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CONSERVATION OF WHITEBARK PINE IN THE CANADIAN ROCKIES: BLISTER RUST AND POPULATION GENETICS

BY

Geoffrey Jonas Stuart-Smith ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial
fulfillment of the requirements for the degree of Master of Science

in

Conservation Biology

DEPARTMENT OF RENEWABLE RESOURCES

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled CONSERVATION OF WHITEBARK PINE IN THE CANADIAN ROCKIES: BLISTER RUST AND POPULATION GENETICS submitted by GEOFFREY JONAS STUART-SMITH in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in CONSERVATION BIOLOGY.

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ABSTRACT

Whitebark pine, a keystone species of the subalpine ecosystem, is relatively unique among conifers in having bird mediated seed dispersal and, as a consequence, its regeneration, stand structure and genetic diversity differ from other conifers. White pine blister rust and, possibly, successional replacement because of fire suppression may be causing a decline in whitebark pine in the Canadian Rockies. Conservation of this species requires information in the following areas, which this study addresses: the levels of blister rust infection and genetic diversity and the importance of disturbances such as fire on regeneration. High blister rust infection was found in the southern Rockies but infection rate declined northward. Genetic diversity was high within populations, $H_e = 0.211$, and population differentiation was moderate, 6.2%. There was no relationship between genetic diversity and latitude nor between genetic and geographic distances. However, an excess of heterozygotes at sites with high blister rust infection was attributed to high mortality removing homozygous individuals. In addition, stand age distributions suggested that disturbances are important in the regeneration process of whitebark pine. Increasing regeneration in stands declining due to blister rust and successional replacement might be achieved through the reintroduction of fire to the ecosystem.

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Chapter I

I Introduction and Thesis Outline

I.1 Introduction

I.1.1 Ecology and Distribution of Whitebark Pine

Whitebark pine (*Pinus albicaulis* Engelm.) is found at timberline throughout the mountainous regions of western North America (Arno and Hoff 1989) (Figure I.1). There are two major distributions of whitebark pine (McCaughey and Schmidt 1990). The first extends through the Cascade Mountains in British Columbia, Washington and Oregon to the Sierra Nevada of central California. The second follows the major ranges of the Rockies from near latitude 55°N to the Wind River Range in western Wyoming including some of the higher interior ranges such as the Columbia Mountains in British Columbia. At the northern-most extent of its range in Southern Alberta and British Columbia, whitebark pine is restricted to small isolated populations on exposed ridges and rocky talus slopes up to approximately 2300 meters elevation (Ogilvie 1990).

Because of its high alpine setting, it was not until 1863 that Engelmann described whitebark pine (Bailey 1975, Engelmann 1863). Since then, our understanding of the ecological role and basic biology of whitebark pine has been limited compared to the accumulated knowledge on more economically important tree species. However, the importance of whitebark pine, the only stone pine in North America (Lanner 1980), is now starting to be appreciated for two reasons. First, many animals, from small rodents and birds to bears, depend on whitebark pine for shelter and on its energy rich seeds as a food source (Arno 1986). Second, whitebark pine may aid in soil stability and prevent erosion (Arno and Hoff 1989). Nevertheless, many basic ecological and genetic questions about whitebark pine remain unanswered.

The importance of whitebark pine to other subalpine organisms is clearly indicated by the relationship that has co-evolved it and Clark's nutcracker. The nutrient rich seeds of whitebark

Figure I.1-1 Distribution of Whitebark Pine in North America. From Arno and Hoff (1989).

pine are wingless and remain in the cone after maturity. While the cones are still attached to the branches, Clark's nutcrackers adeptly harvest the seeds by ripping open the cones with their long pointed beaks. Clark's nutcrackers have a sublingual pouch that can hold up to 150 whitebark pine seeds, an adaptation that is unique among birds (Bock et al. 1973). With a full pouch, nutcrackers fly off to a suitable site where clusters of up to 15 seeds are cached 2 to 3 cm below the soil surface. Nutcrackers feed almost exclusively on whitebark pine seeds when they are available and store the seeds for use throughout the year (Tomback 1978). Caching sites are usually southern exposures where the lower snow depth facilitates seed retrieval during winter months. Nestlings hatch in the spring and are fed almost exclusively from cached whitebark pine seeds (Lanner 1996).

Whitebark pine produces masts of seeds every 3 to 5 years with intervening years having very low or no seed production. In the years with low seed production, nutcrackers have been known to erupt from their usual habitat and travel great distances in search of other food sources (Lanner 1996). One such event was documented in 1976 when nutcrackers appeared in the Cypress Hills of Southern Alberta, over 300 km from their usual habitat (Fisher 1979, Fisher and Myres 1980). While Clark's nutcrackers depend heavily on whitebark pine seeds as a food source, the relationship between whitebark pine and this bird species is mutualistic (Lanner 1996). Without seed caching by nutcrackers, practically no regeneration would occur in whitebark pine. The few seeds that remain in the cones eventually drop to the ground and rot before they germinate if they are not foraged on by rodents (Hutchins and Lanner 1982). Lanner (1996) points to the heavy wingless seeds as evidence of the co-evolution of the bird-pine relationship because wind is no longer an effective method of seed dispersal.

The seeds of whitebark pine are not only important to Clark's nutcracker but also to other animals such as red squirrels and bears (Arno and Hoff 1989). Red squirrels, in order to prolong seed storage time, hoard whole cones in underground middens. Both black and grizzly bears have also been seen climbing into trees to remove cones, but more often, they will simply raid the already concentrated source in squirrel middens. In years with low cone yields, grizzly bears tend to move from the subalpine environment where whitebark pine is found to lower elevations where encounters with humans are more frequent (Mattson et al. 1992). Protection of whitebark pine habitat is important to the survival of viable populations of bears because bear-human encounters often result in bears being relocated or destroyed. Although bears, squirrels and other animals feed on its seeds, only Clark's nutcracker is important to the regeneration process of whitebark pine (Lanner 1996). Because of its importance to many animals, whitebark pine is a keystone component of the subalpine ecosystem, a role that may be in jeopardy due to the introduction of white pine blister rust (*Cronartium ribicola* Fisch.) (Tomback et al. 1995). The combination of blister rust and fire suppression is thought to have caused decline in whitebark pine in some areas (Keane and Arno 1993) and increased the need for an understanding of the ecology and genetics of whitebark pine.

I.1.2 Life Cycle of White Pine Blister Rust

White pine blister rust is thought to be a major factor in the decline of whitebark pine (Tomback et al. 1995). Thought to have originated in Siberia, white pine blister rust was introduced accidentally into western North America via Europe *ca.* 1910 (Peterson and Jewel 1968, Littlefield 1981). Regions of the coastal mountains dominated by western white pine (*P. monticola*) were quickly infected (Bedwell and Childs 1943). Blister rust has since spread throughout the ranges of the soft pines of western North America including sugar pine (*P. lambertiana*), bristle cone pine (*P. longaeva*), limber pine (*P. flexilis*) as well as western white pine and whitebark pine (Liebhold et al. 1995). Recently, blister rust reached interior states such as North and South Dakota and New Mexico (Draper and Walla 1993, Lundquist et al. 1992, Hawksworth 1990) and into the prairie provinces of Canada (personal observation). White pine blister rust has reduced the prominent role of white pines in forest ecosystems throughout North America (Liebhold et al. 1995), and has become an epidemic on whitebark pine in parts of Idaho, western Montana and Wyoming (Arno 1986, Keane and Arno 1993, Keane et al. 1994, Tomback et al. 1995).

Although the presence of blister rust in the Canadian Rockies has been noted in the past (Forest Insect and Disease Survey 1959, 1968, 1970), how widespread the infection is and what proportion of whitebark pines are infected has not previously been investigated. The extent of blister rust infection not only depends on the distribution of whitebark pine but also on shrubs of the genus *Ribes*. The life cycle of white pine blister rust has five stages that alternate between two host species, white pines and *Ribes* spp. (Zillar 1974). After the initial infection of pine needles, hyphae grow down the phloem and enter the branch or stem of the tree. Two to four years later cankers form and rupture the bark surface. Spermagonia, which produce haploid spermatia, form at the advancing edge of the canker. It is thought that insects, attracted to the nectar-like extrusions of the spermagonia, carry the spermatia to other cankers and facilitate cross-fertilization allowing for the production of dikaryotic cells through plasmogamy (Hunt 1985). Where spermatia grew the previous season, aecia develop that produce aeciospores, thick walled spores that are able to withstand desiccation. Aeciospores are carried by wind to the alternate host, *Ribes*, where another stage of the infection begins.

A few weeks after *Ribes* infection (minimum 21 days), uredinia develop on the under-surface of the leaves. Wind spread urediniospores develop from the uredinia. Urediniospores are able only to re-infect other *Ribes*. Thus, even if only a few trees are initially infected, the cycle of re-infection through urediniospores can rapidly spread blister rust throughout the *Ribes* in an area. During periods of hot dry weather or if infection intensity per leaf area is too high, *Ribes* leaves may be dropped thus decreasing the intensity of infection (Kinloch and Dulitz 1991). In the fall, a period of two weeks of cool weather (below 20°C) stimulates the development of telial columns from the aecia (Van Arsdel et al. 1956). The dikaryotic nuclei within the telial columns fuse and undergo meiosis to produce four haploid basidiospores. Due to their thin walls, basidiospores are very sensitive to environmental conditions (Van Arsdel 1965) and thus have been the focus of studies examining the climatic limitations to the spread of blister rust (Peterson and Jewel 1968). Basidiospores require 48 hours of saturated relative humidity (greater than 97%) and cool temperatures (less than 18°C) for release and germination (McDonald et al. 1981). Bega (1960) found that the optimal temperature for germination was 16°C and that exposure to sunlight for more than 5 hours or freezing significantly reduced basidiospore germination. Basidiospores, carried by wind, re-infect pines by entering the stomata on the needles and thus complete the rust life cycle.

As the rust spreads through the phloem, the nutrient supply can be cut off to branches and portions of the upper stem. Flagging, dead needles that droop to one side of the branch, are visible symptoms of branch death due to blister rust infection. Although a blister may become large enough to girdle and kill a tree, infection may not be the direct cause of death. The excretions of the rust that attract insects also attract rodents that chew the bark around the blister removing vascular tissue which may girdle the tree and result in death. These losses of vascular tissue as well as secondary infections are the main cause of mortality. Because the point of infection is the needles, one mechanism of resistance that has been noted in western white pine is the dropping of infected needles before the rust can spread to the branch (Hoff and McDonald 1980). Several such mechanisms of rust resistance have been noted in western white pine and other white pines (Kinloch and Littlefield 1977, Hoff and McDonald 1980, Yokota 1983, Kinloch 1992, Devey et al. 1995, Meagher and Hunt 1996) and selection may be occurring for similar types of resistance in whitebark pine. There must be genetic diversity available within whitebark pine for selection to produce resistant individuals. However, as indicated by the fact that whitebark pine is more susceptible to blister rust than any other white pine (Hoff and McDonald 1993), whitebark pine may have low levels of genetic diversity, at least with respect to certain traits.

The mortality of whitebark pine resulting from blister rust is highly visible and causes direct selection for resistance compared to the more subtle decline and indirect selection caused by successional replacement. Recent studies in northwestern Montana have pointed to fire suppression as the cause of successional replacement of whitebark pine by the more shade tolerant conifers such as Engelmann spruce and subalpine fir (Arno 1986, Keane and Arno 1993, Keane et al. 1994). Many plant species are dependent on disturbance to remove competitors and enable establishment (Bazzaz 1979), as in Rocky Mountain subalpine forests where fire is one of the most important types of regenerating disturbances (Romme and Knight 1981). Although whitebark pine seedlings are able to tolerate moderate shade, they require high light levels to reach maturity and, therefore, depend on disturbance, such as fire, for regeneration (Arno and Hoff 1989). By measuring the basal area covered by whitebark pine, Keane and Arno (1993) have shown that the numbers of whitebark pine have decreased while those of subalpine fir have increased over a 20-year period. As in Montana, there is concern that the past fire suppression practices of the Canadian Parks Service may have altered the natural successional processes and may be contributing to a decline in whitebark pine populations in the Canadian Rockies. The mandate of Parks Canada is to preserve ecosystems in the National Parks in a pristine state so that their ecological integrity is maintained (Canadian Heritage 1994).

In order to achieve this goal, there must be an understanding of the relationship between fire and its effect on the regeneration of whitebark pine.

I.1.3 Genetic Diversity: Key to Future Survival of Whitebark Pine

Decline caused by blister rust and successional replacement may also be resulting in a decline in the genetic diversity of whitebark pine. Although knowledge of the level of genetic diversity is essential to predicting the future survival of an organism, there has been no comprehensive examination of the amount and distribution of whitebark pine's genetic diversity in the Canadian Rockies. Genetic diversity is the template for adaptive evolution and the basic requirement for the long-term survival and success of populations of organisms (Lande and Barrowclough 1987). In order to avoid extinction in the face of environmental change, populations and species must be able to adapt and evolve. By conserving the present level of natural genetic diversity, the potential for future evolutionary change can be preserved (Ellstrand and Elam 1993). The application of genetics to conservation is based on the fundamental assumption that the risk of extinction increases with the loss of genetic variation (Frankham 1995).

Inbreeding, in combination with selection, results in a decrease in genetic diversity. The probability of extinction in laboratory and domesticated animals has been shown to increase with inbreeding (Frankham 1995). Coping with pathogens and herbivores is an important aspect of adaptation that may be impeded with the loss of genetic variation (Falk and Holsinger 1991, Fritz and Simms 1992). The design of an effective conservation strategy for whitebark pine depends on the knowledge of the present level and distribution of genetic variation in this species (Brussard 1990).

A single number describing the genetic diversity of a species is useless without comparable measures of genetic variation in other species. The development of genetic conservation plans for a specific species depends on the accumulation of data on the genetic variation in a wide range of species with contrasting life histories and ecological characteristics (Namkoong 1995). By comparing genetic diversity among species, an understanding can be gained of what level of genetic diversity is biologically meaningful and how much diversity is required for survival. When introduced, it was hoped that allozyme electrophoresis would be a useful method to accumulate background information on genetic diversity in a variety of species (Frankham 1995). Electrophoresis can be used to determine differences at a single locus between individuals (Hartl and Clark 1989). Differences in genotypes which cause amino acid substitutions result in proteins that migrate different distances in an electrical field. By examining a number of loci in many individuals from a variety of locations, the variation in these single-locus differences can be used to estimate the level of genetic variation in a species. Before electrophoresis, the level of genetic variation in a species could not be separated from the interaction between morphological variation due to environmental factors and the genetic makeup of a species. Knowledge of genetic variation has made possible predictions about the potential for survival and evolution of species.

For electrophoresis to be an effective tool for the investigation of genetic variation, allozyme diversity must reflect the variation found within the genome as a whole (Karron 1991). Hamrick (1989) found good correspondence between allozyme data and genetic variation estimates from other sources, such as quantitative traits or DNA. The low cost and relative ease by which many samples can be screened has lead to the accumulation of genetic variability estimates from many organisms, especially plants (for example, Hamrick and Godt 1989). These investigations have revealed higher amounts of genetic variation in plants, especially conifers, compared to other groups of organisms. The discovery of high diversity in taxonomic groups such as conifers has led to the search for the evolutionary processes and mechanisms that maintain diversity.

I.1.4 Basis of Genetic Diversity

Understanding the mechanisms by which genetic diversity is created and maintained is essential to understanding measures of diversity in wild populations and thus to interpreting the level of genetic diversity in whitebark pine. Migration, selection and mutation are the ultimate evolutionary mechanisms affecting genetic diversity within populations and species and understanding these factors will allow for the prediction of genetic diversity (Lande and Barrowclough 1987). Variation in selection cannot be used to predict genetic variability of a species, however, for two reasons. Firstly, selection varies greatly in space and time. Secondly, selection is local and idiosyncratic in how it alters genetic structure (Loveless and Hamrick 1984). Although mutation does have an appreciable effect on the level of diversity on an evolutionary time scale, migration has a much more significant effect on the spatial distribution of variation among and within populations (Lynch 1988).

Differences in migration, or more specifically gene flow, can give rise to differences in genetic variation contained within populations, among populations or among higher regional groupings (Mitton 1995). Gene flow is the successful movement of genes among populations by mating or by migration of seeds or vegetative propagules (Slatkin 1987). Genetic diversity is maintained, in part, by the exchange of rare and unique alleles that results from gene flow among populations. Because gene flow among populations should be greater in the more continuous portions of a species range but decrease in the more marginal population, marginal populations should have lower genetic variation and fewer unique alleles (Slatkin 1987, Ellstrand and Elam 1993). The pattern of genetic diversity among populations that arises from differences in gene flow can be used as a clue to the evolutionary history of a species. For example, correlations of genetic diversity with geographic distance have been used as evidence for the pattern of post-glacial migration of plant species (Critchfield 1984, Millar 1993). Therefore, understanding the factors that affect the pattern of gene flow is essential to understanding the evolutionary history and predicting the future survival of an organism.

The amount of gene flow among populations, in combination with the chance effects of the recombination system of the organism, determine the influence of factors that decrease genetic variation such as genetic drift and inbreeding (Ellstrand and Elam 1993). Genetic drift, the random change in allele frequencies between generations, occurs because gametes transmitted from one generation to the next carry only a subsample of all the alleles present in the parent populations (Crow and Kimura 1970). The genetic structure of small populations with barriers to gene flow, including physical barriers or simple isolation, will be dominated by the influence of genetic drift (Ellstrand and Elam 1993). In the small isolated populations in which whitebark pine is found in the Canadian Rockies, both physical barriers and isolation prevent gene flow. Small populations are more susceptible to genetic drift because there is a higher probability of change in allele frequencies between generations (Nei et al. 1975). Genetic drift also changes the distribution of genetic variation by intensifying differentiation among populations and decreasing the variation within populations through the chance loss of alleles (Slatkin 1987). Populations that are consistently made up of a small number of individuals are most susceptible to the loss of genetic variation through genetic drift. However, any population that experiences occasional fluctuations in population size may experience random loss of genetic variation (Ellstrand and Elam 1993). A population that has experienced a loss of genetic diversity due to a period when its numbers where reduced is said to have experienced a genetic bottleneck (Crow and Kimura 1970).

Similarly, the founder effect, long distance migration by one or a few individuals founding new populations, can result in populations with reduced genetic variation. The probability of a founder event occurring ultimately depends on the ecological characteristics of a species (Carson and Tempelton 1984). The ability of a species to disperse both pollen and seeds will determine the extent of gene flow not only among individual plants within populations but also between populations. Thus, founder effect may have less of an influence on species that are able to disperse large amounts of pollen and seed over long distances. In these types of species, newly founded populations are more likely to receive additional immigrants. The effects of genetic drift associated with isolation can be counterbalanced if migration is frequent (Ellstrand 1992). Because differences in genetic variation among populations can result from differences in gene flow, methods have been developed to estimate gene flow from population differentiation (Wright 1965, Slatkin 1987). One immigrant every second generation or one inter-population mating per generation is sufficient to prevent strong differentiation (Slatkin 1987). The rate of gene flow ultimately determines the extent of genetic variation maintained in a population or a species (Slatkin 1985), so that the level of gene flow can be used to gauge the importance of genetic drift and predict if populations will become more differentiated or loose genetic diversity.

I.1.5 Review of Factors Influencing Species Genetic Diversity

To understand how the life history characteristics of a species affect gene flow, we need to identify the characters associated with different levels of genetic variation (Hamrick et al. 1979). Numerous reviews (Hamrick et al. 1979, Loveless and Hamrick 1984, Hamrick and Godt 1989, Hamrick et al. 1992, Hamrick and Godt 1996a, Hamrick and Godt 1997) have drawn attention to the importance of life history characteristics in determining genetic diversity. Although significant relationships have been established, life history characteristics typically explain only a small portion of the variation in genetic diversity (Hamrick and Godt 1996a). Only 11-30% of the variation in genetic diversity is explained by life history characteristics (Hamrick and Godt 1989, Hamrick et al. 1992, Hamrick and Godt 1996a). For species that cannot be studied directly, predicting genetic diversity from life history characteristics may be the only method of determining the level of genetic diversity. Furthermore, although life history characteristics are not the most important factor determining genetic diversity, it is important to understand how they affect genetic diversity because of their influence on the maintenance and pattern of genetic diversity.

Life history traits that are associated with high levels of gene flow are generally associated with high levels of genetic diversity (Hamrick and Godt 1996a). Breeding system, seed dispersal mechanism and other traits that directly influence gene flow account for the largest proportion of the explainable diversity (Loveless and Hamrick 1984). Hamrick et al. (1989) found that four life history characteristics – breeding system, seed dispersal, life form and geographic range – as well as the taxonomic origin of a species, contributed the most to the proportion of variation explained in genetic diversity. In order to make predictions concerning the maintenance of genetic diversity in whitebark pine, it is necessary to understand how different life history traits affect gene flow and the patterns of genetic diversity associated with these traits. I will therefore briefly discuss how these five characteristics affect genetic diversity in plants in general with some specific references to conifers.

Breeding System

Selfing species have less gene flow, and thus less genetic diversity, compared to outcrossing, wind-pollinated species (Loveless and Hamrick 1984, Slatkin 1987). Outcrossing between closely related individuals (bi-parental inbreeding) is also a form of inbreeding. Most conifers, which experience some inbreeding between relatives, have comparatively high levels of gene flow and genetic diversity due to the high portion of outcrossing and wind dispersal of pollen. In whitebark pine however, the multi-stemmed stand structure that develops from nutcracker seed caches may increase the likelihood of bi-parental inbreeding leading to lower gene flow and genetic diversity compared to other conifers. Lower rates of gene flow resulting from inbreeding also predict a greater probability of population differentiation due to the chance effects of genetic drift or due to site specific selection (Ellstrand and Elam 1993). Less fit individuals that result from inbreeding are generally removed by selection and therefore inbreeding should theoretically purge homozygotes for deleterious recessive alleles. Removal of these alleles from the gene pool may benefit fitness in the long term. However, in species that have recently declined in numbers, inbreeding can produce defects such as infertility, thus further hampering recovery. In species that regularly inbreed, increased inbreeding because of population decline may not produce such drastic consequences. However, some typically inbreeding species also suffer from strong inbreeding depression, indicating the complexity of the relationship between inbreeding, fitness and genetic diversity (Ellstrand and Elam 1993).

Seed Dispersal

More extensive gene flow should result from any adaptation that increases seed dispersal (Slatkin 1987) such as the dispersal of whitebark pine seeds by Clark's nutcrackers. Species with no adaptive traits for seed dispersal, where seeds simply drop under the force of gravity (passive seed dispersal), should have lower levels of gene flow compared to species with winged or animal mediated dispersal (Loveless and Hamrick 1984). Hamrick and Godt (1989) found that species with seeds dispersed in some way by animals - secondary transport, ingestion or attachment - had the highest levels of heterozygosity and least amount of population differentiation. There is often a correlation among specific types of seed dispersal mechanism and other life history characteristics (Hamrick and Godt 1996a). Effective means of seed dispersal are often associated with other traits that are also correlated with high genetic diversity, such as longevity and outcrossing. Successional status, for example, is defined partly by the type of seed dispersal. Isolated populations that result from the tendency of early successional species to disperse into open habitats may consequently have low gene flow. Without gene flow, there is a high probability of genetic drift causing divergence of these populations (Wade and McCauley 1988).

Life-form

High heterozygosity with little differentiation among populations is typical of long-lived woody perennials in comparison to annuals and herbaceous perennials (Hamrick et al. 1992). High diversity may not be an inherent property of long-lived species but a consequence of the suite of traits associated with longevity (Hamrick et al. 1992). Many tree species combine life-history traits that are associated with high genetic variation: long generations, outcrossing, wind pollination, high fecundity, and winged seed dispersal (Hamrick et al. 1979).

Geographic Range

Geographic range and the distribution of individuals within that range explain the largest portion of variation in heterozygosity among plant species (Hamrick and Godt 1989). Widespread species are generally not patchy but distributed continuously or evenly across the landscape, which maintains high levels of genetic variation through gene flow and reduces the impact of genetic drift or bottlenecks compared to more regional or endemic species (Loveless and Hamrick 1984, Slatkin 1987). Hamrick et al. (1979) argue that species with wide geographic ranges require more genetic diversity in order to adapt to the greater number of ecological conditions that might be encountered. However, many species that have large geographic distributions are found in small isolated populations within that range because of factors such as light and nutrient requirements of the species and the topography of the landscape. Even if species in mountainous areas are widely distributed, population differentiation may be high because the patchiness of their distribution creates barriers to gene flow among populations (Guries and Ledig 1981).

Taxonomic Status

Genetic diversity and its distribution are more closely associated with the individual species' life history traits than with their phylogenetic origins per se (Hamrick and Godt 1996b). However, life history characteristics are highly correlated with phylogenetic grouping. Thus, gymnosperms have the highest genetic diversity of any plant species and typically have a low level of population differentiation (Hamrick et al. 1992, Mitton 1995). Most conifers have extensive gene flow and consequently show a lesser degree of spatial differentiation compared to the localized deme pattern of distribution typical of most plant species (Govindaraju 1988, Ellstrand 1992, Adams 1992). The high level of genetic diversity and low differentiation in conifers is due to their life history characteristics (Hamrick et al. 1992). Trees are generally outcrossing, long lived, found in widespread continuous distributions and have wind or animal mediated seed dispersal. The size and stature of trees also aids in pollen and seed dispersal (Harper 1977, Hamrick and Godt 1996b). Conifers produce large amounts of wind-born pollen and are almost exclusively outcrossing (Ellstrand 1992). For example, Adams and Joly (1980) found that pollen could successfully move distances in excess of 100 meters in loblolly pine (*Pinus taeda*).

The seed from most wind-dispersed conifers falls within a few hundred feet of its source (Bush and Smouse 1992). However, seeds harvested and cached by birds have the potential to move kilometres (Mitton 1995). Clark's nutcrackers have been observed caching seeds greater than 20 km from the seed source (Vander Wall and Blada 1977). For conifer species such as whitebark pine, which have bird-dispersed seeds, the caching habits of birds may have important consequences for the genetic structure of the plants they disperse. Clusters of trees, referred to as multi-stemmed trees, are a common sight in tree species that have bird-dispersed seeds. Because caches are commonly made from a single source cone, individuals within clumps may be genetically distinct but highly related (Tomback and Linhart 1990). However, clumps within populations are no more related to each other than clumps between populations because cache location is essentially random (Furnier et al. 1987, Schuster and Mitton 1991, Carsey and Tomback 1994). The population structure developing from nutcracker seed caching behaviour increases the probability of inbreeding within clumps. In stands of wind-dispersed trees, the distance between individuals is inversely correlated with relatedness between them. Since this relationship does not hold for bird-dispersed trees, there is less likelihood of inbreeding if pollen from neighbouring clumps is the source for fertilization. It is presently unclear what overall level of inbreeding results from these two potential mechanisms.

