. Silvae Genetica. 29:100-103.

Monteith, J.L. 1973. Principles of Environmental Physics. American Elsevier Publishing Company Inc. New York.

Neale, D.B. and W.T. Adams. 1981. Inheritance of isozyme variants in seed tissues of balsam fir (Abies balsamea). Can. J. Bot. 59:1285-1291.

Neale, D.B. and W.T. Adams. 1985. Allozyme and mating sytem variation in balsam fir (Abies balsamea) across a continuous elevational transect. Can. J. Bot. 63:2448-2453.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. U.S.A. 70:3321-3323.

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

Nei, M., T. Maruyama and R. Charkraborty. 1975. The bottleneck effect and genetic variability in populations. Evolution. 29:1-10.

O'Reilly, G.J., W.H. Parker and W.M. Cheliak. 1985. Isozyme differentiation in upland and lowland Picea mariana stands in northern Ontario. Silvae Genetica. 34:214-221.

Ohta, T. and M. Kimura. 1969. Linkage disequilibrium due to random genetic drift. Genet. Res. 13:47-55.

Oliver, C.D. 1981. Forest development in North America following major disturbances. Forest Ecol. Manage. 3:153-168.

Pyle, C. 1984. Pre-park disturbance in the spruce-fir forests of Great Smoky Mountains National Park. In: P.S. White ed. The southern Appalachian spruce-fir ecosystem, its biology and threats. NPS, Southeast Regional Office, Research/Resource Management Rpt. No.71. pp. 115-130.

Pyle, C., M.P. Schafale and T.R. Wentworth. 1985. History of disturbance in spruce-fir forests of the SARRMC intensive study sites- Mt. Rogers National Recreation Area, Black Mountains and Great Smoky Mountains. SARRMC -southern Appalachian spruce-fir ecosystem assessment project.

Rheinhardt, R.D. 1984. Comparative study of composition and distribution patterns of subalpine forests in the Balsam Mountains of southwest Virginia and the Great Smoky Mountains. In: P.S. White ed. The southern Appalachian spruce-fir ecosystem, its biology and threats. NPS, Southeast Regional Office, Research/Resource Management Rpt. No.71. pp. 87-99.

Rheinhardt, R.D. and S.A. Ware. 1984. The vegetation of the Balsam Mountains of southwest Virginia: a phytosociological study. Bull. Torrey Bot. Club. 111:287-300.

Rick, C.M., R.W. Zobel, and J.F. Forbes. 1974. Four peroxidase loci in red-fruited tomato species: Genetics and geographic distribution. Proc. Natl. Acad. Sci. 71:835-839.

Roberds, J.H. and M.T. Conkle. 1984. Genetic structure in loblolly pine stands: Allozyme variation in parents and progeny. For. Sci. 30:319-329.

Robinson, J.F. and E. Thor. 1969. Natural variation in Abies of the southern Appalachians. For. Sci. 15:238-245.

Roller, K.J. 1966. Resin canal position in the needles of balsam, alpine and Fraser fir. For. Sci. 15:348-355.

Ross, H.A. and J.L. Hawkins. 1986. Genetic variation among local populations of jack pine. Can. J. Genet. and Cytol. 28:453-458.

Routledge, R.D. 1979. Diversity indices: Which ones are admissible? J. Theor. Biol. 76:503-515.

Schopmeyer, C.S. 1974. Seeds of Woody Plants in the United States. Agriculture handbook No. 450. U.S.D.A. Forest Serv.

Schumaker, K.M. and G.R. Babbel. 1980. Patterns of allozymic similarity in ecologically central and marginal populations of Hordeum dubatum in Utah. Evolution. 34:110-116.

Simon, J.P., Y. Bergeron and P. Gagnon. 1986. Isozyme uniformity in populations of red pine in the Abitibi Region, Quebec. Can. J. For. Res. 16:1133-1135.

Slatkin, M. 1985. Gene flow in natural populations. Annu. Rev. Ecol. Syst. 16:393-430.

Smouse, P.E. and J.V. Neel. 1977. Multivariate analysis of gametic disequilibrium in the Yanomama. Genetics. 85:733-752.

Sokal, R.R. and N.L. Oden. 1978a. Spatial autocorrelation in biology. 1. Methodology. Biol. J. Linn. Soc. 10:199-228.