Environmental variation and adaptation have also been used to explain patterns of genetic differentiation within conifers (Bush and Smouse 1992). For example, Furnier and Adams (1986) found that variation in allele frequencies in *Pinus jeffreyi* seemed to reflect the distribution of ultramafic soils. Guries and Ledig (1981), in provenance trails of *Pinus rigida*, found significant correlations between allele frequencies, morphological variation and climatic variables. However, conifers are much more likely to vary morphologically and physiologically over short distances than they are to vary in allozyme frequency (Bush and Smouse 1992). Although a few studies have found significant correlations between geographic variability in morphological characters and allozyme loci (Grant and Mitton 1977, Lagercrantz and Ryman 1990), there does not appear to be any clear or general relationship between allozyme and environmental variability. Demographic factors, such as population size seem to play a more important role in determining the level of genetic diversity.

A significant relationship between population size and genetic diversity as measured by heterozygosity has been found in a number of plants, especially rare and endangered species (Karron 1987, Van Treuren et al. 1991, Ellstrand and Elam 1993, Prober and Brown 1994, Dolan 1994). The generally small population size in which whitebark pine is found throughout its range, and especially at the northernmost extent of its range in Alberta, would suggest that genetic diversity is low. Although genetic diversity as measured by heterozygosity does not always correlate with population size, the number of alleles per locus often does (Nei 1987, Barrett and Kohn 1991). In populations with no correlation between genetic variation and population size, historical factors such as genetic bottlenecks or founding events may be more important in determining the present pattern of genetic diversity than current population sizes (Raijman et al. 1994).

Past population size, as well as present, are important in determining the influence of evolutionary factors that determine genetic diversity. Variation in population size over time and therefore the influence of population size on genetic diversity depend on the specific ecological characteristics of individual species (Barrett and Kohn 1991). Plant populations of any specific species are small for four reasons (Harper 1977). First, available sites are few and separated by distances beyond the species' normal dispersal ability. Second, the carrying capacity of the site is low for the species. Third, the species is replaced at the site by later successional species after a short period (successional displacement), and fourth, colonization is in its early stages, and full exploitation of the site by the species has not occurred. Disturbance events such as fire, grazing, drought, flood, or insect and disease outbreak may also cause populations to be kept at small sizes during early successional stages. There are likely to be different patterns of genetic variation in small populations that originated in different ways (Barrett and Kohn 1991).

In summary, life history characteristics have been shown to account for a much larger proportion (up to 50%) of the distribution of genetic variation among populations than the magnitude of diversity (between 11 to 30%, Hamrick and Godt 1996a). Species with traits that promote gene flow among populations are frequently associated with low levels of differentiation (Hamrick et al. 1992). Traits that are commonly found in trees, especially conifers, such as wind-dispersed seed and pollen, promote gene flow (Mitton 1995). Species with historically high levels of gene flow may be the most susceptible to loss of genetic diversity through habitat fragmentation (Ellstrand and Elam 1993). Low historical levels of gene flow may have resulted in local adaptation (Frankham 1995). Therefore, determining past and present levels of gene flow is an important contribution to conservation that can be made using electrophoresis of isozymes.

The observed relationships between life-history characteristics and genetic diversity in conifers and plants in general suggest that whitebark pine should have a comparatively low level of genetic diversity. However, some individual traits of whitebark pine may promote maintenance of genetic diversity while others may increase the probability of differentiation and the loss of genetic diversity. Thus, it is difficult to develop a priori hypotheses about the levels of genetic diversity in whitebark pine. Genetic diversity should be maintained by the extremely long life span of whitebark pine; individuals over 1000 years old have been recorded (Luckman et al. 1984). Long-distance seed dispersal between populations due to Clark's nutcrackers should also work to sustain genetic diversity. Wind-dispersed pollen should contribute to the maintenance of high genetic diversity and low population differentiation in whitebark pine. However, the multi-stemmed stand structure in conjunction with the small size and isolated nature in which whitebark pine is found may increase the level of inbreeding and consequently the level of population differentiation (Furnier et al. 1987). Although many conifers are found in continuous populations that cover large geographic areas, others such as whitebark pine are found in small isolated populations despite much larger overall distributions. Compared to more continuously distributed species, there should be low gene flow and high population differentiation in species with patchy distributions such as whitebark pine. The genetic diversity of whitebark pine has been examined in other areas (Yandell 1992, Jorgensen and Hamrick 1997) but how the pattern of genetic diversity that has resulted from its unique combination of traits relates to factors such as blister rust infection, site characteristics or stand demographics has not been investigated.

Knowledge of the life history factors contributing to patterns of genetic diversity in whitebark pine is important for understanding how decline due to blister rust and fire suppression will affect genetic diversity. In whitebark pine, only the outermost branches produce cones. Blister rust greatly reduces cone production because these outermost limbs are those most affected. Thus, even if blister rust does not result in mortality, infection may result in a decline of gene flow and consequently reduced genetic variation. Gene flow may also be reduced by successional replacement as the increased number of individuals of different species in a stand reduce the pollen flow between neighbouring unrelated individuals and increase the probability of inbreeding resulting from within the same cluster or tree. Thus, the factors causing decline in whitebark pine may also be contributing to loss of genetic diversity of the species. An understanding of the processes and interactions among blister rust infection, disturbance and genetic diversity is essential to the design of an effective conservation strategy that will ensure the continued survival of whitebark pine in the Canadian Rockies.

I.2 Thesis Outline and Objectives

This thesis consists of four major sections. Chapter 1 discussed the threats to the survival of whitebark pine, white pine blister rust and fire suppression, and why the conservation of whitebark pine in face of these threats requires an understanding of its level and pattern of genetic diversity. The ecological factors and life history traits that are important in determining the level of genetic variation are also reviewed in Chapter 1 so that an understanding can be gained of what traits are important to determining genetic diversity in whitebark pine.

Chapter 2 will discuss the occurrence and effects of blister rust infection on whitebark pine in the Canadian Rocky Mountains. In addition, the population structure, diameter, height and age distributions of stands, will be examined for the influence of disturbance and the relationship between these characteristics and blister rust infection is explored.

Chapter 3 examines the genetic diversity of these same whitebark pine populations. The level and distribution of genetic variation, as measured by protein polymorphism is documented.

How this variation compares to other species and what characteristics are most likely influencing patterns of variation is discussed. Relationships between genetic variation and site and population characteristics are examined.

Finally, Chapter 4 is a general conclusion wherein results of the previous two chapters are combined to develop an overview of the potential future of whitebark pine. Recommendations are made for the management and conservation of whitebark pine in the Canadian Rocky Mountain Parks to ensure that this species survives and continues to be an essential component of the subalpine ecosystem for the future. Directions for future research are also discussed.

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Chapter II

II Site Characteristics, Demographic Patterns and Blister Rust Infection of Whitebark Pine Stands in the Canadian Rockies

II.1 Introduction

Because there have been no studies focusing on whitebark pine stand characteristics in the Canadian Rockies, the only source of ecological information is broadly based vegetation classification studies such as the biophysical land mapping done by Parks Canada. However, the classification of vegetated areas in the Ecological Land Classification of Banff and Jasper National Parks (Holland and Coen 1982) was based on inferences from characteristics such as slope, aspect and elevation. Therefore, no conclusions can be drawn regarding the characteristics of whitebark pine stands from this type of vegetation classification study.

Due to this lack of information, the characteristics attributed to whitebark pine stands in the Canadian Rockies have been extrapolated from stands in the northern United States. Arno and Hoff (1989) suggest that the ecological role whitebark pine plays in the subalpine community north of Waterton Lakes National Park is different from that in the southern portion of its range. They suggest that whitebark pine is a minor component of the forest ecosystem and limited to southwestern aspects and rocky outcroppings in the northern part of its range. Knowledge of the importance of site characteristics and an understanding of regeneration limitations are essential components of an effective conservation management program for whitebark pine. As such, information and data specific to the populations of whitebark pine in the Canadian Rockies is critical. Despite this importance, the validity of the assumptions made about whitebark pine stands in the Canadian Rockies has not been examined.

Not only is knowledge of whitebark pine site characteristics lacking but the age structure of these stands is also unknown. Understanding the factors involved in regeneration and establishment, which is important in conservation, require knowledge of the age structure. Keane and Arno (1993) suggest that a decline in the area covered by whitebark pine over the last 30 years in Montana is associated with fire suppression. Anecdotal information suggests that whitebark pine is shade intolerant compared to other species found in the subalpine and should therefore display periodic regeneration (McCaughey and Schmidt 1990). Age distribution is indicative of the influence of disturbance on regeneration; even-aged stands, with an origin corresponding to the time of a major disturbance, such as fires or avalanches, indicate a dependence on disturbance for establishment. In contrast, stands composed of individuals of a variety of ages indicate continuous recruitment is possible in the absence of major disturbances. Whether whitebark pine stands have even-aged or continuous age distributions in the Canadian Rockies has not been examined.

Knowledge of the processes involved in whitebark pine regeneration is essential to understanding the effects of fire suppression. If whitebark pine is completely dependent on disturbance for regeneration, fire suppression would be reflected through successional replacement; the low shade tolerance of whitebark pine would result in reduced regeneration and increased average age. Furthermore, if seedling establishment declines as a result of fire suppression, there will also be a reduction in the area covered by whitebark pine because older trees are not replaced.

Through its effect on age structure, fire suppression is one factor that is or potentially may be contributing to the decline of whitebark pine. Another important factor that has already contributed to the decline of whitebark pine in parts of Montana, Idaho and Wyoming is white pine blister rust (*Cronartium ribicola*). The Forest Insect and Disease surveys of western Canada in the 1960's noted the presence of white pine blister rust on whitebark pines in Waterton, Yoho, Kootenay, and Jasper National Parks (Forest Insect and Disease Survey 1959, 1968, 1970). Although the current damage caused by blister rust was thought to be minimal, before this study was undertaken no information was available on the intensity and extent of infection in the Canadian Rockies (Ian Pengelly, personal communication). Furthermore, the dry cold conditions and short summers were thought to limitate the spread of infection into the Canadian Rockies. However, during the last two decades, researchers in the northern United States have discovered high whitebark pine mortality caused by white pine blister rust (Keane and Arno 1993, Kendall 1994) which has lead to a greater concern about the potential impact of blister rust on whitebark pine in the Canadian Rockies. Based on the observation of high rates of infection on whitebark pine in Glacier National Park in northern Montana, Hoff et al. (1994) have predicted that whitebark pine throughout the Canadian Rockies are all highly infected.

Climate may not limit the spread of blister rust in the Canadian Rockies as indicated by the spread of blister rust into the southern United States. During the 1960's and 1970's, the hot and dry conditions south of 42°N were thought to present a barrier to the southward expansion of blister rust (Byler and Parmeter 1979, MacGregor 1969, Peterson and Jewel 1968). However, blister rust is now causing extensive damage to forests and plantations of sugar pine throughout southern California (Kinloch and Dulitz 1990). The existence of different ecotypes of blister rust in Oregon and British Columbia (McDonald et al. 1984) has been implicated in the southward expansion (McDonald 1992). The potential for the development of blister rust ecotypes undermines the assumption that climatic limitations will prevent the development of blister rust to epidemic levels on whitebark pine in the Canadian Rockies. This project was partly initiated in order to document the present extent of blister rust infection so that future monitoring may begin to build an accurate picture of the outlook for whitebark pine.

This chapter examines three aspects of the biology of whitebark pine in the Canadian Rockies: the characteristics of whitebark pine stands, the demographic characteristics of these stands and the level of blister rust infection. How these three areas relate to each other will also be addressed.

II.2 Objectives

1. To determine site characteristics (aspect, slope and elevation) associated with whitebark pine stands in the Canadian Rockies and if these change with latitude.

2. To determine whether the age distribution of whitebark pine stands is continuous or regeneration is clumped. If the age distributions show distinct periods of regeneration with little or no recruitment in the intervening years, regeneration may depend on disturbances such as fire.

3. To determine the extent and pattern of blister rust infection in whitebark pine stands throughout the Canadian Rockies and whether the proportion of individuals infected

correlates with site characteristics (such as slope, aspect, elevation, and latitude), stand age or tree size. In addition, to determine if there is a relationship between the individual probability of infection and the height, diameter, and age.

II.3 Methods

II.3.1 Site Locations

Throughout the Canadian Rockies, from Waterton Lakes National Park to Mt. Robson Provincial Park, 29 sites were visited (Table II.4-1 and Figure II.3-1). Jurisdictions refer to Waterton Lakes, Kootenay, Banff, Yoho, and Jasper National Parks (Parks Canada), Southwestern Alberta Forest District (SW AB), Kananaskis Country Land Use Zone (Alberta Environmental Protection, Land and Forest Service and Natural Resource Service), Cranbrook and Invermere Forest Districts (British Columbia Forest Service) and Mt. Robson Provincial Park (Prince George District, British Columbia Ministry of Environment, Lands and Parks). Although only 12 sites were accessible on the British Columbia, sites were chosen in order to obtain one site approximately every 40km on either side of the Alberta-British Columbia boarder. The localised presence of whitebark pine depends on climate and other regional environmental factors. Therefore, actual sampling locations where chosen through consultation with local resource officers and selected according to accessibility.

II.3.2 Data Collection

Sites were visited between June 20 and September 30, 1996. At each site, a centre point was located where site characteristics - aspect, slope and elevation - were measured. Latitude (northing) and longitude (easting) were also determined for each sampling point (using Universal Transverse Mercator grid reference). Aspect was calculated from the centre along the steepest slope, measured as degrees from north. Slope, measured along the same line as aspect, was calculated in percent using a clinometer. Elevation was also calculated at the centre point using a Casio™ altimeter. 25 trees were randomly located within a quadrat of 200 by 100 meters positioned at the centre point. At each random point, the closest whitebark pine was located and the actual co-ordinates of this tree recorded. For multi-stemmed trees, the number of trunks was recorded and the number of blister rust cankers was noted. The height of each tree

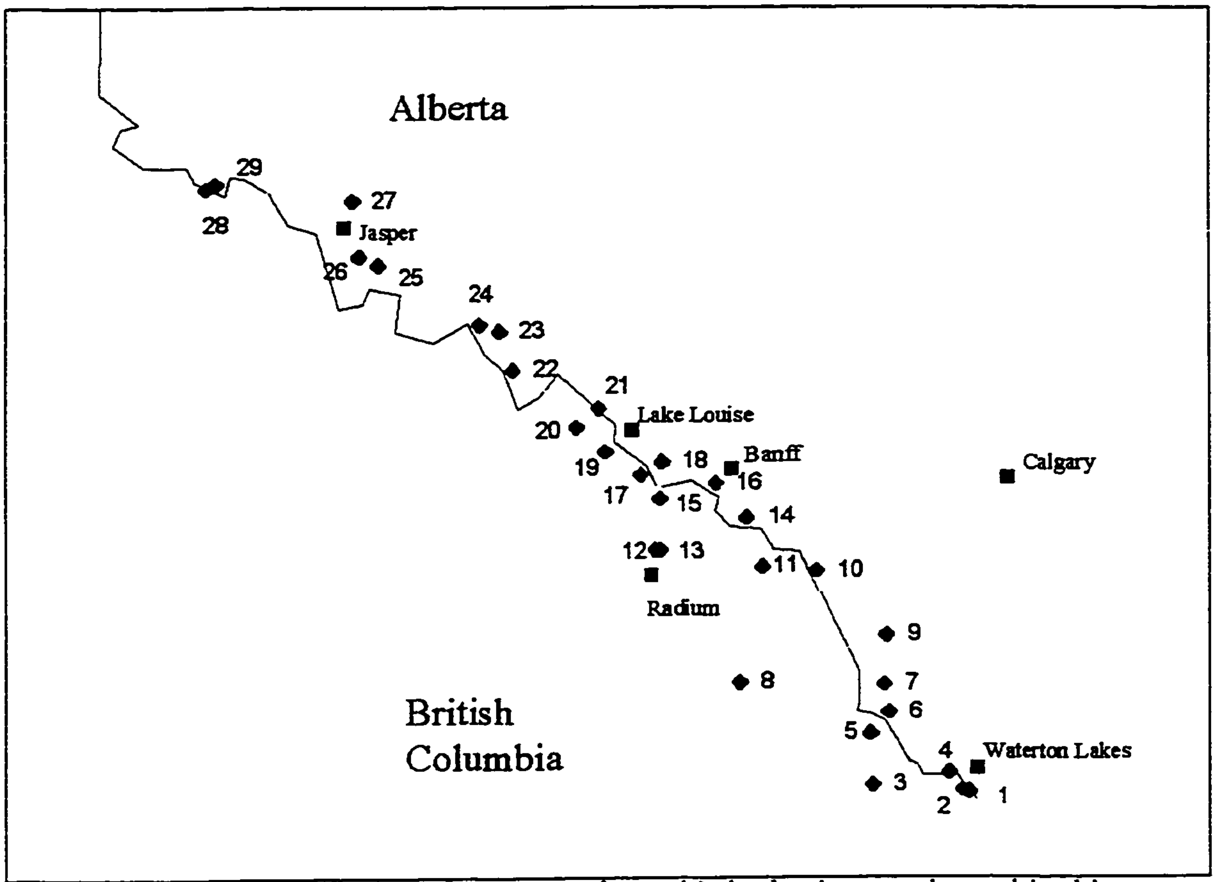

Figure II.3-1 Approximate location of 29 whitebark pine stands used in this study. See Table II.4-1 for names of sites.

was measured to the nearest centimetre for trees less than 2 meters tall and to the nearest half meter for trees over 2 meters. Because many trees were twisted and deformed due to avalanches, rockfall and wind, diameter was measured at the base of each tree. Increment cores were collected from trees that were greater than 4 cm at the base. A clump of needles approximately 2 cm long was collected from each tree for the isozyme analysis (see section III.3.1 for details regarding needle collection).

II.3.3 Data Analysis

Site Characteristics

Regression analysis was used to examine if site characteristics, aspect, slope and elevation, were influenced by latitude. Slope was converted from percent into degrees for this analysis. Because aspect in degrees is not linear, aspects were decomposed into an east-west component and a north-south component by taking the cosine and sine of aspect respectively. The mean aspect was calculated by first taking the average of the north-south and east-west components separately and then taking the arctangent of these averages.

Demographic Analysis

In order to minimize mortality, trees less than 4 cm dbh were not bored. Consequently, of the 723 trees examined, cores were not collected from 284 individuals. In order to estimate the age of individuals that were not bored, a regression model was constructed using the height and diameter of the trees that were bored. The model

$$Age = 7.81 * Height + 3.41 * Diameter$$

had an adjusted r^2 of 0.79. The high proportion of variation accounted for by the model justified the use of this formula to estimate the age of unbored trees. In order to look for periodicity in regeneration the age frequency distributions were plotted for each site.

Blister Rust Infection

The proportion of whitebark pine infected with blister rust was calculated at each site. Regression analysis was used to determine the relationship between the proportion of infected individuals and latitude (northing). Because latitude is related to other site characteristics, longitude (easting), aspect, slope and elevation were also included in the regression. In order to get a more accurate picture of the intensity of infection, the average number of cankers per tree was calculated per site.

In order to test the hypothesis that the age distribution of infected and non-infected individuals was the same, the Kolmogorov-Smirnov two-sample test was employed (Sokal and Rolf 1995). The same method of analysis was also used to test the hypothesis of identical diameter and height distributions between infected and non-infected individuals.

In order to determine if demographic factors are important in determining the probability of blister rust infection, a logistic regression was performed. Because the variables are highly correlated, three separate regressions were performed with the independent variables height, diameter, and age.

II.4 Results

Site Characteristics

The mean aspect of the 29 sites was 204°, which is in a southwesterly direction (Table II.4-1). There was a small but significant correlation between latitude and the east-west component of aspect ($r^2 = 0.17$, $p \leq 0.05$, Figure II.4-1) but not between latitude and the north-south component of aspect.

The average slope was 25.5° with a mean elevation of 2000 m. Slope generally increased with latitude with the exception of the most northern sites (25 to 29, Figure II.4-2). The slope of the most northern sites was lower than that of sites further south. However, even if sites 1 to 24 were considered separately, the correlation between latitude and slope was not significant. In contrast, there was a significant, although low, negative correlation between elevation and latitude ($r^2 = 0.13$, $p \leq 0.05$, Figure II.4-3).

Demographic Analysis

The oldest individual recorded was from site 19 in Yoho National Park. Before heart-rot made counting rings impossible, 680 years were recorded which is in the range of ages for other whitebark pine reported in the Canadian Rockies. Luckman et al. (1984) found whitebark pines over 700 years of age in the Columbia Icefields and Tonquin valley areas of Jasper National Park. The average age across all sites was 72 but with a standard deviation of 86.

At some sites, the age distributions show distinct periods of regeneration whereas others show more continuous regeneration patterns. Site 6, for example, has two distinct groupings of age classes: trees up to 50 years old; trees from 160 to 200 years (Appendix A, Figure 6). This site covered a recent burn and adjacent unburned area. Similarly, site 5 (Appendix A, Figure 5), had two distinct age groupings: trees less than 20 years old; and trees from 130 and 190 years (with the exception of 1 tree in the 70-year age class). Site 28 (Appendix A, Figure 28) showed a

Table II.4-1 The name, location, aspect, slope, elevation and proportion of individuals infected with blister rust (standard error in parenthesis) of 29 whitebark pine stands in the Canadian Rockies. Refer to text for full title of jurisdictions.

Site	Name	Location	Easting (m)	Northing (m)	Aspect (°)	Slope (°)	Elevation (m)	Proportion Infected (SE)
1	Summit	Waterton	717675	5433575	247	19.4	1950	0.72 (0.09)
2	Rowe	Cranbrook	714750	5435675	190	26.6	2000	0.72 (0.09)
3	Cabin	Cranbrook	672950	5438850	326	22.5	1990	0.56 (0.10)
4	Lost	Waterton	708700	5448275	196	15.3	1930	0.44 (0.10)
5	Centre	Cranbrook	672000	5477100	248	41.9	1890	0.69 (0.09)
6	Ironstone	SW AB	680450	5493475	308	18.5	1960	0.76 (0.09)
7	Vicary	SW AB	677575	5514350	160	23.9	1990	0.62 (0.10)
8	Wildhorse	Cranbrook	610760	5516710	243	30.6	2280	0.27 (0.09)
9	Coat	SW AB	679175	5552175	182	19.8	2310	0.56 (0.10)
10	Highwood	Kananaskis	646450	5599800	216	24.3	2030	0.52 (0.10)
11	Joffre	Invermere	621800	5603875	184	23.9	1960	0.36 (0.10)
12	Kindersley	Kootenay	571150	5616725	222	34.2	2225	0.04 (0.04)
13	Sinclair	Kootenay	573200	5617125	212	31.1	1980	0.56 (0.10)
14	Nestor	Kananaskis	614275	5641350	250	40.1	2125	0.00 (0.00)
15	Hawk	Kootenay	573010	5656100	126	24.8	2030	0.28 (0.09)
16	Sulphur	Banff	599600	5666300	240	34.2	2090	0.00 (0.00)
17	Whymper	Kootenay	563550	5673950	148	32.9	2140	0.00 (0.00)
18	Castle	Banff	573725	5683300	195	24.3	1980	0.00 (0.00)
19	Wiwaxy	Yoho	546000	5690550	253	29.7	2095	0.12 (0.07)
20	Whaleback	Yoho	532550	5708850	162	36.0	2035	0.20 (0.08)
21	Dolomite	Banff	542825	5723900	216	30.6	2245	0.00 (0.00)
22	Sarbach	Banff	501725	5752300	89	27.0	1975	0.12 (0.07)
23	Nigel	Banff	495525	5782375	213	27.9	2045	0.16 (0.07)
24	Wilcox	Jasper	486025	5786000	187	28.4	2260	0.08 (0.06)
25	Geraldine	Jasper	436725	5832025	90	2.3	1720	0.20 (0.08)
26	Astoria	Jasper	427975	5839025	358	12.6	1830	0.00 (0.00)
27	Esplinade	Jasper	424000	5879775	126	27.0	1600	0.12 (0.07)
28	Berg	Mt. Robson	354830	5890330	137	14.4	1675	0.40 (0.10)
29	Adolphus	Jasper	359730	5893630	286	14.9	1800	0.20 (0.08)
				Mean	204	25.5	2000	0.30
				Std Error	49	8.7	170	0.26

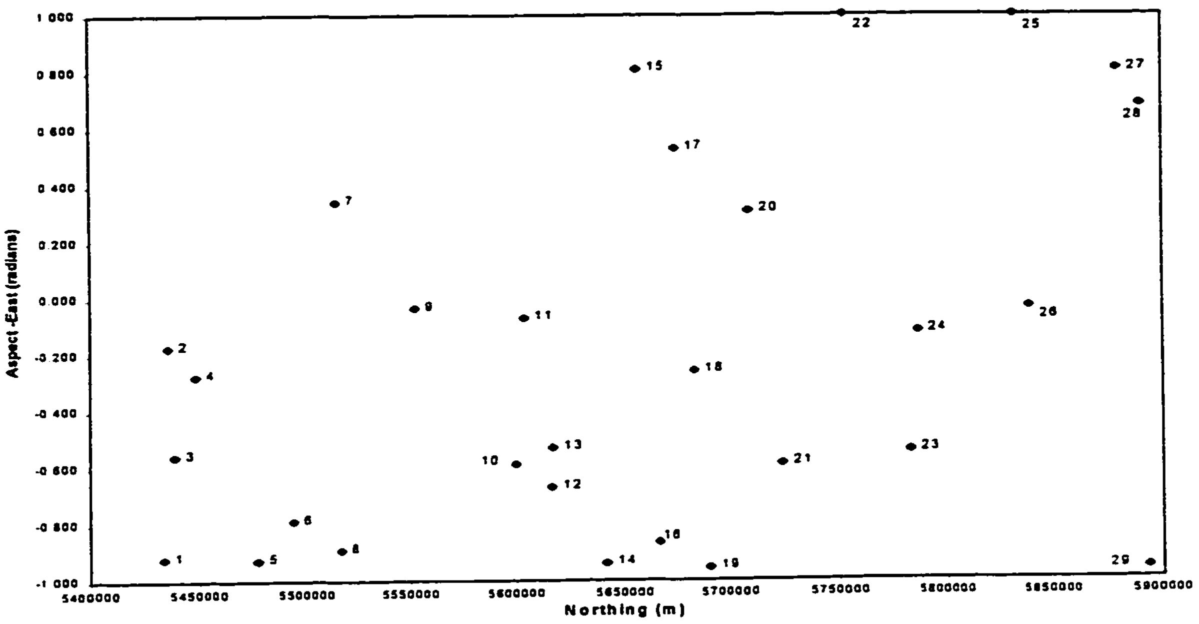

Figure II.4-1 Change in the east-west component of aspect over latitude for 29 whitebark pine stands in the Canadian Rockies.

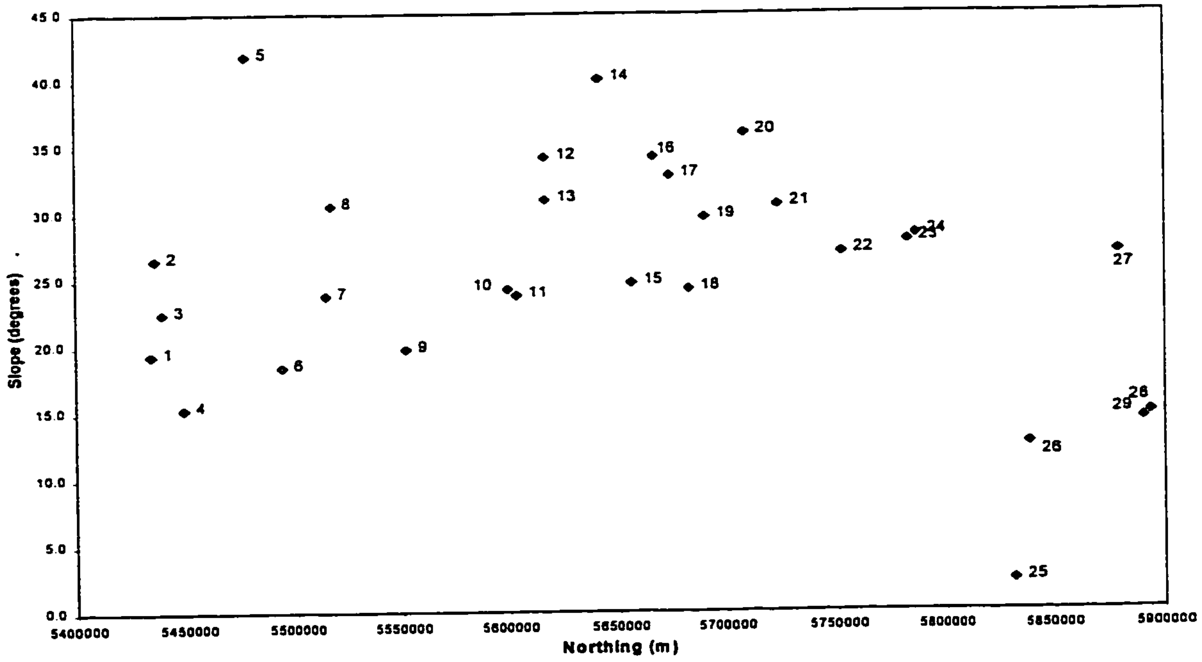

Figure II.4-2 Change in slope with latitude over 29 whitebark pine stands in the Canadian Rockies.

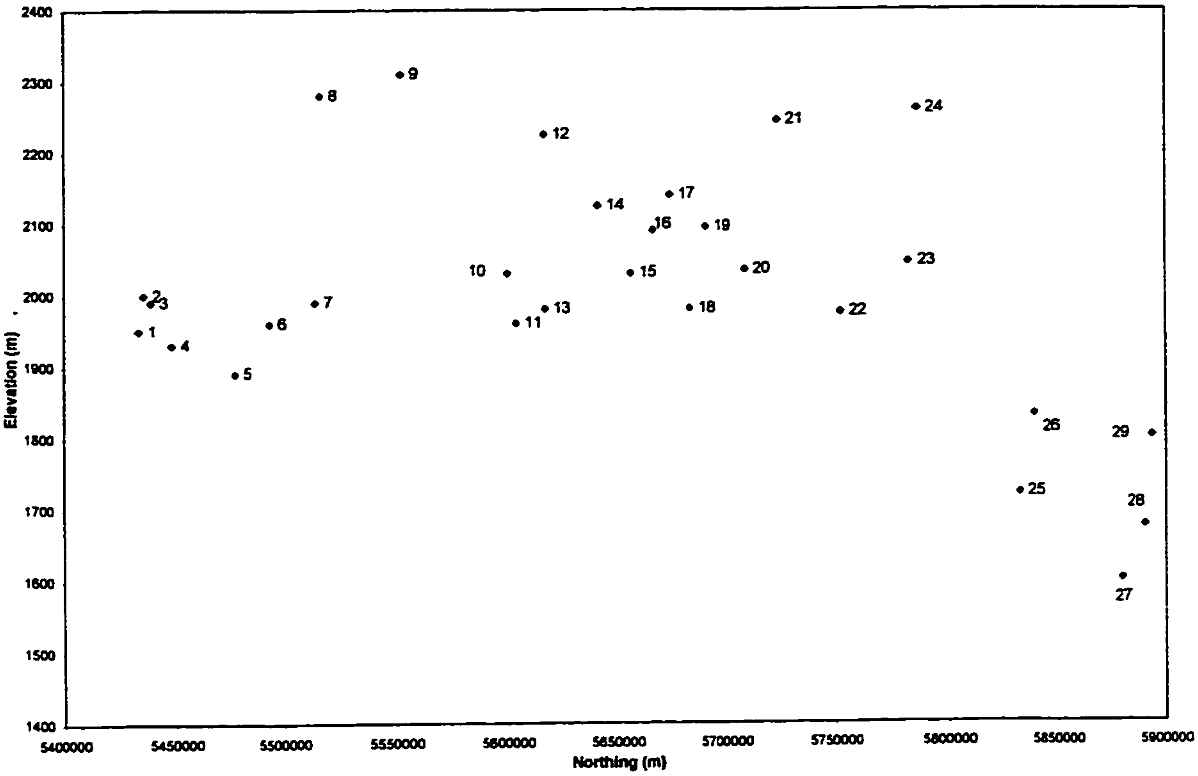

Figure II.4-3 Change in elevation over latitude at 29 whitebark pine sites in the Canadian Rockies.

simple age distribution, as the result of a recent fire. The oldest individual was 40 years old with numbers of trees increasing with decreasing age class. Other sites show patterns that are more complex but still point to periodic regeneration. For example, site 7 (Appendix A, Figure 7) had three groups of ages: less than 20 years, 110 to 180 years, and 300 to 350 years.

Other sites showed a pattern of continuous regeneration. For example, site 27 (Appendix A, Figure 27) had a large range of ages with one or two trees in each age class. The evenness of the frequency of different age classes suggests continuous regeneration although with such a large range there may have been periodic regeneration that was not detectable with only 25 individuals sampled.

Many of the other sites show regeneration patterns that are somewhere in between these two extremes. For example, at site 14 (Appendix A, Figure 14) most trees were in the 140-year age class. But the period of regeneration is not distinct; individuals ranged in age from 0 to 180.

Blister Rust Infection

Latitude and the proportion of individuals infected were strongly correlated ($r^2 = 0.49$, $p \leq 0.01$ Table II.4-1, Figure II.4-4). Because spread of blister rust requires periods of high humidity, the western side of the continental divide might be expected to show higher infection rates (Janz and Storr 1977). However, including easting in the regression model only slightly improved the r^2 (to 0.52) and was not significant. The Canadian Rockies are situated along a northwest to southeast direction. Consequently, easting and northing were highly correlated which may account for the insignificant contribution of easting to the regression. A t-test with 11 pairs of sites situated at approximately the same latitude on either side of the continental divide was also insignificant ($t=0.97$, $p=0.36$) but the low number of pairs suggests that this test is inconclusive.

Infected rates ranged from around 75% in the southern areas to 0 at several sites in Banff and Jasper National Parks (Table II.4-1, Figure II.4-4). Even at sites where no sampled individuals showed signs of infection, the presence of blister rust was noted on other trees. The average rate of infection across all populations was 0.30. The intensity of infection was much greater in southern populations as indicated by the average number of cankers per tree per site (Figure II.4-5). The mean number of cankers per tree across all populations was 0.61 indicating that infected trees are more likely to be infected with more than 1 cankers. The average number of cankers for infected trees was 2.02.

Hunt (1983) found that western white pine stands on sloped sites were more infected than stands in flat areas. However, adding slope to the regression model of infection rate increased the r^2 but its parameter estimate was not significant. Elevation was the only other variable that increased the r^2 and that had a parameter estimate that was significantly greater than zero. With northing and elevation in the regression model, the adjusted r^2 was 0.632 (Table II.4-2).

There was a significant difference in the age distribution of infected and non-infected individuals ($D= 0.32$, $p \leq 0.0001$). The cumulative frequency distribution (Figure II.4-6) showed a higher proportion of individuals in the younger age classes in the non-infected distribution compared to the infected distribution. There was also a significant difference between height ($D= 0.28$, $p \leq 0.0001$) and diameter ($D= 0.31$, $p \leq 0.0001$) distributions of infected and non-

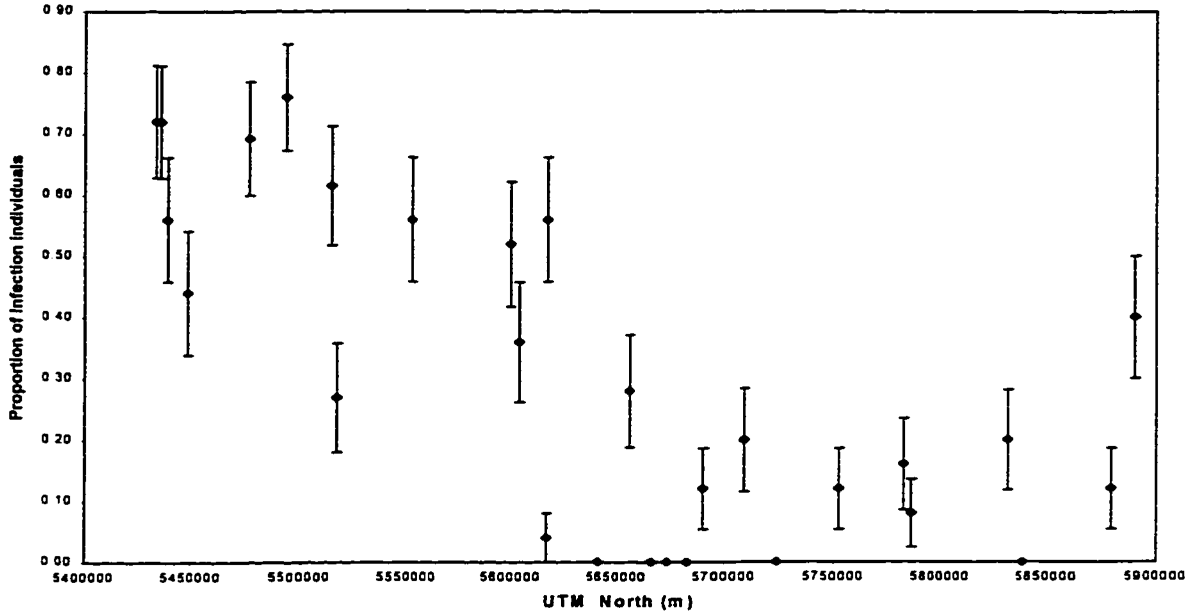

Figure II.4-4 Proportion of whitebark pine infected with *C. ribicola* at 29 locations throughout the Canadian Rockies. Bars represent standard error.

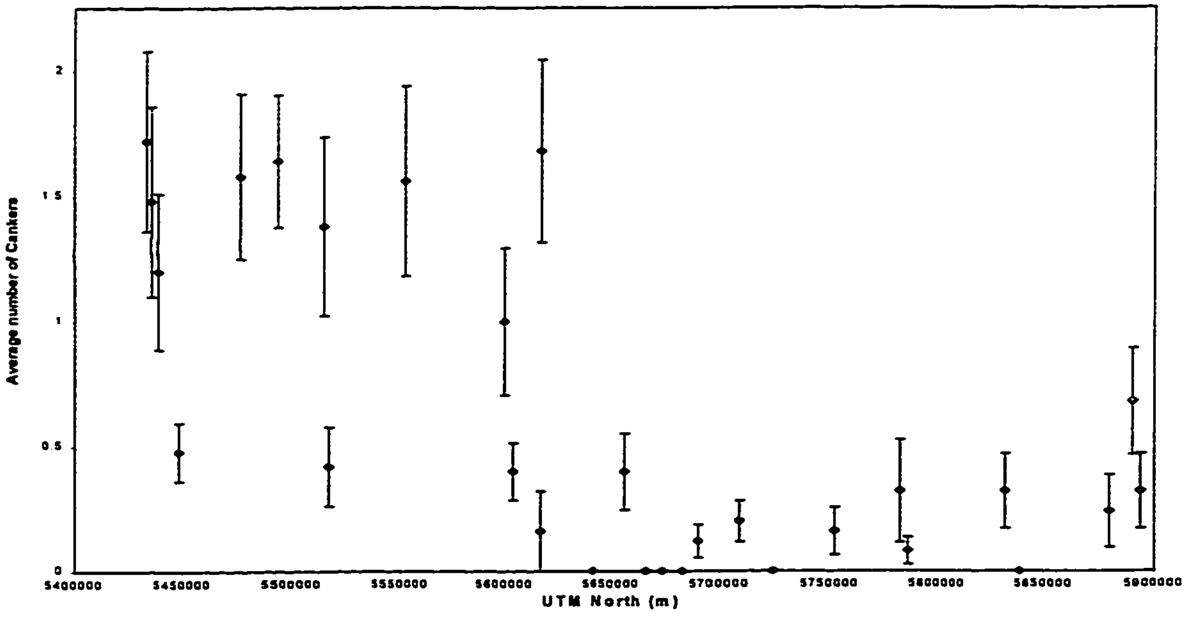

Figure II.4-5 Average number of *C. ribicola* cankers per tree at 29 populations of whitebark pine throughout the Canadian Rockies. Bars represent standard error.

Table II.4-2 Regression analysis with the dependent variable of the proportion of individuals infected with blister rust.

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F	Adjusted r^2
Model	2	1.2550	0.6275	25.0480	0.0001	0.6320
Error	26	0.6513	0.0251			

Variable	DF	Parameter Estimate	Standard Error	T for H_0:Parameter=0	Prob > |T|
Intercept	1	10.4	1.4	7.278	0.0001
Northing	1	-0.0000015	-0.0000002	-6.942	0.0001
Elevation	1	-0.0007	0.0002	-3.819	0.0007

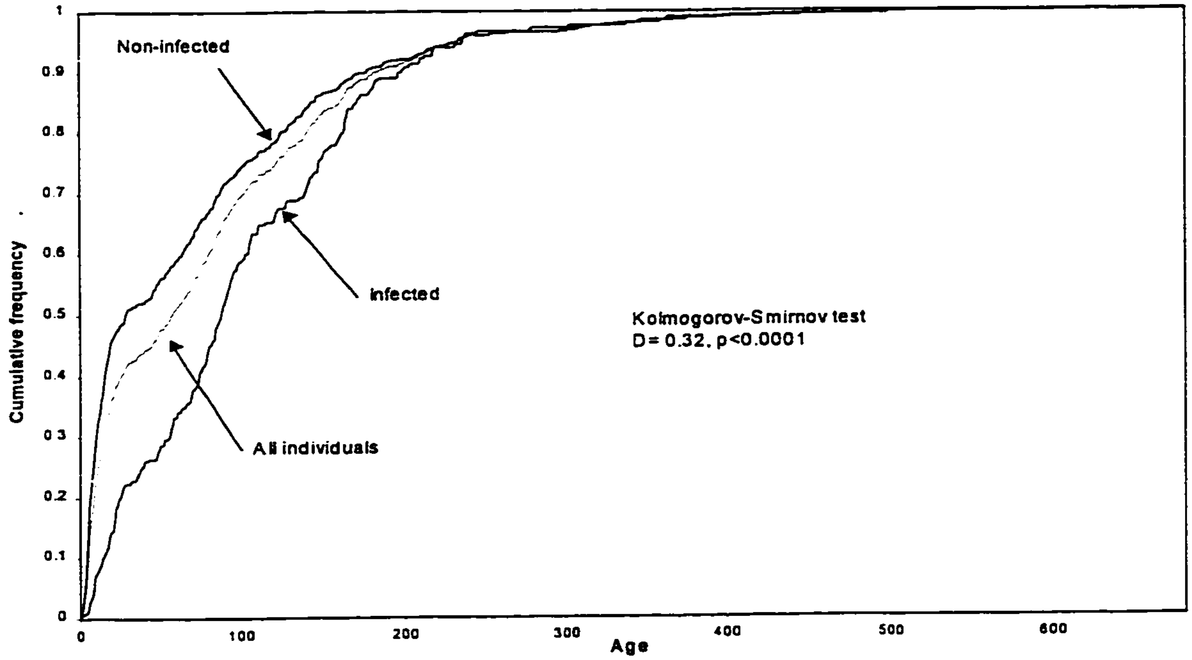

Figure II.4-6 Cumulative frequency of age distribution for blister rust infected and non-infected whitebark pine across all sites.

infected trees with proportionally more non-infected individuals in the smaller height and diameter classes.

Each of height, diameter and age were significantly correlated with infection although these correlations were not large (r^2 between 0.24 and 0.27). The logistic regressions also reflected the relationship between infection and tree size and age (Tables II.4-3 to II.4-5). Height, diameter

Table II.4-3 Maximum likelihood estimates for the logistic regression of infection on the independent variable height.

Variable	DF	Parameter Estimate	Standard Error	Wald χ^2	Pr > χ^2	Standardized Estimate	Odds Ratio
Intercept	1	1.24	0.12	100.7234	0.0001	.	.
Height	1	-0.10	0.02	21.4506	0.0001	-0.20	0.907

Table II.4-4 Maximum likelihood estimates for the logistic regression of infection on the independent variable diameter.

Variable	DF	Parameter Estimate	Standard Error	Wald χ^2	Pr > χ^2	Standardized Estimate	Odds Ratio
Intercept	1	1.2	0.1	101.6568	0.0001	.	.
Diameter	1	-0.023	0.006	17.8341	0.0001	0.185	0.977

Table II.4-5 Maximum likelihood estimates for the logistic regression of infection on the independent variable age.

Variable	DF	Parameter Estimate	Standard Error	Wald χ^2	Pr > χ^2	Standardized Estimate	Odds Ratio
Intercept	1	1.2	0.1	104.6313	0.0001	.	.
Age	1	-0.0042	0.0009	19.7186	0.0001	-0.1972	0.9960

and age were all significant in logistic regressions with infection as the dependent variable. For the model of probability of non-infection with height as the independent variable the chi-squared (χ^2) of the covariates was 21.55 (*d.f.* =1, $p \leq 0.0001$) and the parameter estimates are displayed in Table II.4-3. Similarly, the χ^2 was 18.84 for the model with diameter and 21.49 for age (both $p \leq 0.0001$) with parameter estimates displayed in Table II.4-4 and Table II.4-5 respectively. The parameters for all three variables were negative indicating that the probability of infection increased with increasing size and age.

II.5 Discussion

Site Characteristics

Understanding the ecological conditions required by whitebark pine is essential to its conservation. Because of the high altitude environment and isolated areas in which whitebark pine is found few studies have focused on the ecology of this species. The ecological studies that have been carried out have focussed on whitebark pine stands in the United States. Consequently, there is little information available on the ecological characteristics of whitebark pine in the Canadian Rockies. The site conditions described here show that whitebark pine flourishes under a wider range of characteristics than was anticipated. The average aspect, 204°, is approximately perpendicular to the average angle at which the Canadian Rockies are aligned, 320°. Furthermore, these whitebark pine stands were located on steep slopes. Thus whitebark pine stands were located not only on southwest facing aspects where they receive the most sunlight, have lower snow depth, and are likely to be wind effected, but along slopes where drainage may be highest. Thus, the location of whitebark pine sites may be influenced by climatic factors that result from the specific geological and topographical conditions on southwest facing steep slopes in the Canadian Rockies.

The decline in the elevation of whitebark pine stands with latitude was not unexpected because timberline declines with increased latitude (Arno and Hammerly 1984). Although the exact location of timberline depends on local topography and climate, the greatest factor in the average elevation of timberline is the regional climate resulting, in part, from latitude (Arno and Hammerly 1984). Therefore, the drastic drop in the elevation of sites north of the Columbia Icefields was not expected. The difference in the elevation range between sites north and south of the Columbia Icefields suggests that climate north of this point changes on a regional scale. The distribution of another timberline tree species alpine larch, whose geographic range extends to just south of the Columbia Icefields, supports the hypothesis of large-scale climate change in this area.

Demographics

The age distribution of many sites indicates that disturbances such as fire play an important role in the regeneration process of whitebark pine. The importance of disturbance is evident from the periodic regeneration shown at many sites (Appendix A, Figures 1 to 29). However, the continuous age distributions shown at others suggests that at these sites other factors besides disturbance are important in regeneration. Continuous regeneration may be possible under conditions that, for example, minimize competition from other species. Site 27 (Appendix A, Figure 27) is on a rocky exposed ridge where there is little soil available for seedling establishment. Other species such as Engelmann spruce (*Picea englemannii* Parry) and subalpine fir (*Abies lasiocarpa* (Hook.) Nut.) may not be able to germinate under these types of conditions. Although germination and growth may be slow, whitebark pine can establish on these types of sites in the absence of competition.

Although requirements for germination and seedling growth are essential, seed availability – as dependent on nutcrackers – is the ultimate factor limiting regeneration. Nutcracker caching habits and the periodic cycle of seed masting may be responsible where and when stands develop and may therefore have an effect age distribution. How these factors affect regeneration requires further investigation.

Blister Rust

As expected, the rate of infection declined northward along the Canadian Rockies. High rates of infection were found at sites in the Southwest Alberta Forest District north of Crowsnest pass and the Cranbrook Forest District of southwestern British Columbia. Parks Canada officials had expected high infection only up to the Crowsnest pass but researchers from Montana had predicted high infection throughout the Canadian Rockies (Hoff et al. 1994). Two possible scenarios could be invoked to explain the observed pattern of blister rust infection.

The first possible explanation of the pattern of blister rust infection is that a balance with climatic and ecological factors has been reached that limits the incidence of high infection to sites south of Banff National Park and high mortality from Waterton Lakes National Park south. Because blister rust depends on the availability of both white pines and *Ribes* in order to complete its life cycle, the distribution and density of *Ribes* species may provide a possible explanation for the pattern of infection intensity. Each infected *Ribes* plant is capable of producing thousands of basidiospores (Zillar 1974). There is natural variation in resistance to white pine blister rust among *Ribes* spp. just as there is among white pine species (McDonald et al. 1984, McDonald 1992, Hoff and McDonald 1993). For example, Buchanan and Kimmey (1938) suggest that basidiospore production from *R. viscosissimum* was higher than in *R. lacustra*. Waterton Lakes National Park is the approximate northern limit of the distribution of *Ribes viscosissimum*. The potential for the build up of inoculum and thus the high infection and mortality rate in Waterton Lakes National Park may therefore be a consequence of *Ribes* species distribution. Furthermore, the density of *Ribes* distribution may also determine the level of infection. For example, McDonald et al. (1981) use *Ribes* densities to predict the level of blister rust infection on western white pine. Knowledge of the density of *Ribes* plants may be useful in predicting the intensity and spread of blister rust in the Canadian Rockies.

The second possible explanation is that the disease is spreading northward and that there is potential for high infection and mortality of whitebark pine populations throughout the Canadian Rockies. Although the examination of blister rust levels in this study could not differentiate between the two possible scenarios outlined above, there is some evidence that infection could increase at sites which presently have moderate to low infection. Analysis of climatic data suggests that there are no limitations to the spread of blister rust north through the Rockies (Stuart-Smith, unpublished data). Examination of the availability of climatic conditions required for infection (periods of two days of high humidity and temperatures above 1°C) found no difference in the opportunity for infection at sites in Waterton Lakes National Park compared to areas throughout the rest of the Canadian Rockies. Thus, the high rates of infection that were found in the southern areas may be indicative of the northward spread of the epidemic. Although estimates of mortality were not part of the sampling protocol, a estimates at the sites in Waterton Lakes National Park showed mortality of around 90% whereas High mortality was associated with the high rates of infection at sites in Waterton Lakes National Park but not with the high infection rates of sites further north. Thus, sites with high infection but low mortality simply may not have been infected for a long enough period for significant mortality to occur. Monitoring these sites for significant morality over the next decade will be important for determining whether the epidemic is spreading northward.

The comparatively high blister rust infection detected at the Mt Robson site, much higher than any other nearby sites in Banff and Jasper, may be another indication that blister rust is spreading northward. The Fraser valley that drains the Robson area has a moister climate, as reflected in the vegetation types present, compared to valleys on the east side of the continental divide. Western white pine, infrequently found in the rest of the Rockies, can be found high up the Fraser valley because of the moist climate, and may have and may be acting as an inoculum source for the spread of blister rust. The build up of inoculum on western white pine may have allowed for the more rapid spread of blister rust up the Fraser valley. Thus more time may be required for the build up of inoculum leading to high infection rate for sites south of the Fraser valley that do not have other species acting as inoculum sources.

Evidence from the exploration of factors affecting infection rate in western white pine further suggest that significant infection rates could be found in northern whitebark pine stands in the future. Hunt (1983) found that the rate of infection on western white pine was higher on sites with greater slopes. Slope did not significantly contribute to the explanation of infection rate among the sites examined in this study, which suggests that slope is not a factor limiting the potential for high infection. Sites in Waterton Lakes with high infection rates were on slopes that were in the low range of those sites surveyed indicating that infection rates at the other sites examined could potentially reach higher levels. Van Arsdel (1967) also found that localised wind currents could increase infection rates in eastern white pine in Wisconsin. In the Canadian Rockies, there is great topographical variation and associated variation in microclimate such that localized wind currents and microclimate could also influence infection intensity. Sites in Waterton National Park with high infection do not differ greatly in their topographical profile compared to other areas of the Canadian Rockies. Therefore, microclimate conditions are likely to limit of the spread of blister rust.

Understanding the factors that limit the proportion of individuals infected with blister rust at the stand level depends on an understanding of the mechanism of infection at the individual level. Older and larger individuals showed infection more often than younger individuals

(Kolmogorov-Smirnov test and logistic regression analysis, Tables II.4-3 to II.4-5 and Figure II.4-6). Since mortality due to infection is highest in younger age classes (Hunt 1983), older trees may be more likely to be infected simply because they present a larger area for potential infection. Further, the lack of infection found on younger individuals does not necessarily indicate lower probability of infection but may simply indicate high mortality in these age classes. A single canker may effectively girdle and kill small trees whereas older individuals may be able to withstand a number of cankers before mortality results.