Sokal, R.R. and N.L. Oden. 1978b. Spatial autocorrelation in biology. 2. Some biological implications and four applications of evolutionary and ecological interest. Biol. J. Linn. Soc. 10:229-249.

Soule', M. 1973. The epistasis cycle: A theory of marginal populations. Ann. Rev. Ecol. Syst. 4:165-181.

Steel, R.G.D. and J.H. Torrie. 1980. Principles and Procedures of Statistics. McGraw-Hill Book Company. New York.

Steinhoff, R.J., D.G. Joyce and L. Fins. 1983. Isozyme variation in Pinus monticola. Can. J. For. Res. 13:1122-1132.

Stephenson, S.L. and H.S. Adams. 1984. The spruce-fir forest on the summit of Mt. Rogers in southwest Virginia. Bull. Torrey Bot. Club. 111:69-75.

Stern, K. and L. Roche. 1974. Genetics of Forest Ecosystems. Springer-Verlag, Berlin.

Stutz, H.P. and J.B. Mitton. 1988. Genetic variation in Englemann spruce associated with variation in soil moisture. Artic and Alpine Research. 20:461-465.

Thor, E. and P.E. Barnett. 1974. Taxonomy of Abies in the southern Appalachians: variation in balsam monoterpenes and wood properties. For. Sci. 20:32-40.

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

Turner, M.E., J.C. Stephens and W.W. Anderson. 1982. Homozygosity and patch structure in plant populations as a result of nearest-neighbor pollination. Proc. Natl. Acad. Sci. U.S.A. 79:203-207.

Wallace, B. 1981. Basic Population Genetics. Columbia Univ. Press, New York.

Wallace, B. 1984. A possible explanation for observed differences in the geographical distributions of chromosomal rearrangements of plants and Drosophila. Egypt. J. Genet. Cytol. 13:121-136.

Weir, B.S. 1979. Inferences about linkage disequilbrium. Biometrics. 35:235-254.

Weir, B.S. and C.C. Cockerham. 1973. Mixed self and random mating at two loci. Genet. Res. 21:247-252.

Weir, B.S. and C.C. Cockerham. 1977. Digenic descent measures for finite populations. Genet. Res. 30:121-147.

Weir, B.S. and C.C. Cockerham. 1978. Testing hypotheses about linkage disequilibrium with multiple alleles. Genetics. 88:633-642.

Weir, B.S. and C.C. Cockerham. 1984. Estimating F statistics for the analysis of population substructure. Evolution. 38:1358-1370.

Wheeler, N.C. 1982. Population structure, genic diversity and morphological variation in Pinus contorta Dougl. Can. J. For. Res. 12:595-606.

Wheeler, N.C. and R.P. Guries. 1982. Biogeography of lodgepole pine. Can. J. Bot. 60:1805-1814.

White, P.S. 1984. Southern Appalachian spruce-fir, an introduction. In: P.S. White ed. The southern Appalachian spruce-fir ecosystem, its biology and threats. NPS, Southeast Regional Office, Research/Resource Management Rpt. No.71. pp.1-21.

Witter, J.A. and I.R. Ragenovich. 1986. Regeneration of Fraser fir at Mt. Mitchell, North Carolina, after depredations by the Balsam woolly adelgid. For. Sci. 32:585-594.

Workman, P.L. and J.D. Niswander. 1970. Populations studies on southwestern Indian tribes. II. Local differentiation in the papago. Am. J. Hum. Genet. 22:24-49.

Wright, J.W. 1953. Pollen dispersal studies: Some practical applications. J. For. 31:114-118.

Wright, J.W. 1962. Genetics of Forest Tree Improvement. FAO Forestry and Forest Products Studies. No. 16. Rome.

Wright, S. 1943. Isolation by distance. Genetics. 28:114-138.

Wright, S. 1969. Evolution and the Genetics of Populations. II. The Theory of Gene Frequencies. Chicago.Univ. of Chicago Press.

Wright, S. 1978. Evolution and the Genetics of Populations. Vol. 4. Univ. Chicago Press.

Yeh, F.C. and J.T. Arnott. 1986. Electrophoretic and morphological differentiation of Picea sitchensis, Picea glauca and their hybrids. Can J. For. Res. 16:791-798.