In addition to blister rust, fire suppression may also have contributed to the reduction in area covered by whitebark pine in western Montana (Keane and Arno 1993, Keane et al. 1994). If blister rust has been present in the four mountain parks for less than one whitebark pine generation (likely over 100 years), selection for blister rust resistance could not have caused a shift of the genotypic frequency in the whitebark pine population. Thus, re-introduction of fire into the subalpine community could allow whitebark pine to regenerate under strong selection pressure for blister rust resistance, resulting in a higher proportion of resistant genotypes in the next generation of whitebark pines. By, using fire to reduce the generation time, the process of natural selection may increase the proportion of resistant genotypes in whitebark pine. Whitebark pine was ranked the least resistant out of 11 species of pines susceptible to white pine blister rust, which is indicative of the low resistance found in natural populations (Hoff et al. 1994). Therefore, even if natural selection eventually produces widespread resistance in whitebark pine, the genetic bottleneck (resulting from blister rust and fire suppression caused population reduction) may leave the species susceptible to mortality by other factors.

Mountain pine beetles may also be a factor that has contributed, and may contribute in the future, to the decline of whitebark pine in some areas of Montana and even areas of southern Alberta. The presence of beetles was noted on some dead whitebark pine in Waterton Lakes National Park but data on the distribution of pine beetles and the contribution they have made to this decline were not collected in this study due to time and financial constraints. Determining the relationship between pine beetle infection and regeneration is an important aspect of whitebark pine ecology that needs to be addressed.

II.6 Summary and Conclusions

The conservation of whitebark pine requires the understanding of the ecological characteristics of whitebark pine stands and the role disturbances play in the regeneration process. Whitebark pine regeneration results when the combinations of nutcracker caching habits and micro-site germination and seedling growth requirements are met. The site characteristics, demographic patterns and blister rust infection levels can be summarized as follows. The site characteristics of whitebark pine stands in the Canadian Rockies were generally steep (25°), southwest facing slopes around 2000 m elevation. Clumped age distributions at many sites indicated the importance of disturbances such as fire in regeneration. However, the continuous pattern shown at other sites also indicated that under specific conditions continual regeneration could occur. High blister rust infection and mortality was found in many areas of the southern Canadian Rockies. The probability of infection increased with the size of the individual. The proportion of individuals infected with blister rust declines with latitude and elevation. However, climate may not be a barrier to increased infection in sites that presently have low infection. Differences in the number of *Ribes* host species present and in infection intensity on different *Ribes* species may limit the spread of rust. However, besides this possibility, there are presently no apparent limitations to the spread of blister rust northward.

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Chapter III

III Genetic Variation and Differentiation of Whitebark Pine in the Canadian Rockies

III.1 Introduction

Genetic diversity is essential for adaptation and evolution (Lande and Barrowclough 1987). In order to maintain fitness in the face of selection, there must be sufficient genetic diversity within and among populations. In turn, severe mortality associated with directional selection may result in a decline in genetic diversity. Threats to species survival arise from a diversity of causes, many of which are associated with human influence (Frankham 1995). Specific to this thesis is the whitebark pine ecosystem which has been affected by humans through the accidental introduction of white pine blister rust and, perhaps, through fire suppression. White pine blister rust has caused high mortality and consequently genetic diversity may be in decline (Hoff et al. 1994). Because genetic variation is required for evolution and adaptation, knowledge of the level of genetic diversity and its relationship to site characteristic is essential to the conservation and maintenance of viable populations.

Conifers, which are generally long-lived, outcrossing, wind pollinated and have large geographic ranges, can consequently have high genetic variation within species and populations, but relatively little differentiation among populations (Hamrick et al. 1992). As a conifer, whitebark pine has these traits, which predicts that it should have high genetic variation but some of its unique characteristics may affect the level and pattern of genetic diversity. Seed dispersal by Clark's nutcrackers and the small isolated nature of whitebark pine populations in the Canadian Rockies (Ogilvie 1990), however, may decrease gene flow among populations and result in greater differentiation among populations (Brussard 1990). Furthermore, founder effects, genetic bottlenecks and genetic drift, evolutionary forces which are more likely in small populations, all result in increased differentiation among populations and are often accompanied by loss of genetic diversity (Ellstrand and Elam 1993, Frankham 1995).

Inbreeding, another consequence of population isolation and low gene flow, may further increase differentiation among populations (Hamrick 1982, Slatkin 1987). Politov et al. (1994) found high inbreeding in the Eurasian stone pines. Based on analysis of 50 individuals from only two populations, Furnier and Dancik (1990) suggest that inbreeding is high in whitebark pine. Thus, the size and isolation of whitebark pine populations suggests that they should have low genetic diversity and high differentiation among populations.

Based on a survey of 30 stands, Jorgensen and Hamrick (1997) estimate that whitebark pine's genetic diversity and population divergence are lower than that of most conifers. In contrast, Yandell (1992) studied 14 populations from the southwestern portion of the range and found allozyme diversity levels similar to other pines but higher levels of population differentiation than in most conifers. In both studies, populations in mountain ranges that are separated by hundreds of kilometres over which there is effectively no gene flow were combined to estimate genetic diversity. For example, the probability of seed or pollen transfer between whitebark pine populations in the Cascade ranges of Washington and Oregon and the populations found in the Rockies of Montana, Idaho and Wyoming is effectively zero (Figure I.1). The populations I studied in the Canadian Rockies represent a more continuous profile of whitebark pine distribution, despite their small size and isolation. Thus, I expected high genetic diversity but low population differentiation in comparison to other parts of the range of whitebark pine.

Since the end of the last glacial period 10-15 000 years ago, whitebark pine is thought to have migrated into the Canadian Rockies from refugia in the southwestern United States (Critchfield 1984). A consequence of this migration may have been reduced genetic variation in the founding populations. A decline in genetic diversity with latitude would support this hypothesis of post-glacial migration.

Whitebark pine populations in the Canadian Rockies are at the margin of the species distribution (Arno and Hoff 1989). Marginal populations may have to endure environmental conditions that would not be common throughout the rest of the range and may have lower genetic diversity due the lower gene flow and founder effects. The studies of whitebark pine by Jorgensen and Hamrick (1997) and Yandell (1992) did not relate patterns of genetic variation to environmental or demographic factors.

Fire suppression and blister rust mortality may also have effected the level and pattern of genetic diversity in whitebark pine populations in the Canadian Rockies. The effect of fire suppression should be to increase the average age of stands and reduce the variation in ages. Populations with little age variation should have lower genetic diversity than populations with a mixed age structure because the overlap of generations maintains genotypes that would otherwise be lost (Frankham 1995). Blister rust mortality may have reduced the number of genotypes and eventually lead to a reduction in genetic diversity. If rust resistance is related to the degree of homozygosity, the selection resulting from rust mortality may be reducing genetic variation. Therefore, in order to determine the factors that affect the level and pattern of genetic diversity, it is important that ecological and demographic information be considered in conjunction with genetic data (Lande 1995).

III.2 Objectives

The three objectives of this chapter were as follows.

1. To determine if the caching behaviour of Clark's nutcrackers, and the small isolated populations in which whitebark pine are found in the Canadian Rockies, have resulted in a lower level of genetic variation and greater differentiation among populations compared to wind-dispersed and more widespread conifers.

2. To determine if genetic diversity in whitebark pine declines from the United States border northward through the Rockies. Such a decline would support the hypothesis that whitebark pine has migrated from southern refugia since the end of the last ice age.

3. To determine if there is a correlation between environmental and/or demographic variables and genetic diversity and to suggest what mechanisms determine these relationships.

III.3 Methods

III.3.1 Needle Collection and Preparation

Needles were collected from 25 randomly sampled trees at each site as described in section II.3. Needles were stored on ice within hours of their collection. Within seven days after collection, needles were frozen at -70°C. Needle tissue was prepared for electrophoresis following the method outlined in Mitton et al. (1979). Needles were cut into pieces and ground with a mortar and pestle in liquid nitrogen until a fine powder was produced. The liquid nitrogen cooled the mortar so that when approximately 2 ml of grinding buffer (Mitton et al. 1979) was added to the ground tissue the liquid would partially freeze. This slurry was packed into 2.5 ml Eppendorff tubes that were centrifuged. Samples were returned to the -70°C freezer until electrophoresis. In order to increase efficiency and reduce the number of times samples were thawed and refrozen, later samples were prepared beforehand by soaking wicks in the samples and then refreezing them until electrophoresis.

III.3.2 Electrophoresis Procedures.

Gels were made using a 12.5 % (w/v) starch solution. Three buffer systems were employed in electrophoresis. The Pol buffer is a discontinuous tris-citrate system based on a recipe by Poulik (1957) as modified by Schuster et al. (1989) (Mitton personal communication). The gel buffer was made by mixing 18 ml of 10.5 g monohydrate citric acid/L solution with 24 ml of 23.0 g Tris /L solution diluted to 300 ml for a single gel. The concentration in the final gel solution, in which the pH was not adjusted, was 3 mM citric acid and 15 mM Tris. The electrode buffer was made by mixing 4 g NaOH and 18.55 g boric acid per L and then adjusting the pH to 8.6 with 1.0 M citric acid. The Hist buffer is a recipe modified by King and Dancik (1983) from recipes by Florence (1981) and Namkoong et al. (1979). Gels were made from a 1:5 dilution of the following solution: 0.05 M L-Histidine-HCl and1.40 mM EDTA adjusted to pH 7.0 with 1.0 M Tris. The electrode buffer for the Hist system was from a solution of 0.125 M Tris adjusted to pH 7.0 with 1.0 M citric acid. The TC buffer is a continuous tris-citrate buffer recipe described in Selander et al. (1971). The gel buffer was made from a 0.008 M Tris/ 0.003 M citric acid solution: 1.4521 g Tris and 0.8646 g anhydrous citric acid in 100 ml distilled water and adjusting the pH to 6.7 with 1.0 M NaOH. Gels were made from a 1:15 dilution of the gel buffer solution. The electrode buffer was made by mixing 27 g Tris and 16.52 g anhydrous citric acid per litre of distilled water to produce a 0.223 M Tris and 0.086 M citric acid solution. A 1.0 M solution of NaOH was used to adjust the pH of the electrode buffer to 6.3.

The conditions for horizontal starch gel electrophoresis were as follows. Gels were run for 20 minutes at half voltage and then dewicked before running at full voltage. TC gels were run for approximately 5 hours at 200 V, Pol gels for 6 hours at 300 V, and Hist gels for 4 hours at 300 V. Gels were stopped when the marker dye had moved 6 cm from the origin. While gels were running, they were cooled by placing a Pyrex dish containing an icebath over the top of the gel.

The three buffer systems were used to resolve the following 16 putative allozyme loci representing 12 enzyme systems (Table III.3-1). For all enzymes except UDP, enzyme stain recipes were adapted from Cheliak and Pitel (1984). The enzyme stain recipe for UDP was from Harris and Hopkinson (1976) as modified by Mitton (Personal communication). Mendelian inheritance was assumed based on patterns previously demonstrated for whitebark pine and other conifer species (Guries and Ledig 1982, Mitton et al. 1979, King and Dancik 1983, Furnier et al. 1986, Schuster et al. 1989).

Table III.3-1 Enzymes, Enzyme Commission numbers (E.C. #), buffer systems used for starch gel electrophoresis and abbreviations of putative loci. See text for explanation of buffer system abbreviations.

Enzyme	E. C. #	Buffer System	Putative loci
Aldolase	4.1.2.7	HIST	*Ald*
Malate dehydrogenase	1.1.1.37	HIST	*Mdh-1,2*
Shikimate dehydrogenase	1.1.1.25	HIST	*Skdh-1*
Fluorescent esterase	3.1.1.2	POL	*Fe-1,3*
Glutamate dehydrogenase	1.4.1.2	POL	*Gdh*
Menadione reductase	1.6.99.2	POL	*Mnr-2*
Phosphoglucomutase	5.4.2.2	POL	*Pgm-1,2*
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	TC	*G3pdh*
Isocitrate dehydrogenase	1.1.1.42	TC	*Idh*
Phosphoglucose isomerase	5.3.1.9	TC	*Pgi-1,2*
6-phosphogluconate dehydrogenase	1.1.1.44	TC	*6-Pgd*
Uridine diphosphoglucose pyrophosphorylase	2.7.7.9	TC	*Udp-1*

III.3.3 Statistical Analysis

Genetic Diversity, Differentiation and Distance Calculation and Dendrogram Construction

There are a variety of ways to measure genetic diversity from allozyme data but no consensus as to which is the best or most correct method available. A discussion of the different approaches to the problem of genetic data analysis and the justification for the methods chosen here is presented in Appendix B.

Genetic diversity statistics were calculated using a combination of the following programs: Genepop version 1.2 (Raymond and Rousset 1995), Popgene version 1.21 (Yeh, Yang and Boyle 1997), Biosys release 1.7 (Swofford and Selander 1989), Phylip version 3.572c (Felsenstein 1995) and programs written by myself in Visual Basic for Microsoft Excel 97. The following statistics were calculated at the species and population level across all loci: proportion of loci polymorphic (P), average number of alleles per locus (A), average number of alleles per polymorphic locus, effective number of alleles (A_e), expected heterozygosity (H_e), and observed heterozygosity (H_o) (see appendix B for references regarding these statistics). Observed, expected and total heterozygosities (H_O, H_S, H_T) were also calculated at each locus. Wright's fixation indices, F_{IS}, F_{IT}, and F_{ST}, as well as the estimate of gene flow, *Nm*, were calculated at each locus and at the species level. F_{IS} was also calculated at each locus for each population. Deviation from Hardy-Weinberg equilibrium (significance of F_{IS}) was tested using Fisher's exact test (Rousset and Raymond 1997) within each population at each locus, at each locus and over all loci. Significance level was determined from the number of tests performed using the Bonferroni technique (Lessios 1992). Population differentiation was also tested using an exact test but because of the computational requirements, the Markov Chain algorithm was used to estimate the significance level (Goudet et al. 1996, Raymond and Rousset 1995). As suggested by Lessios (1992), the significance level was divided by the number of tests performed (Bonferroni technique). Nei's (1972, 1978) unbiased standard genetic distance was used to estimate genetic distances between sites. A Mantel test was used to test for a significant relationship between the genetic and geographic distances (Raymond and Rousset 1995). The Neighbor-Joining (NJ) method (Saitou and Nei 1987) was used to construct a dendrogram from the genetic distances. The jackknifing procedure with 10 000 resamples was used to test the 'stability' of the NJ tree (Felsenstein 1995).

Redundancy Analysis

Principle component analysis (PCA) can be used to separate sites according to the variance in allele frequencies if the allele frequencies at each site are treated as species variables (Ter Braak 1988). As Jorgensen and Hamrick (1997) point out, because the variance is used to discriminate among populations in PCA, the contribution of rare alleles is minimal. Redundancy analysis (RDA) was used in order to determine if the discrimination among sites based on allele frequencies correlates with environmental variables. Principle component analysis and redundancy analysis were carried out using the CANOCO package (Ter Braak 1988). The environmental variables used in the redundancy analysis include: latitude (northing), aspect, slope, elevation, percent of individuals infected with blister rust, the oldest tree found at the site, the variance in ages within each site, the average diameter, the average height, and an index of avalanche probability (Table III.3-2). The aspect was divided into north-south by taking the sine of the angle of aspect and into the east-west component by taking the cosine. Two additional variables, average age of the stand and easting, were initially included in the analysis but high variance inflation factors indicated they were highly correlated with other variables (Ter Braak 1988). Because latitude and longitude were highly correlated ($r=-0.97$), only the latitude (northing) was included in the analysis. Average age was also highly correlated with maximum and variance of the ages as well as the average height and diameter. Because the age of trees not bored was estimated from the height and diameter, this correlation is expected. Therefore, the average age of the stand was removed from the analysis. After these two environmental variables were removed, variance inflation factors were small (<20, Ter Braak 1988). The Monte Carlo permutation resampling method of CANOCO was used in order to test the significance of the primary axis and to test the significance of the overall effect of the environmental variables on the species.

Table III.3-2 Environmental variables used in the redundancy analysis and the abbreviations used for these variables in Figure III.4-2.

Variable	Description	Abbreviation
Diameter	Average diameter as measured at the base of the tree	Diameter
Variance Age	Variance in age distribution at each site	Var Age
Maximum Age	Age of oldest tree at each site	Max Age
Height	Average height of trees at each site	Height
Elevation	Elevation above sea level at the centre of the site	Elev
North	Latitude in UTM co-ordinates of the centre of the site	North
Aspect	Aspect taken at the centre of the site. Divided into a north and east component	ANorth and AEast
Avalanche	Relative rating of the potential for avalanches at the site	Aval
Blister rust infection	Percent of individuals infected with blister rust	% BR
Slope	Slope taken at the centre of the site	Slope

Multiple Regression Analysis: Factors Important to Genetic Diversity

PCA and redundancy analyses are useful in order to differentiate between sites based on allele frequencies and site characteristics. However, in order to determine if environmental and demographic factors as well as blister rust infection relate to the level of inbreeding (F_{IS}) and measures of genetic diversity (H_S, H_O, A and P), multiple regression analysis was used. The demographic and environmental variables used in the RDA (Table III.3-) except for avalanche were entered into a multiple regression model. Avalanche potential is not a continuous variable and was therefore not included in this analysis. Because multiple regression takes into account the correlation between independent variables, the two other variables that were not included in the RDA were also entered into the analysis: easting and average age. The model that best explained the variation in the genetic indices was chosen by examining all of the possible models and selecting the one with the highest adjusted r^2.

III.4 Results

The genetic diversity statistics for species and populations were respectively: proportion of loci polymorphic 56.25%, 50.25%; average number of alleles per locus 2.06, 1.64; average number of alleles per polymorphic loci 2.89, 2.14; effective number of alleles 1.43, 1.38; expected heterozygosity 0.224, 0.211; and observed heterozygosity 0.218, 0.218 (Table III.4-1). The

Table III.4-1 Genetic diversity statistics in 29 populations of whitebark pine calculated from allozyme frequencies at 16 putative loci. P: percent of loci polymorphic; A: average number of alleles per locus; A_e: effective number of alleles per locus; H_o: observed heterozygosity; H_e: SE; standard error of the mean.

Site	P	A	A_e	H_o(SE)	H_e(SE)
1 Summit	43.8	1.56	1.35	0.203 (0.077)	0.187 (0.059)
2 Rowe	43.8	1.69	1.35	0.197 (0.071)	0.194 (0.056)
3 Cabin	43.8	1.56	1.40	0.206 (0.083)	0.201 (0.062)
4 Lost	50.0	1.69	1.42	0.238 (0.073)	0.230 (0.060)
5 Centre	50.0	1.63	1.43	0.221 (0.076)	0.232 (0.060)
6 Ironstone	50.0	1.69	1.46	0.254 (0.084)	0.235 (0.063)
7 Vicary	43.8	1.69	1.35	0.217 (0.077)	0.194 (0.056)
8 Wildhorse	50.0	1.69	1.34	0.168 (0.058)	0.197 (0.053)
9 Coat	56.3	1.63	1.43	0.246 (0.073)	0.237 (0.058)
10 Highwood	56.3	1.63	1.40	0.193 (0.064)	0.219 (0.058)
11 Joffre	43.8	1.56	1.41	0.241 (0.079)	0.213 (0.063)
12 Kindersley	50.0	1.56	1.41	0.231 (0.067)	0.227 (0.059)
13 Sinclair	43.8	1.50	1.39	0.225 (0.079)	0.204 (0.062)
14 Nestor	56.3	1.63	1.35	0.213 (0.072)	0.206 (0.055)
15 Hawk	56.3	1.69	1.37	0.203 (0.071)	0.214 (0.056)
16 Sulphur	56.3	1.69	1.38	0.267 (0.076)	0.217 (0.056)
17 Whymper	56.3	1.63	1.39	0.227 (0.066)	0.221 (0.056)
18 Castle	56.3	1.63	1.39	0.239 (0.071)	0.216 (0.057)
19 Wiwaxy	43.8	1.56	1.26	0.123 (0.059)	0.158 (0.049)
20 Whaleback	50.0	1.69	1.32	0.162 (0.075)	0.183 (0.052)
21 Dolomite	43.8	1.63	1.35	0.229 (0.073)	0.201 (0.056)
22 Sarbach	50.0	1.63	1.35	0.226 (0.073)	0.206 (0.055)
23 Nigel	56.3	1.63	1.42	0.218 (0.073)	0.231 (0.059)
24 Wilcox	56.3	1.81	1.43	0.274 (0.082)	0.225 (0.059)
25 Geraldine	56.3	1.75	1.41	0.239 (0.074)	0.228 (0.057)
26 Astoria	37.5	1.50	1.28	0.144 (0.065)	0.162 (0.054)
27 Esplinade	56.3	1.75	1.43	0.190 (0.059)	0.221 (0.060)
28 Berg	50.0	1.63	1.40	0.274 (0.083)	0.225 (0.059)
29 Adolphus	50.0	1.69	1.42	0.248 (0.082)	0.224 (0.061)
Mean	50.2(5.7)	1.64(0.1)	1.38(0.05)	0.218 (0.073)	0.211 (0.058)
Species Value	56.3	2.06	1.43	0.218	0.224

Table III.4-2 Fixation indices for individuals relative to the subpopulation at 9 polymorphic loci (F_{IS}). (*, $p \leq 0.05$, exact test of Hardy-Weinberg equilibrium, Rousset and Raymond 1995). '-': locus fixed for one allele.

Site	*Mdh*	*Fle-3*	*Mnr*	*Pgm-2*	*Idh*	*Pgi-1*	*Pgi-2*	*6Pg*	*Udp*
1 Summit	0.21	-0.09	-0.69*	0.22	-	-0.48	0.22	-	0.78
2 Rowe	-0.20	0.07	-0.81*	0.28	0.00	-0.24	0.33	1.00	0.60
3 Cabin	0.35	-0.07	-0.75*	0.46	0.00	-0.88*	0.69	-	0.55
4 Lost	0.03	-0.18	-0.58	0.44	0.00	-0.45	-0.21	-0.14	0.80*
5 Centre	0.28	-0.19	-0.70*	0.41	-0.02	-0.50	0.36	1.00*	0.36
6 Ironstone	-0.26	-0.51	-0.81*	0.08	-0.04	-0.10	0.84*	1.00	0.08
7 Vicary	-0.03	0.24	-0.82*	-0.34	-0.02	-0.41	0.09	1.00	1.00
8 Wildhorse	0.33	0.27	-0.70*	-0.17	-0.09	0.21	0.53	1.00	0.62
9 Coat	0.10	0.23	-0.69	-0.06	-0.17	-0.58	0.01	0.78	0.58
10 Highwood	0.03	0.32	-0.47	0.10	0.34	-0.28	0.20	1.00	1.00
11 Joffre	-0.26	0.10	-0.75*	-0.17	-	-0.48	0.27	-	0.42
12 Kindersley	0.22	-0.08	-0.69	0.04	0.00	-0.24	0.32	-0.16	0.44
13 Sinclair	-0.36	-0.15	-0.81*	0.06	-	-0.34	0.61	-	0.44
14 Nestor	0.14	-0.33	-0.70	0.11	-0.03	-0.29	0.09	0.65	0.77
15 Hawk	0.06	-0.11	-0.66	0.51	-0.12	-0.55	0.55	-0.04	0.92*
16 Sulphur	-0.26	-0.36	-0.88*	-0.08	-0.07	-0.21	-0.05	-0.03	0.14
17 Whymper	-0.18	-0.03	-0.75*	0.05	0.19	-0.14	0.42	-0.09	0.37
18 Castle	0.18	0.04	-0.81*	-0.17	-0.04	-0.32	0.51	-0.09	0.05
19 Wiwaxy	-0.04	1.00*	-0.81*	0.34	0.00	-0.04	-0.02	-0.09	0.92*
20 Whaleback	0.34	-0.33	-0.75*	0.86	-0.02	-0.29	0.47	1.00*	1.00*
21 Dolomite	0.42	-0.04	-0.88*	0.03	0.00	-0.31	-0.32	-0.01	0.20
22 Sarbach	0.18	-0.15	-0.88*	0.33	-0.05	-0.50	0.18	-0.02	0.36
23 Nigel	-0.07	0.49	-0.53	0.08	-0.11	-0.88*	0.76*	-0.04	0.64
24 Wilcox	-0.12	0.07	-0.81*	0.01	-0.07	-0.88*	0.02	-0.20	0.43
25 Geraldine	-0.17	-0.39	-0.81*	0.16	-0.11	-0.23	-0.20	0.05	1.00*
26 Astoria	0.34	0.10	-0.88*	0.47	-	-0.17	-	0.00	0.90*
27 Esplinade	0.18	0.64*	-0.63	-0.11	-0.07	-0.17	0.35	0.21	0.54
28 Berg	-0.18	-0.19	-0.73	0.27	0.32	-0.96*	-0.13	0.00	0.13
29 Adolphus	0.04	0.36*	-0.81*	0.15	0.00	-0.96*	0.39	-0.03	0.13

Table III.4-3 Genetic diversity and population differentiation statistics at each locus *, $p<0.05$ indicates significantly different from 0.

Locus	Alleles	H_O	H_S	H_T	F_{IS}	F_{IT}	F_{ST}	Nm
Mdh-2	2	0.368	0.389	0.393	0.054	0.064	0.030	8.08
Fle-3	3	0.540	0.554	0.572	0.025*	0.055	0.051*	4.61
Mnr	3	0.882	0.505	0.496	-0.747*	-0.777	0.003	74.45
Pgm-2	3	0.347	0.399	0.411	0.129	0.154	0.051*	4.68
Idh	3	0.117	0.117	0.121	0.005	0.031	0.046*	5.16
Pgi-1	2	0.647	0.452	0.478	-0.431*	-0.352	0.075*	3.09
Pgi-2	2	0.284	0.389	0.466	0.271*	0.392	0.184*	1.11
6Pg	4	0.100	0.138	0.142	0.280*	0.297	0.044*	5.45
Udp	4	0.201	0.425	0.513	0.527*	0.608	0.189*	1.07
Averages		0.218	0.211	0.224	-0.035*	0.030	0.062*	3.77

following seven loci were monomorphic in all 29 populations: *Ald*, *Fle-1*, *Gdh*, *G3pdh*, *Mdh-1*, *Pgm-1* and *Skdh-1*.

At an adjusted significance level of 0.0002 (=0.05/253 tests performed), 38 of the 253 (15.0%) loci within populations showed significant departures from Hardy-Weinberg equilibrium (Table III.4-2). Of these departures, 13 were heterozygote deficiencies and 25 showed an excess of heterozygotes, 20 of which were at the *Mnr* locus. At an adjusted significance level of 0.006 (=0.05/9), F_{IS} values at the locus level were significantly different from zero, therefore showing either an excess or deficiency of heterozygotes compared to Hardy-Weinberg equilibrium across all populations, at the *Fle-3*, *Mnr*, *Pgi-1*, *Pgi-2*, *6Pg*, and *Udp* loci (Table III.4-3). Although most of the significant excesses and deficiencies were either in the 6 most southern sites or in the 10 most northern sites, departures were not consistent across loci (Table III.4-2).

All individual loci showed significant population differentiation (exact test) with 0.3% (*Mnr*) to 18.9% (*Udp*) of variation found among populations (Table III.4-2). F_{ST} estimates were significantly different from 0 at all loci except *Mdh-2* and *Mnr* for both allele or genotype frequencies. There was also significant population differentiation over all loci with 6.2% of variation found among populations. The level of diversity within populations, 93.8%, is higher than expected given the isolated nature of these populations.

Gene flow among all populations, *Nm*=3.77, was just below the threshold ($Nm \geq 4$) thought to be required to counteract genetic drift (Hartl and Clark 1989). Genetic distances among sites were small, (-0.004 to 0.075; average of 0.019), and the largest distance was between population 2 (Rowe, Waterton Lakes NP) and 20 (Whaleback, Yoho NP). There was a significant but very low correlation between geographic distance and Nei's unbiased standard genetic distance (r^2=0.028, p=0.02, Mantel test with 10 000 permutations).

The dendrogram based on genetic distances did not correspond well to actual geographic relationships (Figure III.4-1). The low percentage of trees with the same topology, indicated by high variation among jackknifed replicates, further supports the lack of relationship between genetic and geographic distance. Site 16 (Sulphur) separated out from the rest of the populations in over 90% of the replicate trees. However, all other branches were similar in less than 51 % of the replicate trees. The dendrogram showed three main groups: populations in the northern region of the study area at the top of the dendrogram; populations from the southwestern British Columbia in the middle cluster; and populations from southwestern Alberta at the bottom of the dendrogram. However, not all populations within these groupings followed this pattern and these groupings only loosely describe the dendrogram separation. Many sites within the second and third groupings do not correspond to this classification (for example, sites 2, 6 and 10 are from the Alberta side of the divide but grouped into the southern BC cluster). Populations 11, 21 and 29 were closely aligned with the two southern clusters but separate from them.

PCA and RDA analysis

The PCA and RDA separated populations on the basis of allele frequencies and showed that this separation could be accounted for by the environmental variables. The first and second axis of the PCA accounted for 40.5% and 32.1% of the variance in allele frequencies respectively; the first four axes together accounted for 87.4% of the total variation (Figure III.4-2A). In the RDA, the first two axes explained similar amounts of variation as in the PCA (31.6% and 10.9% respectively Figure III.4-2B) but the first four axes accounted for only 50.6% of allelic variation. The species-environmental correlation was 0.890 for the first axis and 0.596 for the

```
                                 +-----------------------------Nestor      14
                                 !
                                 !                       +-----Wiwaxy      19
                                 !                  +27.7
                                 !              +23.63   +-----Astoria     26
                            +26.3!              !   !
                            !    !         +20.41   +----------Whaleback   20
                            !    !         !    !
                            !    !         !    !        +-----Geraldine   25
                            !    !    +33.62    +------23.92
    +-------------------22.08    !    !    !             +-----Esplinade   27
    !                       !    +30.73    !
    !                       !         !    +-------------------Castle      18
    !                       !         !
    !                       !         +------------------------Whymper     17
    !                       !
    !                       +----------------------------------Sarbach     22
    !
    !                            +-----------------------------Kindersley  12
    !                            !
    !                            !                       +-----Wilcox      24
    !                            !                   +34.27
    !                            !              +26.40   +-----Rowe        2
    !                       +37.68              !    !
    !                       !    !         +12.89    +---------Cabin       3
    !                       !    !         !    !
    !                       !    !         !    !        +-----Wildhorse   8
    !                       !    !    +46.38    +------23.88
    !                   +35.64   !    !    !             +-----Highwood    10
    !                   !   !    +49.33    !
    !                   !   !        !     +-------------------Centre      5
+92.02                  !   !        !
!   !                   !   !        +-------------------------Ironstone   6
!   !                   !   !
!   !                   !   +----------------------------------Hawk        15
!   !                   !
!   !                   !                                +-----Summit      1
!   !              +12.04                            +50.94
!   !              !    !                       +23.80   +-----Vicary      7
!   !              !    !                       !    !
!   !              !    !                  +23.80    +---------Sinclair    13
!   !              !    !                  !    !
!   !              !    !             +23.80    +--------------Berg        28
!   !         +20.33    !             !    !
!   !         !    !    !         +17.10   +-------------------Nigel       23
!   !         !    !    !         !    !
!   !         !    !    +------23.80   +-----------------------Lost        4
!   !    +29.87    !              !
!   !    !    !    !              +----------------------------Coat        9
!   !    !    !    !
!   !    !    !    +-------------------------------------------Adolphus    29
!   +20.88    !
!        !    !
!        !    +------------------------------------------------Joffre      11
!        !
!        +-----------------------------------------------------Dolomite    21
!
+--------------------------------------------------------------Sulphur     16
```

Figure III.4-1 Neighbor-joining dendrogram, based on Nei's unbiased standard genetic distance, of 29 whitebark pine populations in the Canadian Rockies. The numbers at the nodes indicate the percent that the group consisting of the populations which are to the right of that node occurred out of 10 000 jack-knifed replicates. Note that this is an unrooted tree. Branch lengths are not related to the genetic distance between populations.

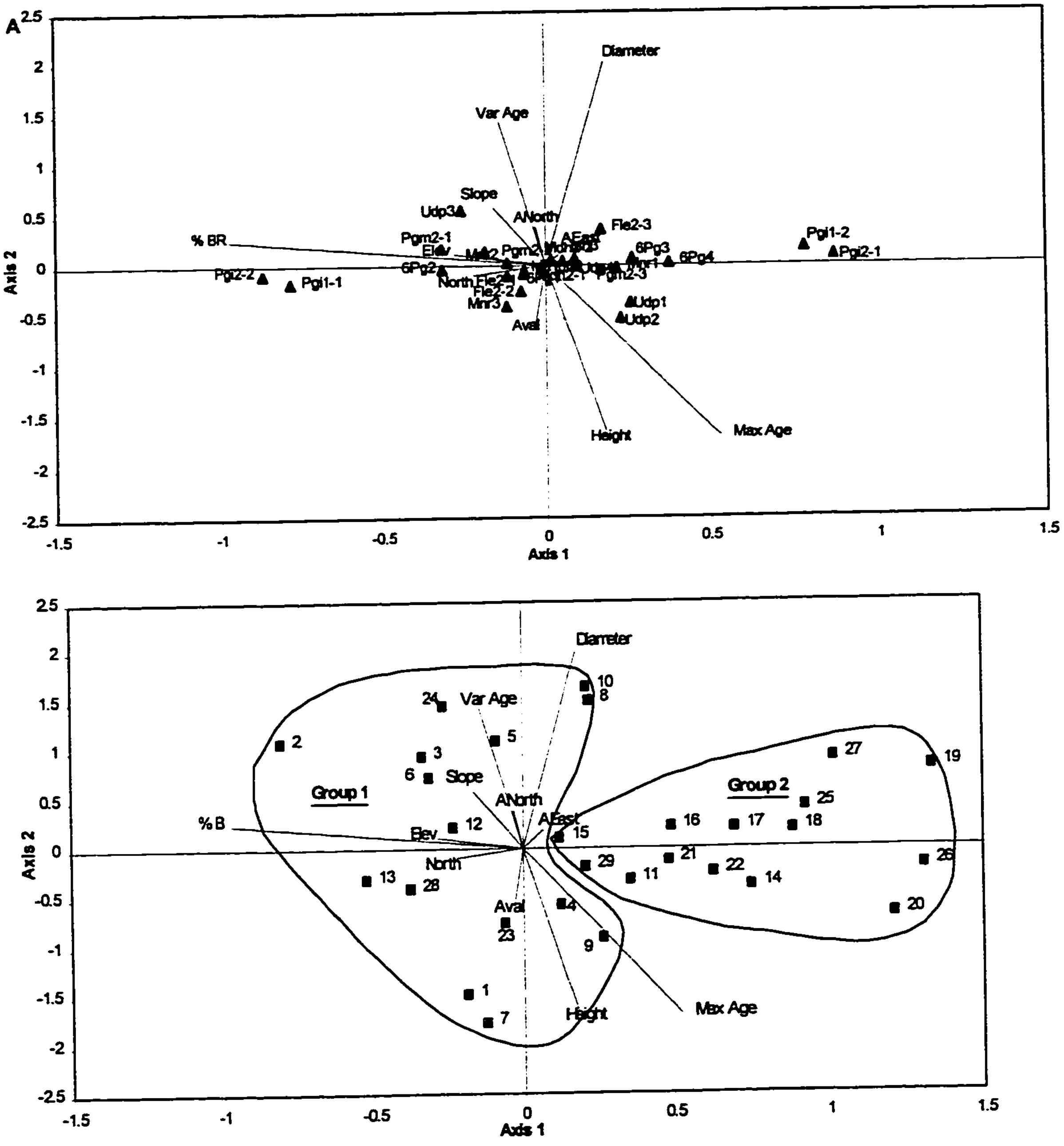

Figure III.4-2 First two principle axes from the redundancy analysis on 9 polymorphic loci with environmental scores and A) allele scores, B) site scores. The end of an environmental vector is associated with high values for that variable. The labels used to identify the environmental variables are listed in Table III.4-3.

second axis. The first axis in the RDA was significant (F=7.87, $p \leq 005$) and the environmental variables in the RDA over all axes were also significant (F = 1.86, $p \leq 001$, permutation Monte Carlo test, Ter Braak (1988)).

The *Pgi-1-1* and *Pgi-2-2* alleles separated with blister rust infection on the negative end of the first axis (Figure III.4-2A). Populations with high infection rates also separated on this side of the first axis whereas populations with low infection were positioned on the positive side of the first axis (Figure III.4-2B). The second axis was highly influenced by maximum age, along with diameter, height and variance in age. The site characteristic variables, slope, aspect and geographic location did not contribute much to the explanation of allelic variation, as compared to the demographic variables. The low variation explained by the second axis (10.87%) indicates that the differences in allele frequencies do not cause a significant separation of loci along this axis and that the separation of variables along this axis may not reflect significant relationships.

The RDA roughly separated populations into two groupings along the first axis (Figure III.4-2B). Populations in the first group are in the southern portion of the study area (except populations 23, 24 and 29, which are from the northern part of the study area but separated out with this group). Populations in this group generally had higher blister rust infection. The second group includes populations from the northern section of the study area that generally had lower blister rust infection. Because the second axis was not significant, only the separation on the first axis was used to determine the groupings.

Multiple Regression Analysis of Genetic Diversity

There was a high (negative) correlation between blister rust infection and the within population inbreeding coefficient, F_{IS} (r^2=0.94, Figure III.4-3). This reflects an excess of heterozygotes in populations with high infection. Most of the variation in the multiple regression analysis of F_{IS} was explained by blister rust infection (Table III.4-4). Based on the adjusted r^2, the multiple regression model that best explained the variation in F_{IS} included blister rust infection rate, average diameter and average height. Despite the high positive correlation between diameter and height (r^2 = 0.78), the multiple regression indicates that these variables correlate to F_{IS} in opposite ways when all other variables are held constant. Environmental and demographic

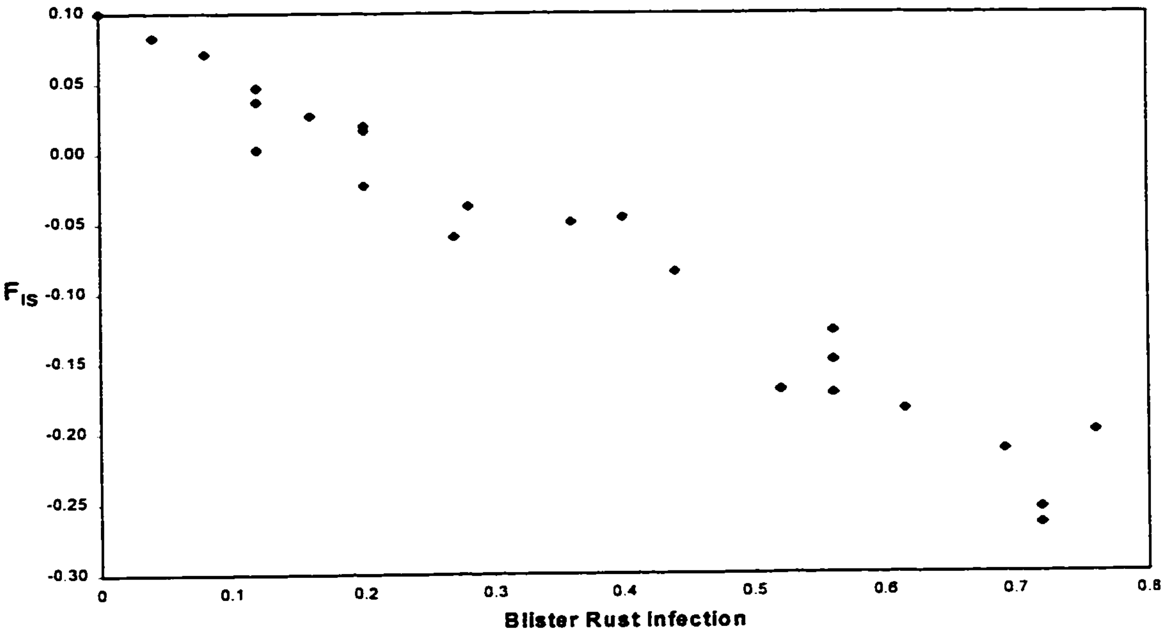

Figure III.4-3 Relationship between F_{IS} and the proportion of individuals infected with blister rust. Note that 5 populations had no infected individuals.

Table III.4-4 Multiple regression analysis of Wright's inbreeding coefficient, F_{IS}. DF; degrees of freedom.

Source	DF	Sum of squares	Mean Square	F Value	Adjusted r^2	Prob>F
Model	3	39.25719	13.18573	412.561	0.9778	0.0001
Error	22	0.79296	0.03172			

Variable	DF	Parameter Estimate	Standard Error	T for H_0: Parameter = 0	Prob > |T|
Intercept	1	1.09	0.09	12.020	0.0001
Infection Rate	1	-4.6	0.1	-34.078	0.0001
Diameter	1	-0.04	0.01	-3.672	0.0011
Height	1	0.12	0.04	2.769	0.0104

variables did not explain much of the variation in other genetic diversity measures. Models of heterozygosity had r^2s in the range of 0.40 to 0.52 and models of A and P had r^2s of only 0.13 and 0.21 respectively. Therefore, only the multiple regression of F_{IS} will be considered further.

III.5 Discussion

Genetic diversity was higher than expected (H_T = 0.224) based on the assumption that gene flow was low among isolated populations of whitebark pine in the Canadian Rockies. The comparatively low level of population differentiation, 6.2%, and moderate estimate of *Nm*, 3.77 migrants per year, also suggests that gene flow is relatively high among populations. Although whitebark pine has many of the life history traits the are generally associated with high genetic diversity, long-life span, large geographic range, outcrossing, and wind pollination, the small and isolated nature of whitebark pine populations suggested that gene flow, and consequently genetic diversity, would be low. The observed level of genetic diversity suggests that gene flow from both wind-dispersed pollen and bird mediated seed dispersal must be high enough to counteract the effects of isolation.

Also contrary to expectation, there was little correlation between genetic diversity and latitude, lending no support to the hypothesis of post-glacial migration northward through the Rockies. Seed dispersal by wind results in a predictable pattern of genetic relationship between source and progeny populations; newly founded populations must have been derived from neighbouring populations (Mitton 1995). However, with bird mediated seed dispersal, the pattern of relationship may be different; the genetic parent of a newly founded population does not necessarily have to be the closest source but could a mix of neighbouring populations. Thus, bird dispersal may obscure the evidence of founder effect.

The level of genetic diversity in whitebark pine populations in the Canadian Rockies (0.224) was much higher than the average of 0.16 found by Hamrick et al. (1992) in a survey of 93 different of pines. In a study of 14 whitebark pine in the southwestern United States, Yandell (1992) found genetic variation similar to that found in this study, 0.204 (Table III.5-1). In contrast, Jorgensen and Hamrick (1997), who sampled throughout the range of whitebark pine from the Sierra Nevada of California to two populations in the Alberta Rockies, found heterozygosity of only 0.102.

Table III.5-1 Comparison of genetic diversity (expected heterozygosity at the species level, H_{es}) and population differentiation (F_{ST}) among other pine species. S: Number of populations studied. Loci: The number of allozyme loci examined including monomorphic loci. '-': Information was not available.

	S	Loci	H_{es}	F_{ST}	Reference
Stone pines					
Pinus albicaulis	29	16	0.224	0.062	This study
P. albicaulis	30	20	0.102	0.034	Jorgensen and Hamrick 1997
P. albicaulis	14	13	0.204	0.088	Yandell 1992
P. koraiensis	3	16	-	0.041	Krutovskii et al. 1990b
P. pumila	3	16	-	0.025	Krutovskii et al. 1990b
P. pumila	5	22	0.255	0.056	Goncharenko et al. 1993a
P. pumila	18	19	0.271	0.170	Tani et al.1996
P. sibirica	9	19	0.159	0.022	Krutovskii et al. 1990a
P. sibirica	8	20	0.176	0.075	Goncharenko et al. 1993b
Other Pines					
P. resionosa	5	5	0	0	Fowler and Morris 1977
P. resionosa	7	23	0	0	Mosseler et al. 1991
P. Torreyana	2	59	0.008	1.000	Ledig and Conkle 1983
P. muricata	19	26	0.085	0.141	Millar 1983
P. clause	21	26	0.100	0.054	Parker and Hamrick 1996
P. washoensis	3	26	0.150	0.016	Niebling and Conkle 1990
P. rigida	11	21	0.152	0.023	Guries and Ledig 1982
P. banksiana	3	21	0.118	0.024	Dancik and Yeh 1983
P. banksiana	4	22	0.174	0.018	Gauthier et al. 1992
P. contorta	32	42	0.125	0.034	Wheeler and Guries 1982
P. contorta	5	21	0.187	0.018	Dancik and Yeh 1983
P. contorta	21	66	0.190	0.057	Yang and Yeh 1993
P. monticola	28	12	0.191	0.148	Steinhoff et al. 1983
P. flexilis	30	24	0.219	0.101	Jorgensen and Hamrick 1997
P. pungens	20	21	0.242	0.135	Gibson and Hamrick 1991
P. jeffreyi	14	20	0.248	0.138	Furnier and Adams 1986
P. sylvestris	18	21	0.286	0.033	Goncharenko et al. 1994
P. ponderosa	11	13	0.289	0.015	Hamrick et al. 1989
P. longaeva	5	14	0.380	0.038	Hiebert and Hamrick 1983

The low heterozygosity values of Jorgensen and Hamrick (1997) are puzzling because their estimates are roughly half of those obtained by Yandell (1992) or myself for sites that appear to be the same. For example, Yandell (1992) estimated expected heterozygosity at 0.208 for a stand from Mt. Rose in the Sierra Nevada whereas Jorgensen and Hamrick (1997) obtained an estimate of 0.120 for this same site. There was a similar discrepancy between estimates for two populations in the Cascade Range (Mt. Lassen and Mt. Shasta). For two sites in the Canadian

Rockies (Parkers Ridge and Mt. Edith Cavell), Jorgensen and Hamrick (1997) obtained heterozygosity estimates of 0.095 and 0.088 respectively compared to my result of 0.231 and 0.162 (sites 23 and 26). For three populations in the Rockies of Montana, Idaho and Wyoming, Yandell (1992) found heterozygosities similar in magnitude (average 0.216) to the values found in this study.

There are several possible explanations for the lower levels of heterozygosity found by Jorgensen and Hamrick (1997). Firstly, Jorgensen and Hamrick's (1997) are slightly lower because they did not use Nei's (1977) correction for heterozygosity. However, this correction would only increase their estimate by a few percent. Secondly, estimates of heterozygosity depend on the loci examined (Nei 1986) and because different loci were examined, heterozygosity estimates may not be comparable between studies. Thirdly, electrophoresis conditions differed, further complicating comparison between studies because electrophoresis conditions effect the number of alleles detected and thus heterozygosity estimates (Bonhomme and Selander 1978, Coyne et al. 1978). Under the electrophoretic conditions used by Jorgensen and Hamrick (1997), the common alleles may not have separated and thus were indistinguishable. Comparison of the proportion of polymorphic loci among studies suggests that this may be the case. Jorgensen and Hamrick (1997) found 25% of loci were polymorphic at the species level (frequency cut off of 95%) which is much lower than the 71% that gymnosperms have on average (Hamrick et al. 1992), the 46% found by Yandell (1992) and 56% in my populations.

Jorgensen and Hamrick (1997) did, however, find a high number of alleles at polymorphic loci compared to Yandell (1992), this study and other gymnosperms (Hamrick et al. 1992). What proportion of loci examined by Jorgensen and Hamrick (1997) were polymorphic depended on the frequency at which a locus was considered polymorphic. Changing the frequency limit from 95% to 99% dramatically increases the number of polymorphic loci in their study from 25% to 85% whereas there is only a difference of a few percent in Yandell's (1992) and this study. This suggests that they found, as Jorgensen and Hamrick (1997) point out, many alleles that were unique to specific populations. Although electrophoretic conditions may have contributed to the difference, the magnitude of the discrepancy in heterozygosity level is so large that it is unlikely to be the only reason.

A more likely factor causing this discrepancy is the definition of a stand, and consequently, the area over which samples were collected. A Wahlund effect could explain the low level of heterozygosity obtained by Jorgensen and Hamrick (1997) if sampled from a comparatively large sample area (although they did not state the size of area sampled). If individuals from two genetically distinct populations are pooled, the proportion of heterozygotes is lower than expected from mean allele frequencies; a condition referred to as the Wahlund effect (Liengsiri et al. 1990). Due to the nature of the landscape in which whitebark pine is found, where the potential for disturbance events such as avalanches or fire is frequent, boundaries between genetically distinct stands may not always be apparent. For example, differentiation between stands separated by an avalanche path would be possible if the two populations were founded from different sources. This type of fine scale genetic differentiation has been demonstrated in a number of wind-dispersed tree species (Mitton 1995). Furthermore, because individual whitebark pines within two populations were shown no more related than individuals within a population (Furnier et al. 1987), there is potential for large differences in heterozygosity between neighbouring stands and if they are pooled.

High heterozygosity has been found in other pines that also have discontinuous distributions. In Japan, *P. pumila* occurs above timberline in disjunct populations where mountainous terrain presents barriers to gene flow. However, heterozygosity levels there (Tani et al. 1996) are as high as in the more continuous portion of *P. pumila*'s range in Russia (Goncharenko et al. 1994, Table III.5-1). Japanese populations have much higher population differentiation compared to Russian populations of *P. pumila* and to other pines in general. This suggests that, although there is little gene flow among populations, other evolutionary forces, such as selection, and the ecological characteristics of *P. pumila* maintain high levels of heterozygosity within Japanese populations.

Another pine with high heterozygosity despite a discontinuous distribution is *P. longaeva*. Despite restriction to small isolated populations in mountainous areas, *P. longaeva*, with among the highest level of heterozygosity of any pine species (Table III.5-1), has very little variation among populations (Hiebert and Hamrick 1983). The lack of population differentiation may reflect a more continuous historical distribution of this species. As recently as the end of the last ice age (10-15 000 years ago), continuous populations of *P. longaeva* covered large portions of the unglaciated southwestern United States. As the climate changed, *P. longaeva* was left isolated in patches at higher elevations but the pattern of genetic diversity still reflects its previously continuous distribution. With increasing evolutionary time, inbreeding resulting from the lack of gene flow among populations is expected to decrease genetic variation within these populations and increase the differentiation among them (Hiebert and Hamrick 1983).

Sediment cores show that whitebark pine type pollen constituted a much higher proportion of the pine pollen present in the Canadian Rockies at that time then it does now suggesting that whitebark pine may have been more prevalent in the period directly after glaciers receded (Beaudoin 1986, Luckman and Kearney 1986, Reasoner and Hickman 1989). The ability of whitebark pine to colonize recently burned areas or newly uncovered glacial deposits relates to its dependence on Clark's nutcrackers and likely is the basis of its rapid colonization in the post-glacial period (Baker 1990). However, other species of pine and conifers were also present at that time. This evidence does not suggest there were continuous stands of whitebark pine covering the recently uncovered Canadian Rockies but may indicate more continuous distribution, and thus higher rates of gene flow at that time. Because of the long life span of whitebark pine, the high heterozygosity and low differentiation may simply reflect its previous more continuous distribution These populations may now be isolated and experiencing genetic drift that will eventually cause them to diverge, as has occurred in *P. longaeva*. If climate change causes the continued rise of timberline, whitebark pine populations may become further reduced and isolated, exacerbating any decline of the species genetic diversity.

A higher frequency of non-random mating (departure from Hardy-Weinberg equilibrium) was found here compared to other studies of whitebark pine and of other pines. The overall deficiency of heterozygotes with the majority of departures being deficiencies and lack of relationship between geographic location and the pattern of deficiencies was consistent with the findings of Yandell (1992) and Jorgensen and Hamrick (1997). Although most adult conifers show random mating (Hardy-Weinberg equilibrium) or have an excess of heterozygotes (Bush and Smouse 1992), heterozygote deficiencies have been found in other wind-dispersed pines (e.g. *Pinus rigida* (Guries and Ledig 1982), *P. banksiana* and *P. contorta* (Dancik and Yeh 1983)). Brown (1978) offered three possible explanations for this phenomenon that would apply to whitebark pine: inbreeding, Wahlund effect, and selection induced micro-scale differentiation.

Pines generally have relatively high levels of gene flow (Mitton 1995, Hamrick et al. 1992) as was found here for whitebark pine. Therefore, the first possible explanation, that inbreeding has reduced the number of heterozygotes, is unlikely the cause of heterozygote deficiencies. Because nutcracker caches are commonly from a single cone source, the multi-stemmed stand structure of highly related clumps that has evolved may have predisposed whitebark pine to high inbreeding (Furnier and Dancik 1990). Whitebark pine may therefore be able to tolerate higher levels of inbreeding than wind-dispersed pines because of this predisposition (Brussard 1990). Inbreeding results in a higher proportion of homozygotes and thus higher levels of heterozygote deficiencies might be expected in whitebark pine.