Yeh, F.C. and Y. A. El Kassaby. 1980. Enzyme genetic variation in natural populations of Sitka spruce (Picea sitchensis (Bong. Carr.). I. Genetic variation patterns among trees from ten I.U.F.R.O. provenances. Can. J. For. Res. 10:415-422.

Yeh, F.C., W.M. Cheliak, B.P. Dancik, K. Illingworth, D.C. Trust and B.A. Pryhitka. 1985. Population differentiation in lodgepole pine, Pinus contorta ssp. latifolia. Can. J. Genet. Cytol. 27:210-218.

Yeh, F.C., M.A.K. Khalil, Y.A. El Kassaby and D.C. Trust. 1986. Allozyme variation in Picea marianna from Newfoundland. Can. J. For. Res. 16:713-720.

Yeh, F.C. and C. Layton. 1979. The organization of genetic variability in central and marginal populations of lodgepole pine Pinus contorta spp. Latifolia. Can. J. Genet. Cytol. 21: 487-503.

Yeh, F.C. and D. O'Malley. 1980. Enzyme variations in natural populations of Douglas-fir, Pseudotsuga menziesii (Mirb.). I. Genetic variation patterns in coastal populations. Silvae Genetica. 29:83-92.

Zar, J.H. 1984. Biostatistical Analysis. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

Zavarin, E. and K. Snajberk. 1972. Geographical variability of monoterpenes from Abies balsamea and A. fraseri. Phytochemistry. 11:1407-1421.

Appendix A

Recipes for Stains and Buffers

The formulations for the electrode buffers, gel buffers and enzyme stain recipes are given below;

Electrode Buffers

A) 223 mM Tris*
86.15 mM citric acid
pH 6.2

B) 0.06 M lithium hydroxide**
0.3 M boric acid
pH 8.1

Gel Buffers

A) 35 ml of electrode buffer diluted to one liter

B) 0.03 M Tris
0.005 M citric acid
1% electrode buffer
pH 8.5

* buffer system A was modified from Neale and Adams (1981)
** buffer system B was modified from Cheliak and Pitel (1984)

Slices from buffer system A were stained for 6-PGD, IDH, and MDH. Slices from buffer system B were stained for PGM, GOT, LAP and GDH. The stain recipes were taken from Cheliak and Pitel (1984) and are as follows;

PGM

25 ml	0.2 M Tris-HCl, pH 8.0
150 mg	glucose-1-phosphate
0.25 ml	glucose-1,6-diphosphate solution(0.1mg/ml water)
25 units	glucose-6-phosphate dehydrogenase
0.5 ml	1% $MgCl_2$ (w/v)
0.5 ml	NADP
0.5 ml	MTT
0.5 ml	PMS

GOT

Stain Recipe

2 mg	pyridoxal-5'-phosphate
50 mg	fast blue BB salt
25 ml	substrate solution*

*Substrate solution

5.30 g	L-aspartic acid and
0.70 g	a-ketoglutaric acid dissolved in 1 litre of 0.2 M Tris-HCl, pH 8.0

LAP

25 ml	0.2 M Tris-HCl pH 8.0
25 ml	0.2 M maleic anhydride
0.5 ml	10% MgCl
25 mg	L-leucine b-napthylamide-HCl
35 mg	fast black K salt

GDH

25 ml	0.2 M Tris-HCl, pH 8.0
200 mg	L-glutamic acid
0.5 ml	NAD
0.5 ml	NBT
0.5 ml	PMS

6-PGD

25 ml	0.2 M Tris-HCl, pH 8.0
20 mg	6-phosphogluconic acid
0.5 ml	NADP
0.5 ml	NBT
0.5 ml	PMS

IDH

25 ml	0.2 M Tris-HCl, pH 8.0
100 mg	DL-isocitric acid
0.5 ml	1% MgCl (w/v)
0.5 ml	NADP
0.5 ml	NBT
0.5 ml	PMS

MDH

12.5 ml	0.2 M Tris-HCl pH 8.0
12.5 ml	0.5 M DL-malic acid, pH 7.0
0.5 ml	NAD
0.5 ml	NBT
0.5 ml	PMS

The vita has been removed from the scanned document