If chronic inbreeding has been present in the evolutionary history of whitebark pine, high heterozygosities as were found here would not be expected to be as high as was found in this study. Two other stone pine species that are also bird-dispersed, *P. sibirica* and *P. pumila*, were found to be in Hardy-Weinberg equilibrium or to have an excess of heterozygotes (Krutovskii et al. 1990b, Goncharenko et al. 1993a, 1993b). However, heterozygote deficiency was not expected with these species because their distributions are more continuous and they are less likely to be found in multi-stemmed forms (Politov and Krutovskii 1994). Furnier and Dancik (1990) suggested high inbreeding in whitebark pine based on unpublished progeny analysis of whitebark pine seeds but to date there are no data with which to directly assess the amount of inbreeding in whitebark pine and whether it is higher than in wind-dispersed pines.

A Wahlund effect is a second possible explanation for the observation of heterozygote deficiencies (Brown 1978). Inbreeding between related individuals closely spaced within a stand could result in genetic structure with a population. Differences in allele frequencies within a population of ponderosa pine have been found on the scale of tens of meters (Linhart et al. 1981). Parker and Hamrick (1996) suggest that heterozygote deficiency in populations of *P. clausa* could be explained by the patchy distribution of individuals on that landscape after regeneration and survival of fire. It is possible that this effect occurs frequently and goes undetected in studies of genetic structure in plants, especially in trees where stature and geographic range may cause overestimation of population size (Bush and Smouse 1992). Bird mediated seed dispersal results in seeds travelling further on average from the parent source and thus makes this scenario less likely for whitebark pine. However, as discussed in conjunction with sampling scale, the possibility of population differentiation on a small scale may arise because of disturbances such as fire or avalanches. More detailed studies of how genetic variation changes across the landscape within a single area are needed before this explanation can be validated.

The third possible cause of heterozygote deficiency discussed by Brown (1978) is that micro-scale genetic differentiation has occurred within populations in response to heterogeneous selection pressures on loci linked to allozyme loci. This type of variation has been reported in other pines (e.g. Mitton et al. 1977, Linhart et al. 1981, Dancik and Yeh 1983, Lagercrantz and Ryman 1990). Environmental heterogeneity, especially in the mountains, can occur on a very small scale; factors important to seedling establishment and growth, such as snow depth, can vary substantially over the scale of metres (Ellstrand 1992). With the high amount of genetic variation found in pines, it seems possible that selection, acting at this level, could cause genetic structure on a small scale.

The multiple regression analysis points to another possible explanation for the observation of heterozygote deficiencies. Blister rust infection rate was the most important factor in determining the extent of departure from Hardy-Weinberg equilibrium. There are two possible hypotheses: firstly, increased inbreeding may have allowed for the expression of rare alleles or unique genotypes that express resistance against blister rust infection. Rare alleles are often recessive but inbreeding may allow for their expression. The possible negative effects of inbreeding, such as decreased fitness, may be offset by the increase in rust resistance.

The second possible hypothesis is that mortality caused by the rust has selected against homozygotes. As discussed in Chapter I, the basis for predicting the evolutionary success of organisms based on genetic diversity is the assumption that heterozygosity relates to fitness. The level of heterozygosity of an individual and therefore its fitness may be related to its ability to withstand or be resistant to blister rust infection. Mortality of homozygous individuals would result in a population with an excess of heterozygotes and thus a reduced inbreeding coefficient (F_{IS}). This hypothesis is supported by the observation of high mortality in heavily infected populations. Although mortality was not measured at all sites, an estimate of 90% mortality due to blister rust was calculated at sites in Waterton Lakes National Park where blister rust infection rate was high (sites 1 and 2). The high rate of mortality in heavily infected populations suggests that selection, resulting from blister rust mortality, has reduced the number of homozygotes and thus altered the genetic structure of these stands. Thus, the pattern of genetic diversity present in whitebark pine may no longer reflect historical patterns of gene flow and migration but the degree of mortality and selection caused by blister rust infection.

Mountain pine beetle infection, which was noted in areas of Waterton Lakes National Park, may also be contributing to the change in genetic structure of whitebark pine populations in southern Alberta. Selection of more heterozygous whitebark pine may be occurring as a result of pine beetle infection and mortality. How the presence of pine beetles and blister rust interact to determine mortality has not been investigated although studies in Montana and Idaho suggest that the combination of these factors is responsible for the high mortality observed (Keane and Arno 1993). Whether pine beetles have been a significant factor in whitebark pine decline needs to be further investigated before a complete picture of the future survival of whitebark pine can be determined.

The lack of relationship between genetic and geographic distances is a consistent finding among the three studies of genetic variation in whitebark pine (Yandell 1992, Jorgensen and Hamrick 1997, this study). Two possible explanations would account for the absence of a strong correlation between genetic and geographic distances and the discrepancy between phenograms and physical geographic locations (Figure III.4-1). Firstly, nutcracker seed dispersal results in a non-linear process of population founding because more than one population can act as the seed source for a new population. The second possibility is that mortality from blister rust has changed the allele frequencies so that they no longer reflect their genetic origins. Evidence of a high correlation between genetic and geographic distance in *P. pumila*, another bird-dispersed pine in Japan (Tani et al. 1996), supports the second scenario. Because blister rust is thought to have evolved on pines in eastern Asia (Millar and Kinloch 1991), *P. pumila* may have evolved a natural level of resistance and thus its genetic structure does not reflect blister rust mortality. These observations support the possibility that blister rust mortality has changed the genetic structure of whitebark pine populations in the Canadian Rockies and consequently masked post-glacial migration relationships among populations.

Allelic richness is thought to decline with founding events although the level of heterozygosity may not be affected (Ellstrand and Elam 1993). Because rare alleles contribute relatively little to total heterozygosity, their loss through founding events and drift has little affect on heterozygosity (Nei et al. 1975). Cwynar and Macdonald (1987) supported this theory with the observation that the time since founding in populations of *P. contorta* correlated with the allelic diversity but not heterozygosity. Based on this assumption, Jorgensen and Hamrick (1997) expected that whitebark pine populations in areas that were glaciated during the last ice-age, and thus have been founded more recently than populations in unglaciated areas, should have less allelic diversity. However, no difference was found in the average number of allele or heterozygosity between the two groups of populations. Similarly, the average number of alleles in populations examined here was not well correlated with latitude (r = 0.18) or the level of blister rust infection. This lends further support to the conclusion that, because of nutcracker seed dispersal, the founding of populations is not linear. It is therefore unlikely that populations of whitebark pine in the Canadian Rockies were founded from a single unique source.

Although the most likely movement of seed and pollen in the Rockies would be in a linear north-south direction, the situation seems to be much more complex. Seed and pollen dispersal across the Rocky Mountain trench is likely severely limited. Nutcrackers can, however, transport seeds in excess of 20 km (Vander Wall and Blada 1977) making it possible that some populations in the Canadian Rockies were founded from seed sources in the Columbia Mountains. The use of more sensitive molecular techniques, such as micro-satellites, might help elucidate the pattern of gene flow among populations and aid in reconstructing the evolutionary history of whitebark pine. However, an understanding of the magnitude of genetic variation and the extent of gene flow, the knowledge provided by this study, are the most important considerations for conservation and management decisions.

Although latitude did not correlate well with expected heterozygosity, some patterns in the level of heterozygosity are worth noting (Figure III.5-1). Although the variation is high, there is a general trend of decreasing heterozygosity over latitude for most populations, with the exception of a few southern sites that have low heterozygosity (sites 1, 2 and 3). A rough estimate at these sites set mortality at approximately 90% where some of the highest rates of blister rust infection were observed. I hypothesize that blister rust mortality has been selective

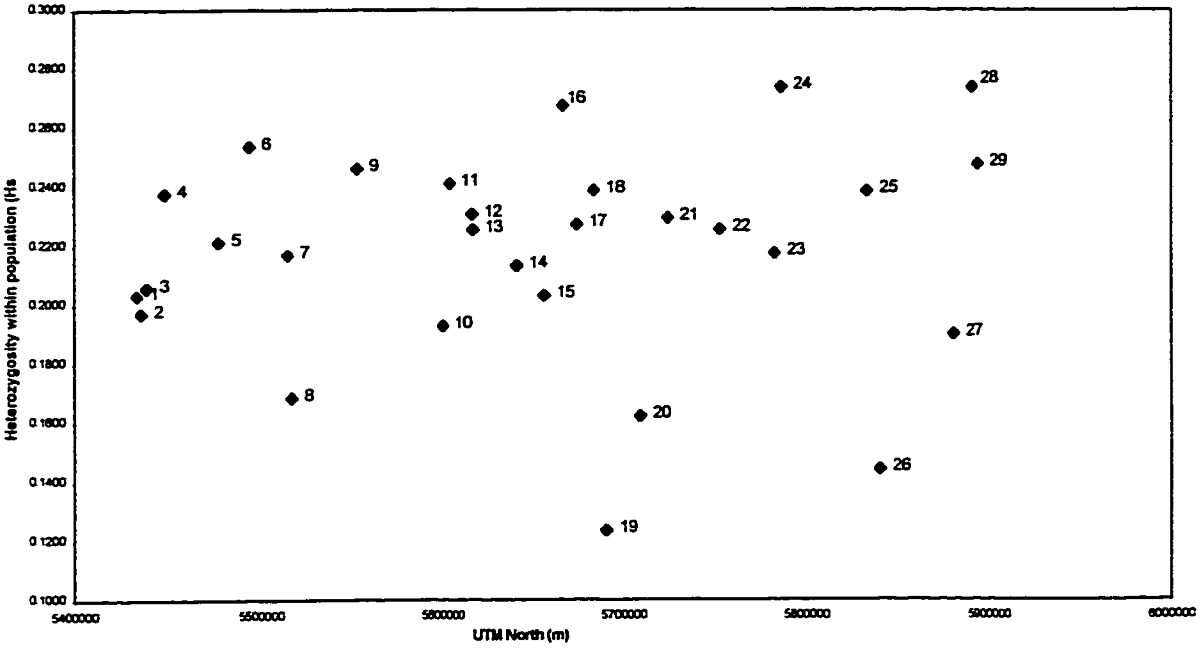

Figure III.5-1 Heterozygosity within each population averaged across 9 polymorphic loci plotted against latitude. Site numbers correspond to those in Table II.4-1.

for homozygotes so that genotypic frequencies have become skewed to a much higher proportion of heterozygotes than expected under Hardy-Weinberg equilibrium (thus a negative F_{IS}). Observed heterozygosity would increase consequently but expected heterozygosity may actually decline. Some sites had a high infection rate but still seemed to have relatively high expected heterozygosity (e.g. site 6). This may be a reflection of a relatively recent northward spread of blister rust through the Rockies. With the high rate of infection at site 6, mortality would eventually be expected to reach the same level as in sites 1 to 3. If my hypothesis is correct, homozygotes would be purged from the population, and expected heterozygosity would decline.

Expected heterozygosity was negatively correlated with maximum age ($r=-0.57$, $p \leq 0.001$) and may contribute to the explanation of the pattern of heterozygosity. Sites 19 and 20 have among the oldest individuals in the study, over 500 and 600 years respectively, and the lowest levels of expected heterozygosity (Figure III.5-1). Site 28, on the other hand, had the lowest maximum age and among the highest levels of expected heterozygosity (Figure III.5-1). Nutcracker dispersal, may contribute to a large number of genotypes being present in younger stands but selection likely reduces this over time. If this is true, management, such as prescribed burns, designed to increase regeneration could contribute to the maintenance of genetic diversity in populations.

The ordination (RDA) also supports the relationship between blister rust infection and genetic diversity (Figure III.4-2). The correlation of the two *Pgi* loci with high levels of blister rust infection suggests that there may be a link between these alleles and susceptibility to rust that may be worth investigating. Allelic diversity across all loci in general may be important in the ability of whitebark pine to evolve resistance to white pine blister rust (Hoff and Hagle 1990).

Different strains of blister rust have been found in Western North America and resistance for one strain may not predict resistance for another (Hoff et al. 1994). Furthermore, although the level of population differentiation was not as high as was expected based on population isolate, it is still significant and suggests that if resistant stock are developed from one source area they may not be transplanted successfully over large geographic distances. Differentiation among whitebark pine populations may simply be due to drift and migration and thus not reflective of adaptive variation. However, allozyme diversity may be reflective of adaptive variation (Mitton 1995). Therefore, caution should be taken in the collection and transfer of genetic resources from different geographic locations.

Genetic diversity was also related to characteristics such as diameter and height of trees within stands (Table III.4-4). The significant contribution of average diameter and height to the multiple regression of F_{IS} (Table III.4-4) may indicate a dependence of genetic diversity on the way a stand develops. The influx of seeds dispersed by nutcrackers from a variety of source stands may result in high genetic diversity after a disturbance. As this stand matures and competition reduces the number of individuals, genetic diversity may also decline. Support for this hypothesis is found in the significant correlation between maximum age and expected heterozygosity (r=-0.59, $p \leq 0001$), suggesting that young stands may have greater genetic variation. Therefore, factors that affect the demographics of a stand, such as disturbances and subsequent regeneration, may contribute to the level of genetic variation.

Information on the distribution of genetic diversity among and within populations is essential for designing conservation strategies to conserve genetic diversity (Lande and Barrowclough 1987). For species with high diversity among populations, we require a conservation strategy to protect as many populations as possible (Falk and Holsinger 1991) because this may indicate that local populations have adapted to different conditions. In the future, if *ex situ* conservation programs are necessary for whitebark pine, sampling should include individuals from across the species range to ensure that ecologically relevant variation is preserved (Soule 1986). Species with a high portion of genetic variation within populations and little among populations may not require the protection of as many populations but for these, gene flow among populations may be important in maintaining genetic diversity (Ellstrand 1992). In this case, the protection of contiguous populations may be required. Conserving only a few populations could result in the loss of variation important for future adaptive potential and survival of the species. Although population differentiation in whitebark pine is not as high as would be predicted based on population structure, there is still a substantial proportion of genetic diversity contained among populations. The moderate level of gene flow may have been important in maintaining genetic diversity, and suppressing population differentiation in whitebark pine. The ecological characteristics of whitebark pine suggest a high potential for gene flow as long as populations are not isolated from one another beyond the maximum distance that nutcrackers travel or pollen is carried by wind. The decline in numbers of whitebark pine brought about by white pine blister rust and fire suppression as well as changes in treeline resulting from future climate change could increase the population isolation. Future management decisions should thus address the requirement for maintenance of gene flow.

III.6 Summary and Conclusions

The level of genetic diversity in whitebark pine populations in the Canadian Rockies was higher than was expected and the differentiation among populations lower than expected based on the isolated nature of these populations. Although there is a significant portion of genetic diversity found among populations (6.2%), gene flow may be high enough to counteract the effects of genetic drift and isolation. The lack of relationship between genetic and geographic distance may reflect the unique ecological features of whitebark pine, nutcracker seed dispersal and resulting multi-stemmed stand structure. Bird dispersal may result in populations with more than one parent source and consequently, there is no evidence for the post-glacial migration of whitebark pine from southern refugia. Blister rust infection and mortality as well as demographic factors contribute to the explanation of the pattern and amount of genetic diversity. Redundancy analysis and multiple regression emphasize the link between genetic diversity and blister rust infection. The close relationship between $\bar{F}_{IS}$ and the level of blister rust infection suggest that the rust has selectively removed homozygotes and that the genetic structure of whitebark pine has been effected by this epidemic. This change may have obscured the relationship between distance and genetic diversity and the pattern of post-glacial migration. Although selection associated with blister rust mortality may be temporarily increasing heterozygosity in individual populations by removing homozygotes, genetic diversity will likely eventually decline over time. Management programs designed to increase regeneration, such as prescribed burns, may help maintain genetic diversity while increasing the chance for recombination and selection to produce resistant individuals.

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Chapter IV

IV Conclusions, Recommendations and Future Research

IV.1 Conclusions

The findings of this study can be summarized as follows. Firstly, the conclusions from Chapter 2 are that whitebark pine stands are generally found on steep southwest facing slopes in the Canadian Rockies. Furthermore, stands in the southern part of this area have a high proportion of infection and have experienced heavy mortality due to white pine blister rust. The proportion of whitebark pine infected with blister rust declines northward through the Canadian Rockies. However, the potential for high infection does not appear to be limited by climatic or stand conditions and presents the possibility of high mortality in northern areas.

The conclusions from Chapter 3 concerning the genetic diversity and structure of whitebark pine stands can be summarized as follows: a higher level of genetic diversity was found in whitebark pine populations in the Canadian Rockies than was expected based on the comparison of the life history, ecological characteristics and genetic diversity levels with other pine species. Furthermore, the level of differentiation among populations was similar to that of other more widespread conifer species. Two explanations could account for this pattern. First, whitebark pine may have been historically more widespread in its distribution which would have allowed for high levels of gene flow. Secondly, the mechanisms involved in pollen and seed transfer are effective enough to maintain a higher level of gene flow than was expected simply based on the isolated nature of whitebark pine populations. Furthermore, selective mortality from blister rust has removed the more homozygous individuals from heavily infected populations and changed the genetic structure of these stands.

Increasing habitat destruction and fragmentation continues to put pressure on species sensitive to extinction. Without effective conservation programs global biodiversity will continue to decline. The survival and persistence of species is dependent on availability of genetic variation. Therefore, being able to assess genetic variation and understand the factors responsible for its level and distribution within species, such as this study has provided for whitebark pine in the Canadian Rockies, is important in the design and implementation of effective conservation programs. Based on the information gained from this study, the next section will outline recommendations for the management and conservation of whitebark pine in the Canadian Rockies that will help ensure this species remains an integral part of the subalpine ecosystem. The final section in this chapter will discuss the questions that still remain to be answered concerning the biology of whitebark pine and new questions that have arisen as a result of this study.

IV.2 Recommendations for Management and Conservation

1. Disturbances such as fire play an important role in whitebark pine regeneration as indicated by periodic regeneration found at many sites. Thus, fire could be used as a potential tool to increase regeneration where blister rust and successional replacement have resulted in decline. However, continuous regeneration was observed at some sites. Therefore, the specific regeneration pattern at a site in the past does not necessarily constrain how it would or could regenerate in the future. Thus, the use of fire as a management tool must be based no only on the consideration of the potential for increased regeneration, but also on site specific characteristics that might suggest the intensity or patchiness of the burn. The age distribution pattern and the site characteristics should be considered in order to determine the best use of fire at a specific site.

2. Because this is the first estimation of blister rust infection in the Rockies, whether epidemic levels of infection are spreading or the present level reflects a balance between infection and factors that limit the spread of blister rust cannot be determined. Therefore, continued monitoring of infection levels is essential to determining if the scenario of high infection throughout the area can be expected.

The importance of *Ribes* to the life cycle of blister rust suggests that a suitable method to control its spread would be to eliminate *Ribes* shrubs. This approach was taken in the United States but most of these attempts failed due to the long period of *Ribes* seed dormancy and the ability of *Ribes* to regenerate from suckers (Hunt 1983, Liebhold et al. 1995). Not only is this solution too costly and time consuming to be an effective method of control but, because National Park Policy places the protection of ecological integrity as a first priority, *Ribes* elimination is not an available option. Other methods of control such as pruning and excision of cankers has been used with some success in plantations of western white pine (Hagle et al. 1989, Hunt 1991). These methods are not only ecological intrusive but also labour and cost intensive and may therefore not be feasible in the National Parks. More ecologically sound methods of controlling the level of infection may be found by returning the cycle of disturbances such as fire to its natural regime.

3. In order to maintain genetic diversity in whitebark pine, and thus the potential for future evolutionary change, efforts must be taken to ensure that continued gene flow is maintained. As our national parks become remnant islands where managers attempt to maintain functioning ecosystems, the issue of gene flow among populations will become more important. Therefore, attempts must be made to ensure that whitebark pine populations on provincial lands survive and flourish so that minimum levels of gene flow are maintained throughout the Rockies.

4. Blister rust may be selecting for more heterozygous individuals by removing the more homozygous individuals from the population. The overall level of genetic diversity may also be declining due to blister rust but only future monitoring will be able to determine what the effects of blister rust infection have been. If this level of selection continues, allelic frequencies will be affected and genetic diversity could decline. There are two options to prevent extinction of whitebark pine in areas that presently have high mortality and to aid regeneration at sites where it has declined as a result of blister rust. *Ex situ* breeding programs could be used to develop resistant individuals for introduction into high mortality areas. Resistance breeding programs have been the major thrust of blister rust control in plantation species such as western white pine over the last 20 years and could be used as the basis for breeding programs for whitebark pine (McDonald et al. 1994). Using resistantly breed trees creates a population with reduced genetic variation and increases selection pressure for virulence in disease species (Hagle et al. 1989). Therefore, resistant individuals must be used sparingly in order to be an effective control. Whitebark pine has shown some natural resistance to blister rust (McDonald 1992, Hoff and McDonald 1993) and breeding programs would probably produce resistant stock. However, this solution would decrease genetic diversity, increase selection pressure

(McDonald et al. 1981) as well as leaving the population open to decline through other factors such as mountain pine beetle (*Dendroctonus ponderosae*) or successional replacement (Arno and Hoff 1989).

Secondly, the process of natural selection could be aided by increasing regeneration through management initiatives such as prescribed burns. The expression of rare alleles and unique mutations often requires sexual recombination. By increasing regeneration, unique combinations of genes conferring different mechanisms of resistance may be produced. The use of fire may increase the potential of germination and present opportunities for selection. Successional replacement may be reducing the number of genotypes that natural selection can act upon not only by reducing the number of individuals that are present in a stand but by reducing the opportunities for regeneration.

IV.3 Future Research

To ensure the continued survival of whitebark pine in the subalpine ecosystem the following three areas must be priorities for future research:

1. In order to determine if high mortality from blister rust can be expected not only in the southern areas but also throughout the Rockies, monitoring of blister rust levels must be initiated. This study has established base line levels with which future levels can be compared. Furthermore, a more detailed picture of the pattern of blister rust infection may reveal climatic or physical factors that determine the probability of infection. Permanent plots should be set up that can easily be revisited and where detailed data on climate and micro-site factors can be collected. They type of data that should be collected are, for example, relative humidity and temperature at a time scale of hours or minutes. The transfer of blister rust from *Ribes* to pines requires specific humidity and temperature conditions (Van Arsdal 1965) and understanding what these conditions are in the Canadian Rockies will help predict the potential for epidemic levels of infection in whitebark pine populations in the northern Rockies and other areas. The number of cankers per tree was used here as an indication of infection intensity but determining the extent of defoliation at the individual level would give a more accurate picture of the effects of rust and aid in the indication of disease spread.

2. Although whitebark pine has been shown to have a comparatively high level of genetic diversity, the relationship between blister rust infection and the pattern of genotypes in whitebark pine stands indicates that there is potential for decline in genetic diversity. Because this study is the first to examine the level of genetic diversity in whitebark pine in the Canadian Rockies, I cannot determine whether genetic diversity has been eroded in whitebark pine as a result of blister rust. Furthermore, a pattern of post-glacial migration could not be detected in these populations but may be present. More sophisticated molecular techniques could be used to determine this pattern so that it could be removed from the determination of what the effects of blister rust have been on the level of genetic diversity in whitebark pine.

3. Although the importance of disturbances such as fire in whitebark pine regeneration are indicated by the age distribution patterns at many stands, the mapping of whitebark pine stands in much greater detail, including micro-topographical and micro-climate differences would enable the determination of the factors important in the regeneration process. Further study on relationship between disturbances and regeneration would increase the understanding of the process of regeneration, which is essential to maintaining viable populations of whitebark pine. Furthermore, understanding how the population dynamics of Clark's nutcrackers determines the level of whitebark pine regeneration is required because of the essential role that the birds play in seed dispersal.

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Appendix A: Age Distributions from 29 Whitebark Pine stands Throughout the Canadian Rockies

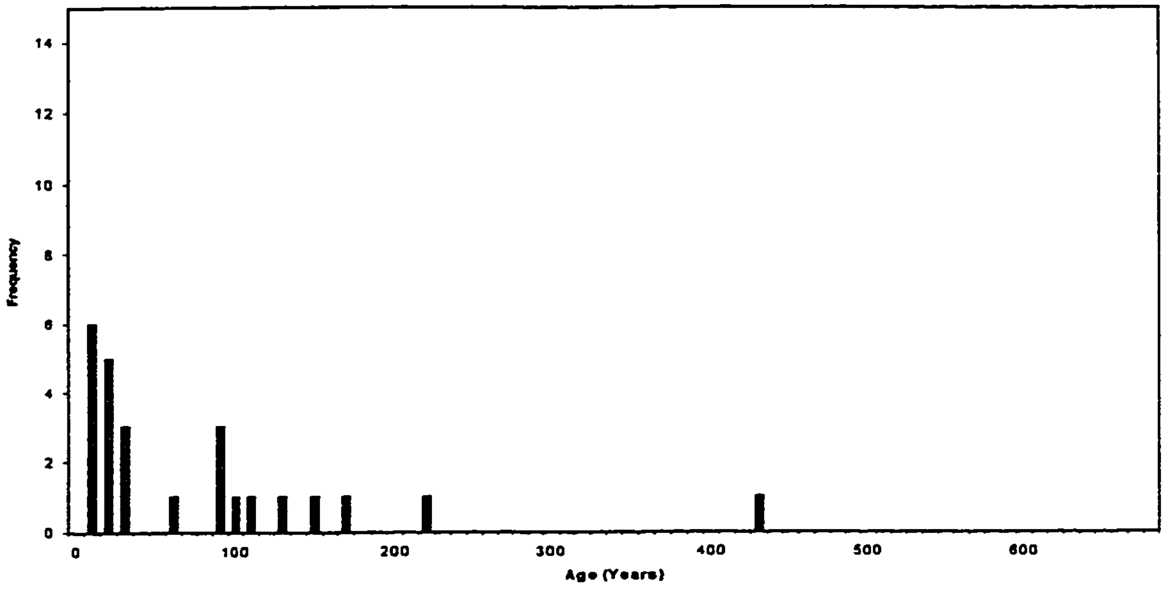

Figure 1 Age distribution at site 1, Summit, Waterton Lakes National Park, AB

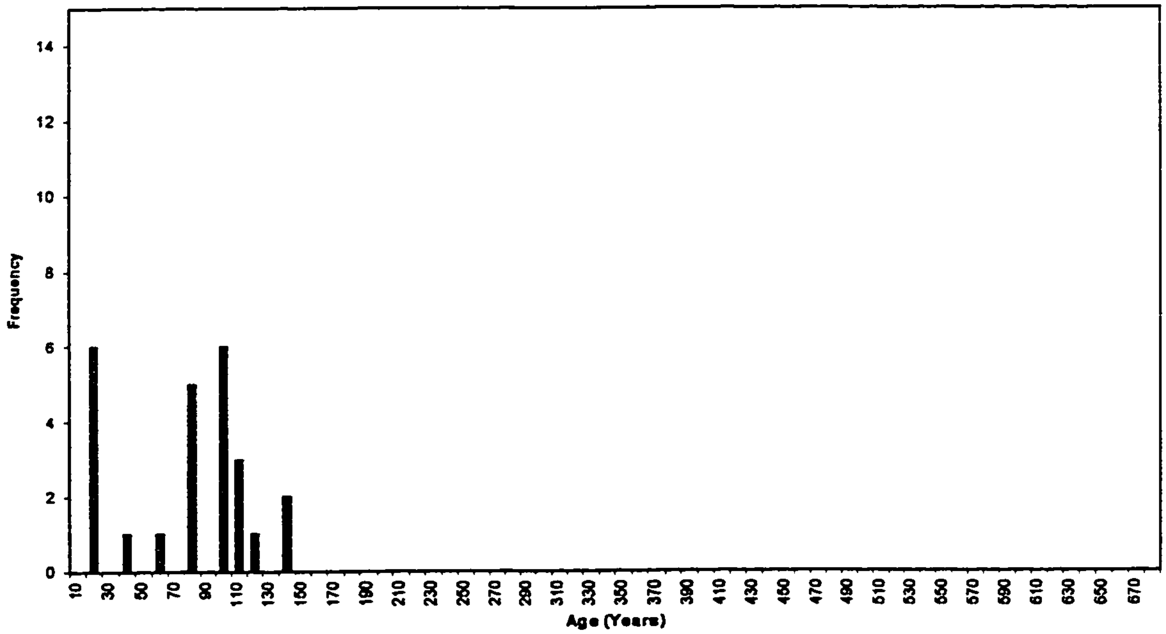

Figure 2 Age distribution at site 2, Rowe, Cranbrook Forest District, BC

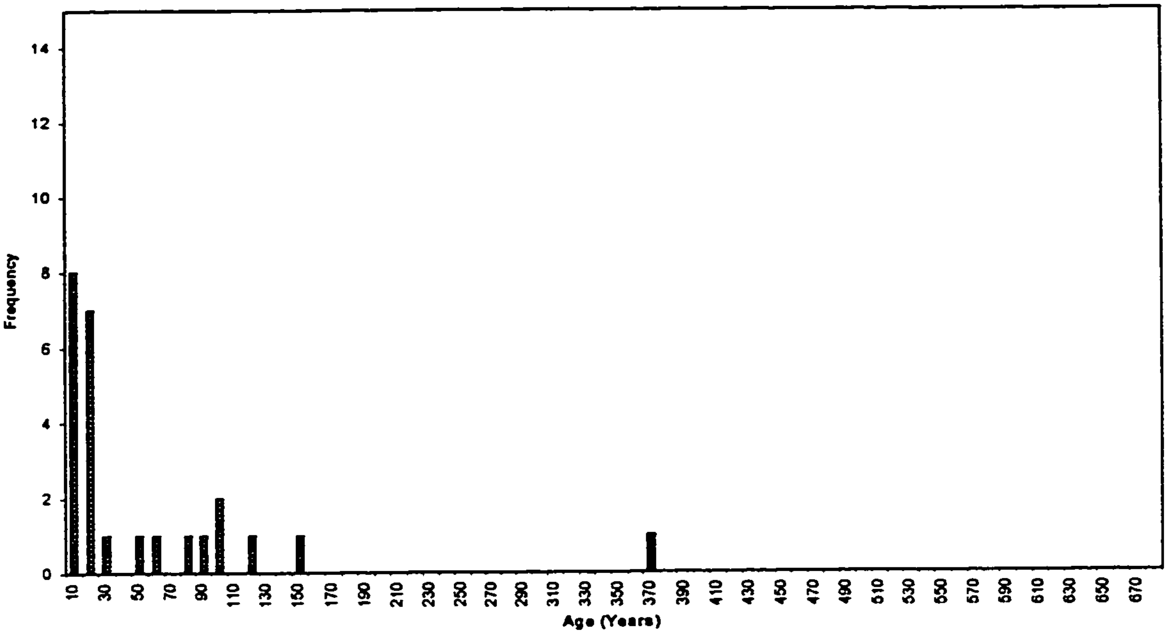

Figure 3 Age distribution at site 3, Cabin, Cranbrook Forest District, BC

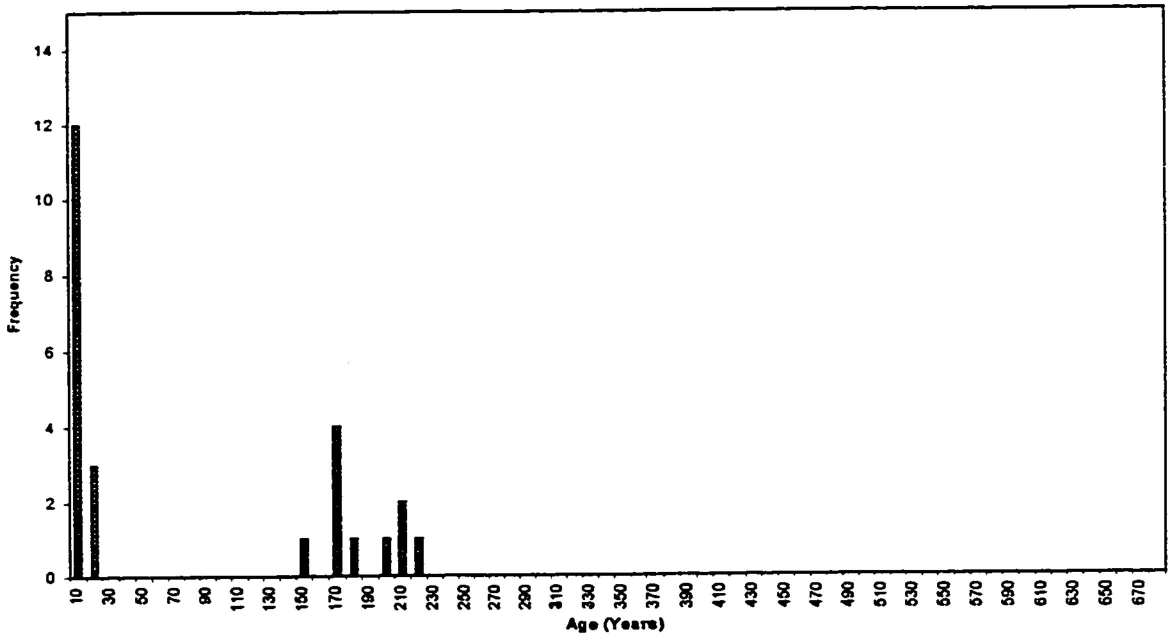

Figure 4 Age distribution at site 4, Lost, Waterton Lakes National Park, AB

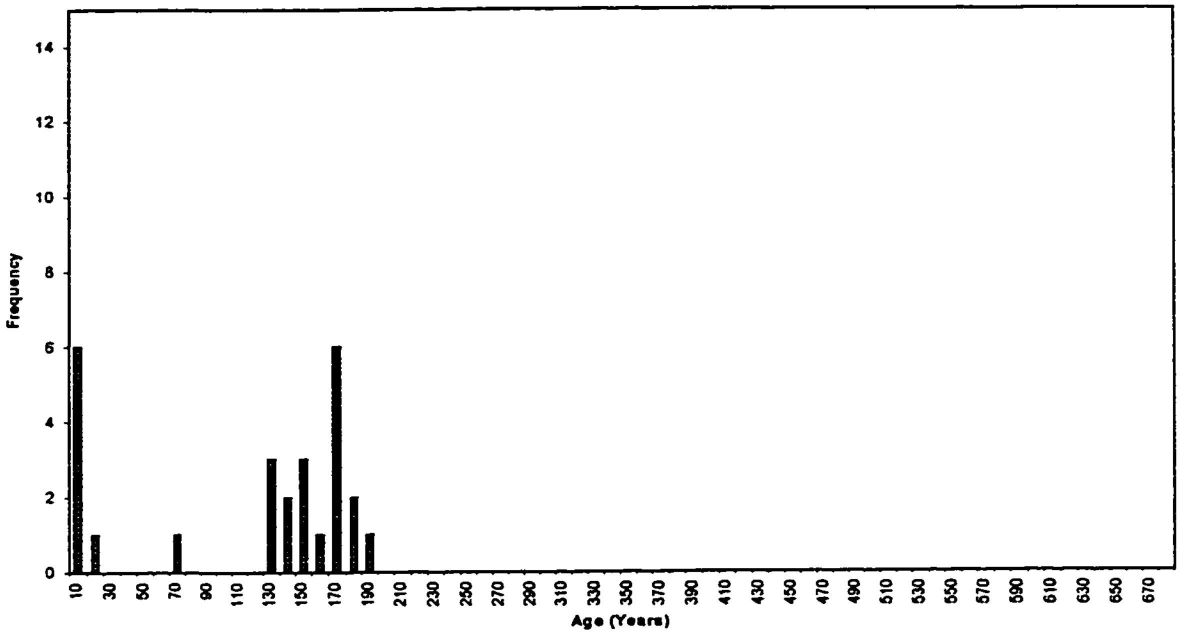

Figure 5 Age distribution at site 5, Centre, Cranbrook Forest District, BC

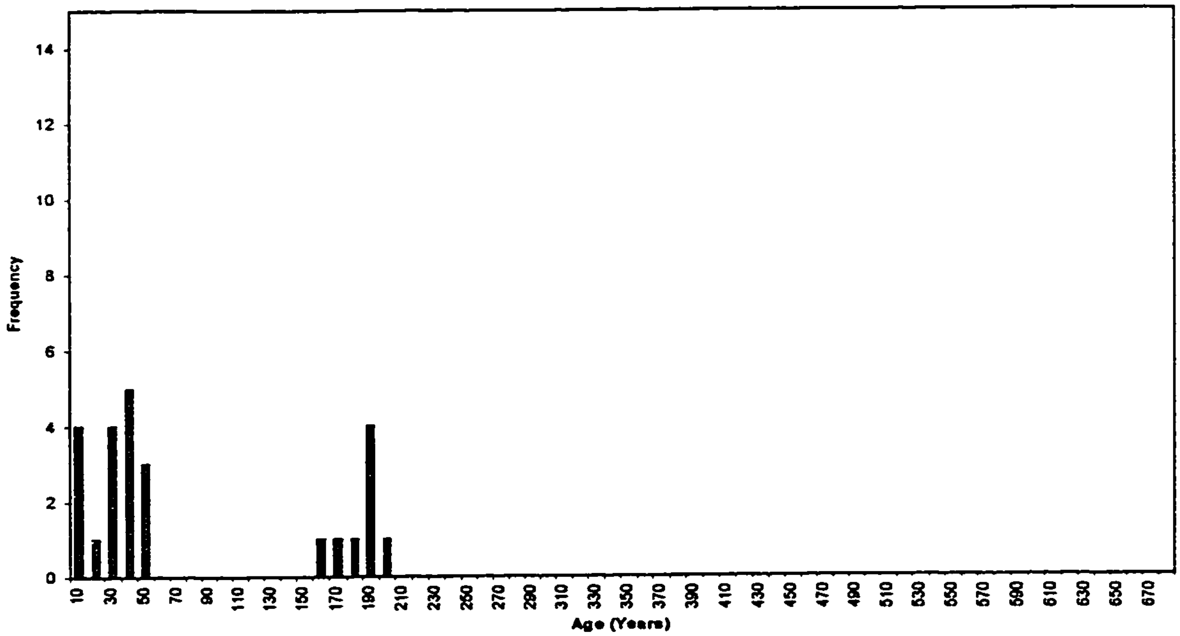

Figure 6 Age distribution at site 6, Ironstone, SW Alberta Forest District, AB

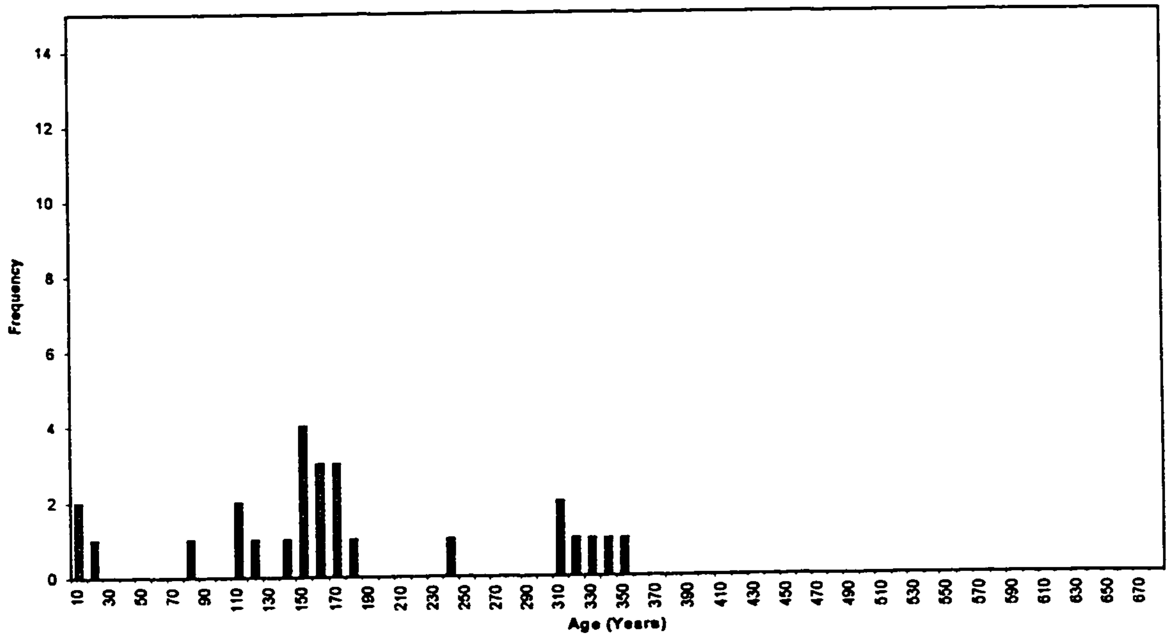

Figure 7 Age distribution at site 7, Vicary, SW Alberta Forest District, AB

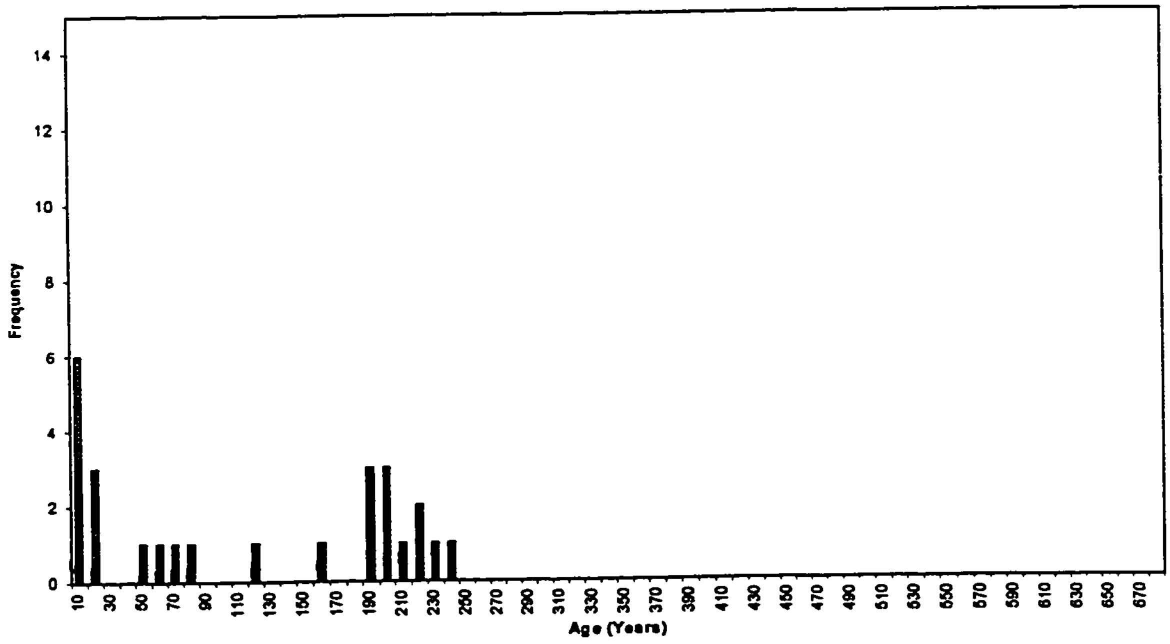

Figure 8 Age distribution at site 8, Wildhorse, Cranbrook Forest District, BC

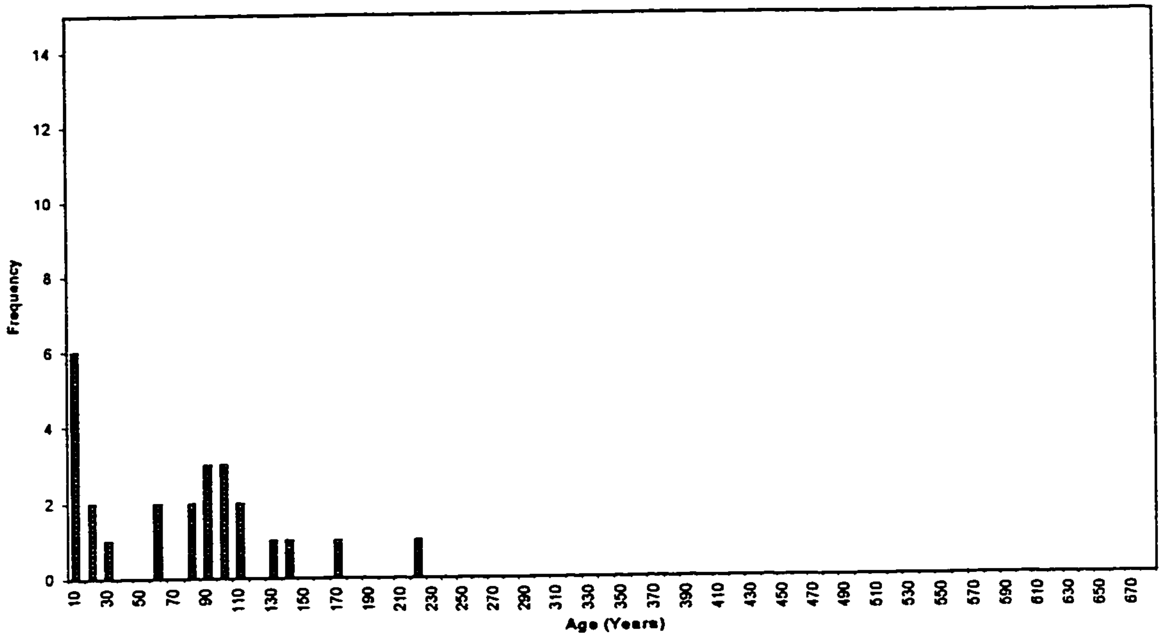

Figure 9 Age distribution at site 9, Coat, SW Alberta Forest District, AB

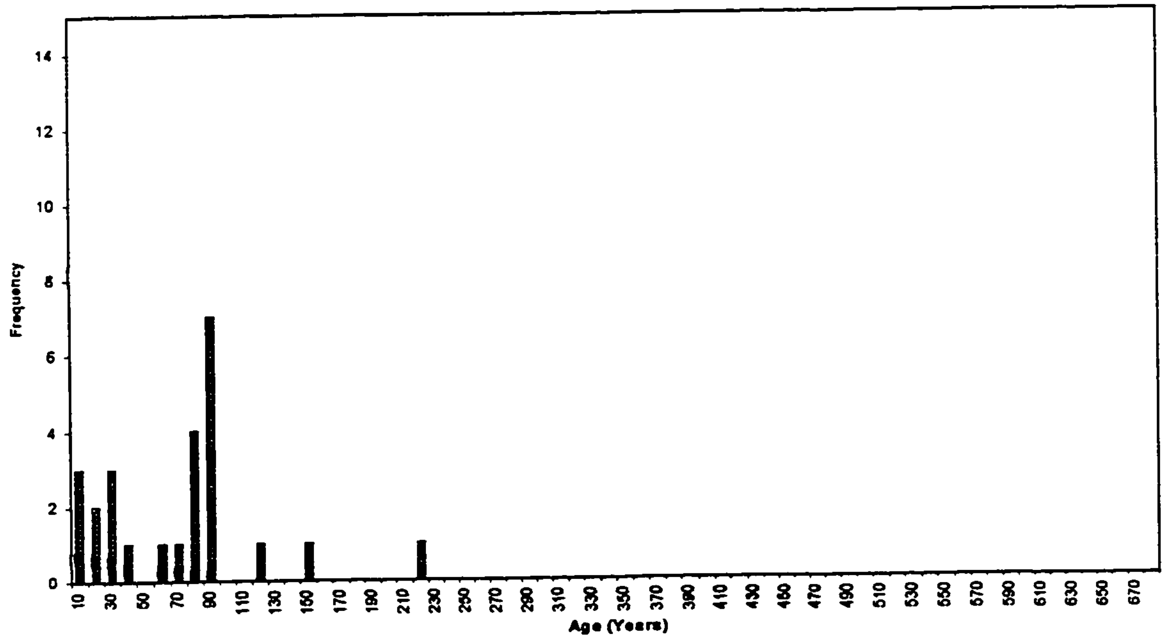

Figure 10 Age distribution at site 10, Highwood, Kananaskis Country, AB

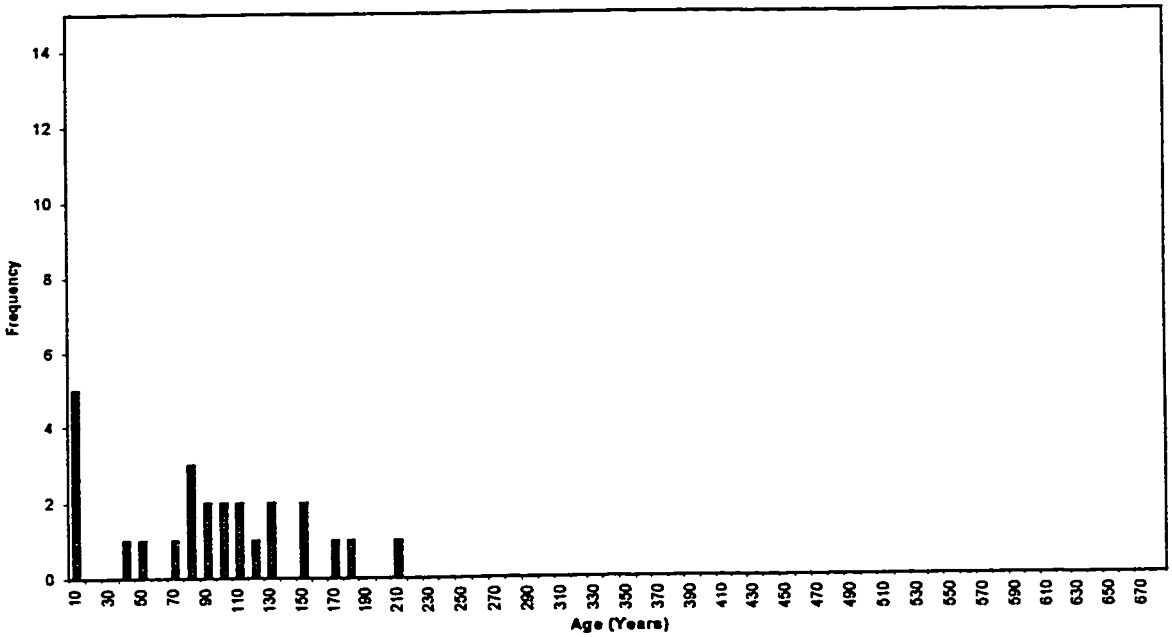

Figure 11 Age distribution at site 11, Joffre, Invermere Forest District, BC

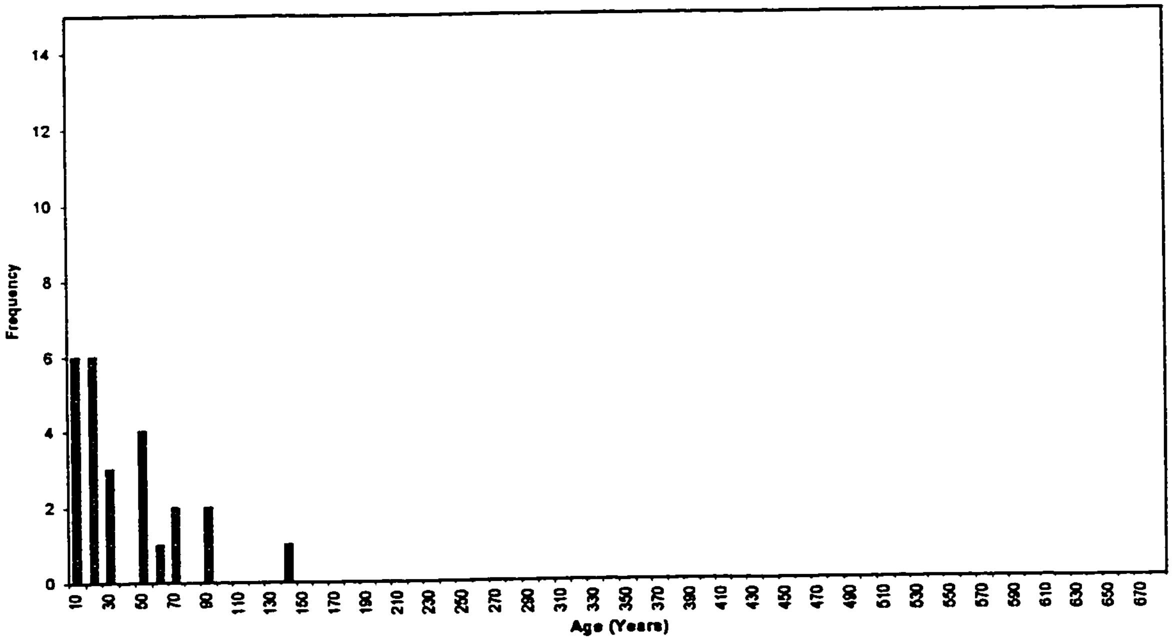

Figure 12 Age distribution at site 12, Kindersley, Kootenay National Park, BC

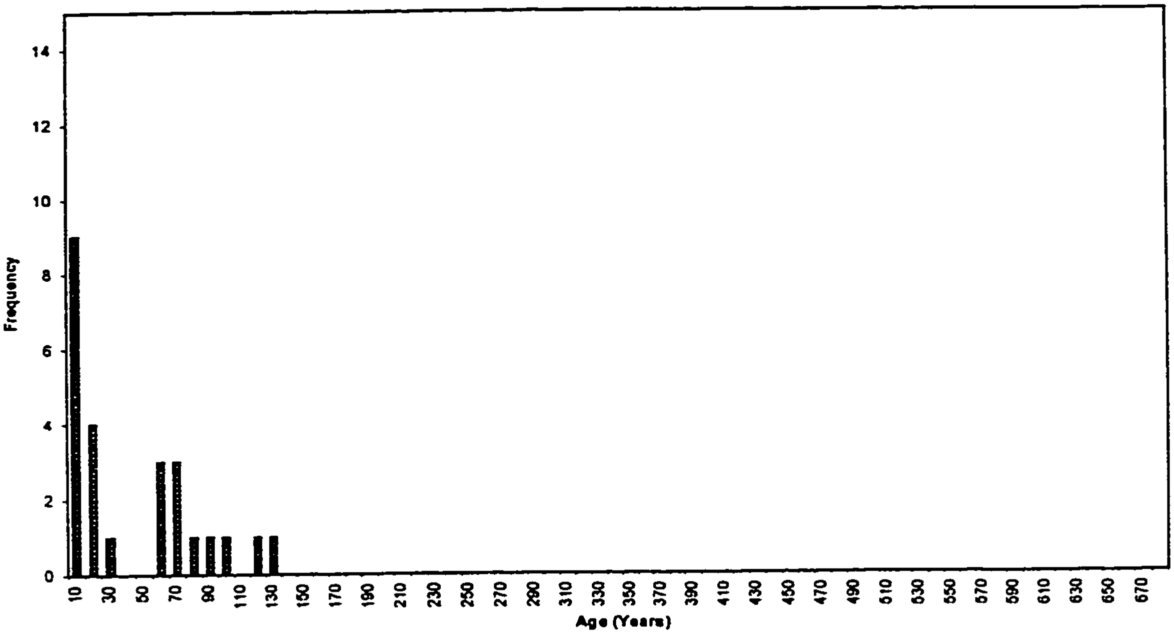

Figure 13 Age distribution at site 13, Sinclair, Kootenay National Park, BC

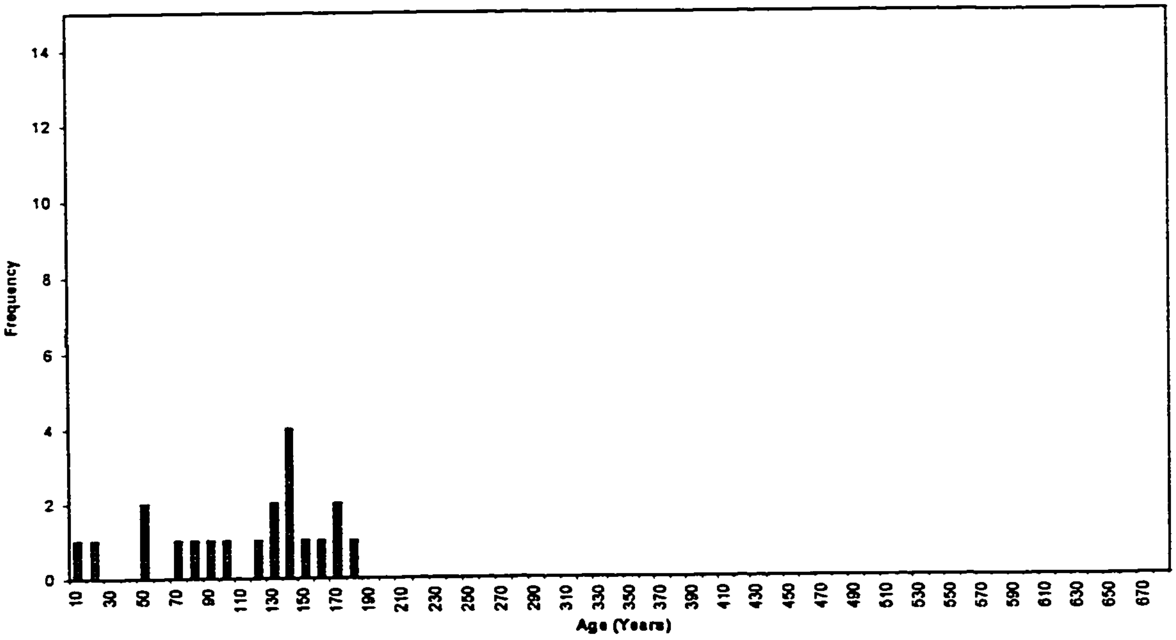

Figure 14 Age distribution at site 14, Nestor, Kananaskis Country, AB

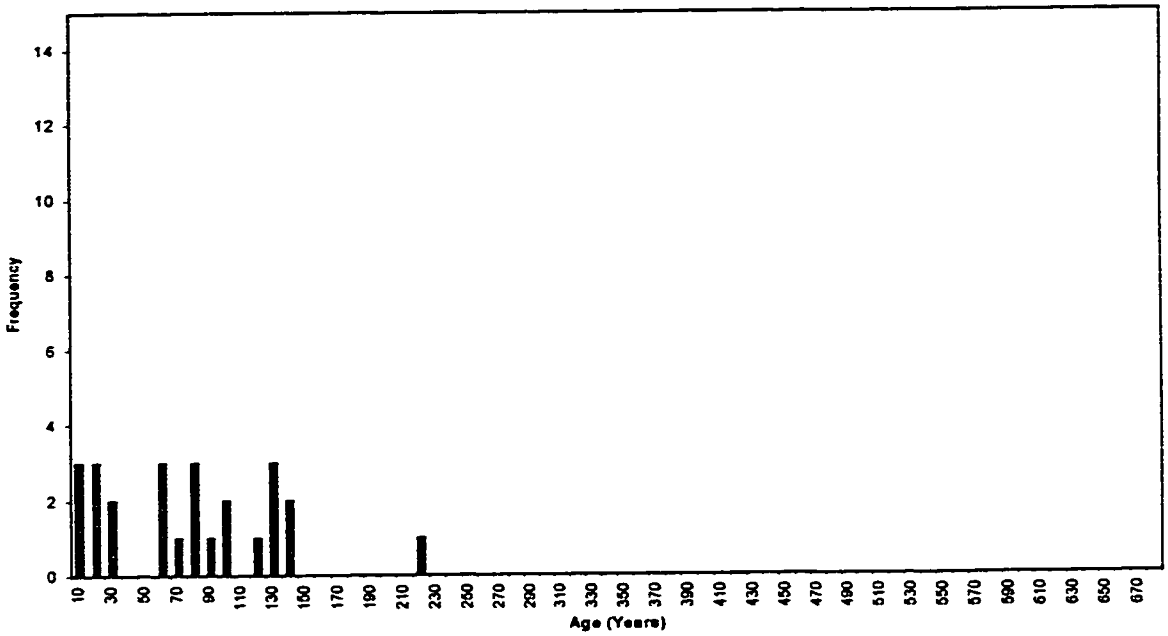

Figure 15 Age distribution at site 15, Hawk, Kootenay National Park, BC

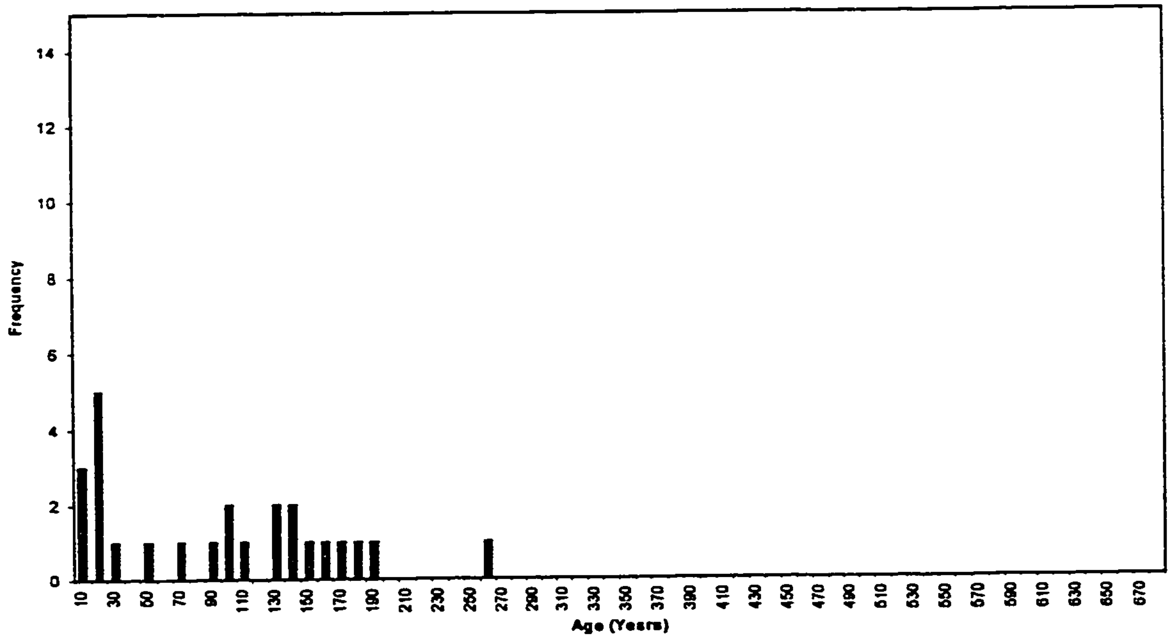

Figure 16 Age distribution at site 16, Sulphur, Banff National Park, AB

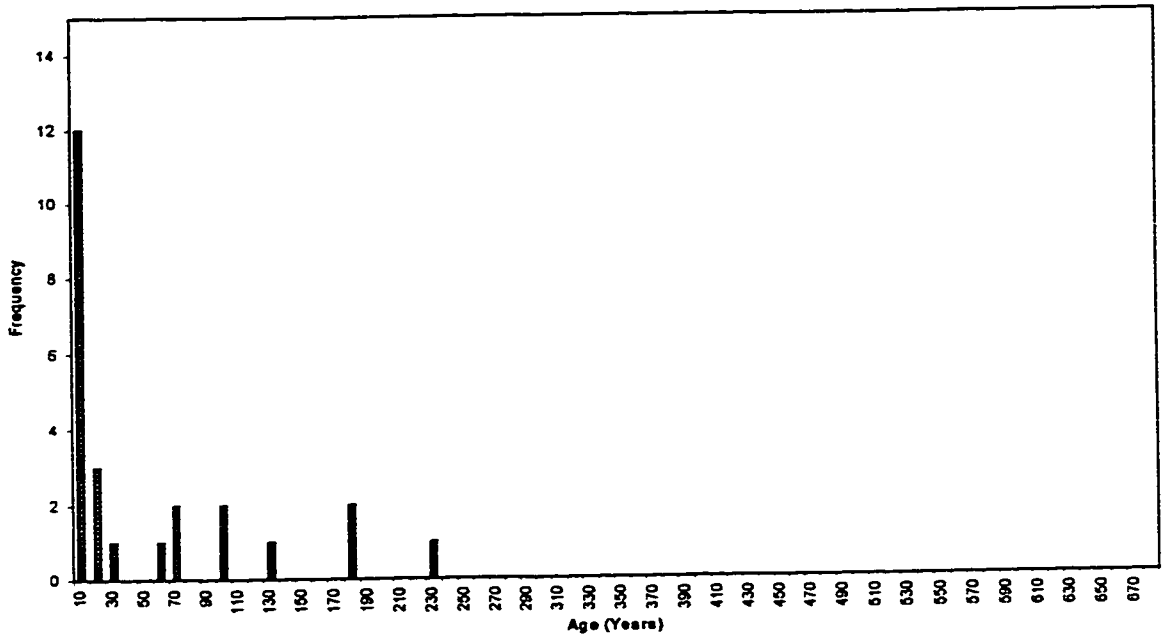

Figure 17 Age distribution at site 17, Whymper, Kootenay National Park, BC

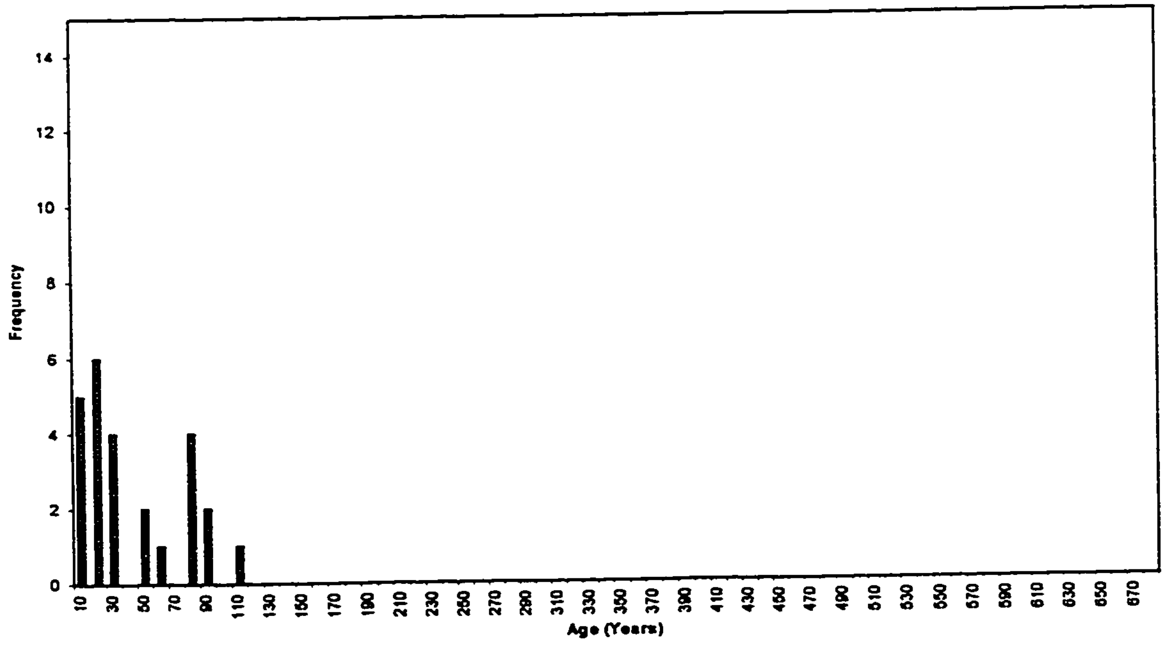

Figure 18 Age distribution at site 18, Castle, Banff National Park, AB

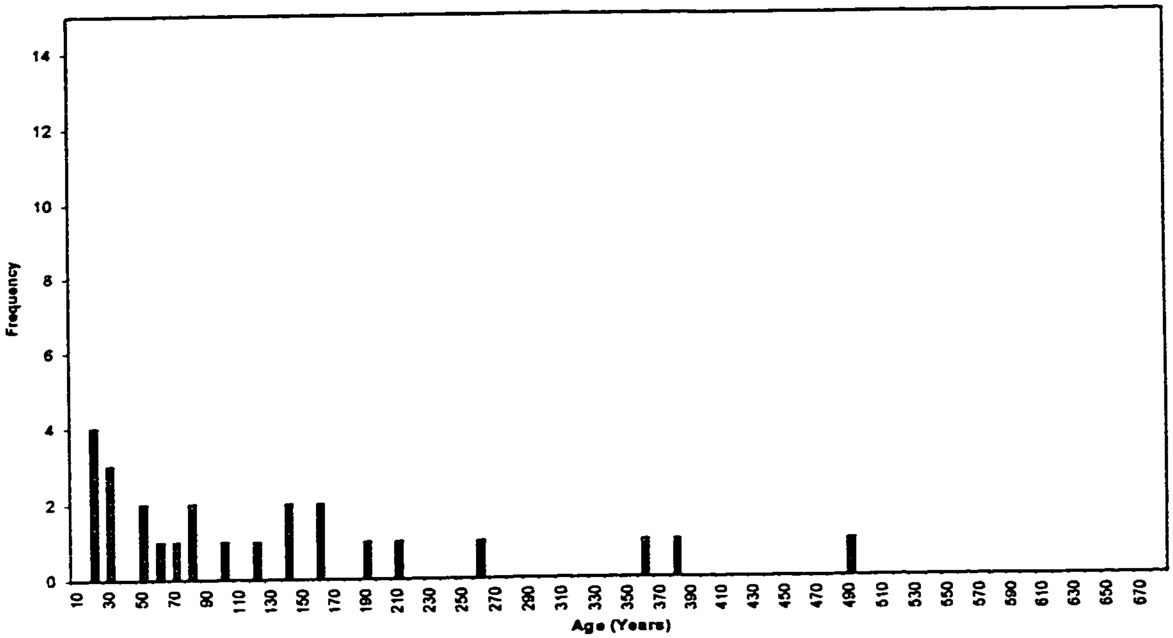

Figure 19 Age distribution at site 19, Wiwaxy, Yoho National Park, BC

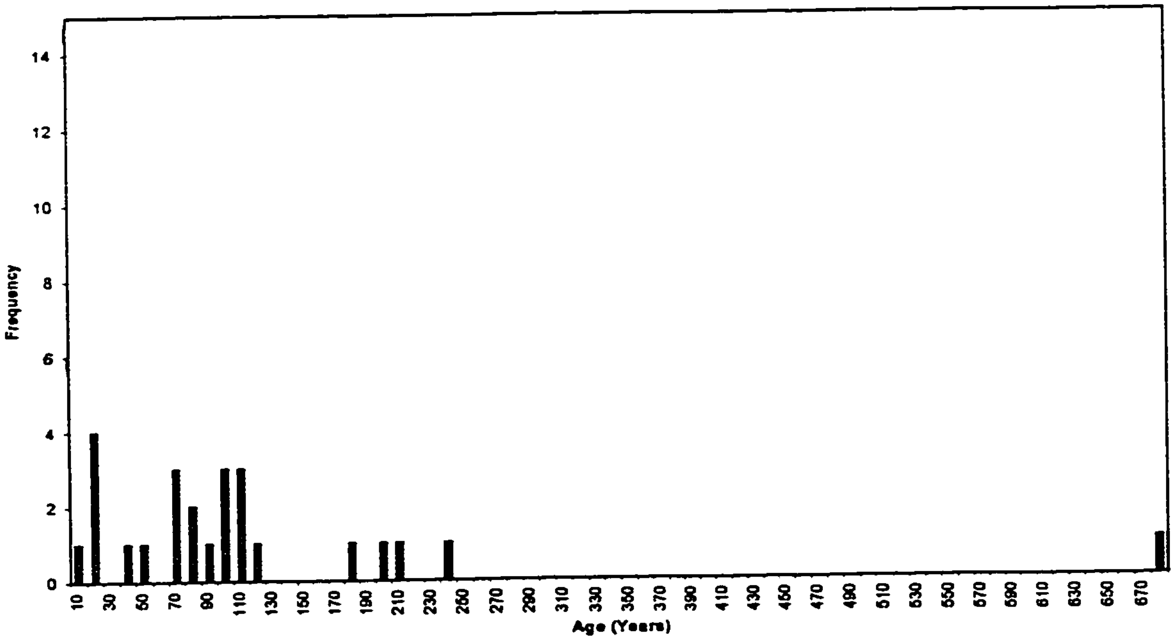

Figure 20 Age distribution at site 20, Whaleback, Yoho National Park, BC

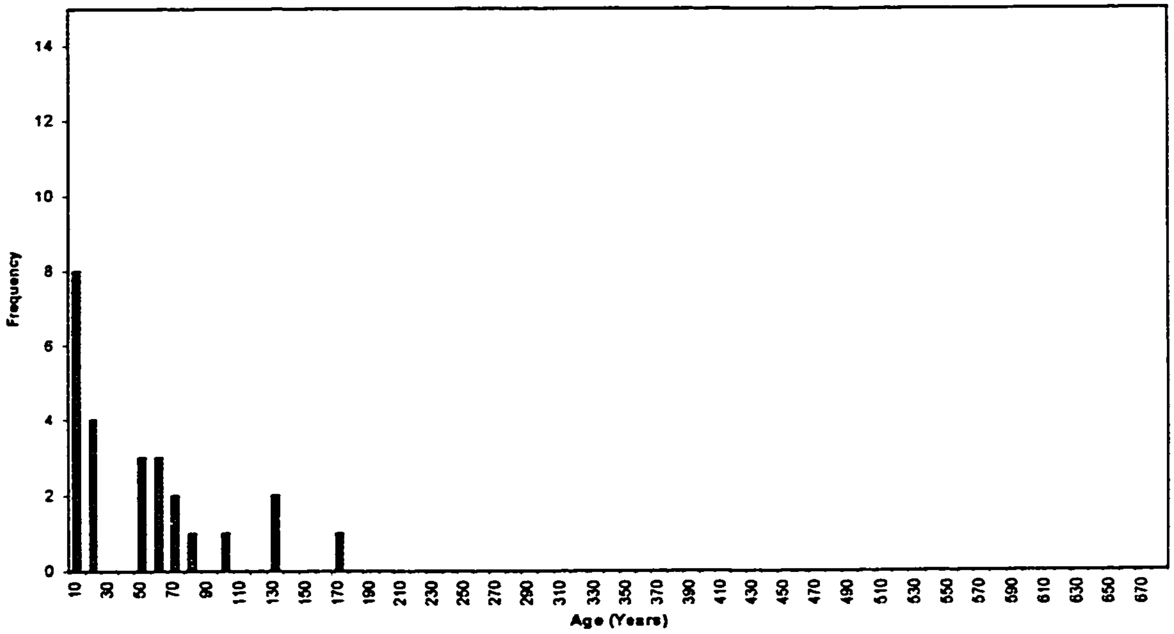

Figure 21 Age distribution at site 21, Dolomite, Banff National Park, AB

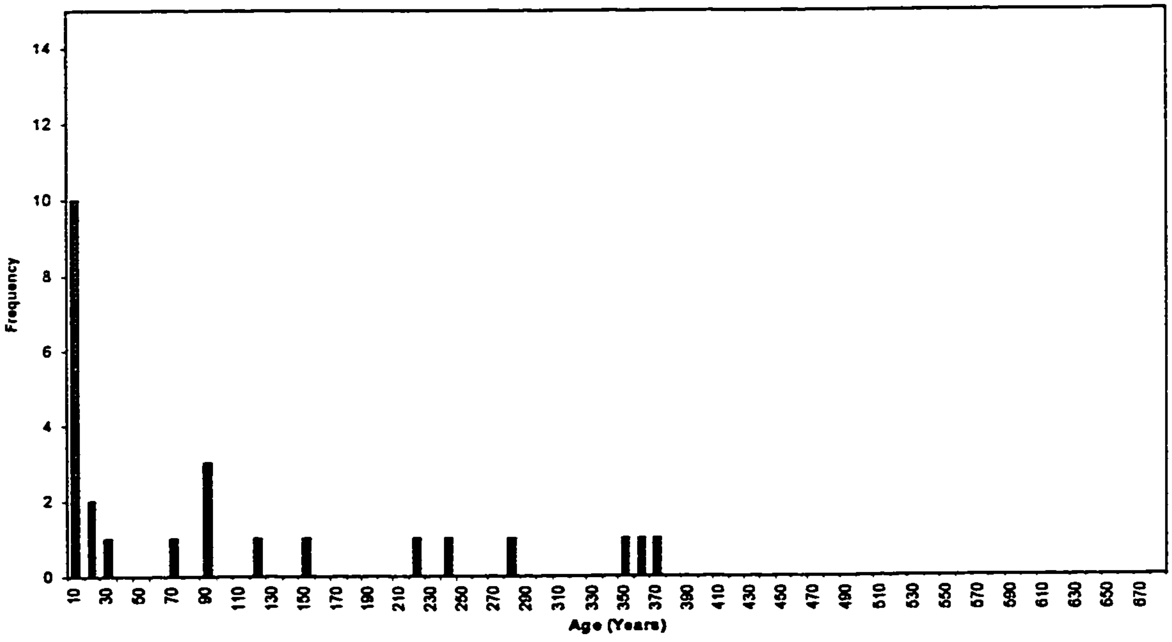

Figure 22 Age distribution at site 22, Sarbach, Banff National Park, AB

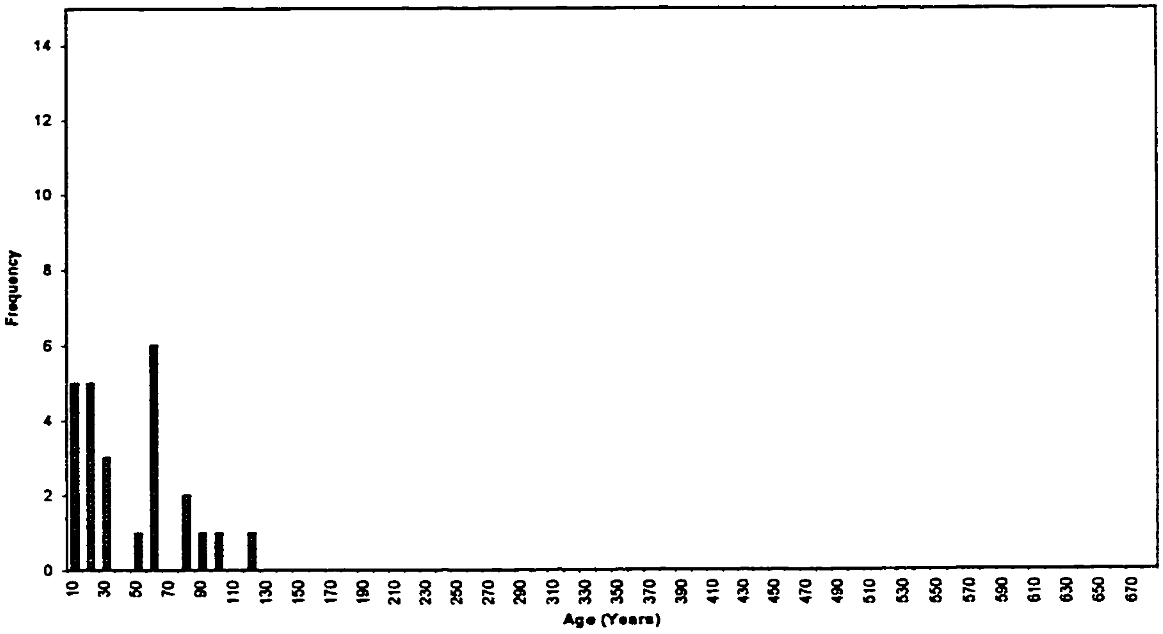

Figure 23 Age distribution at site 23, Nigel, Banff National Park, AB

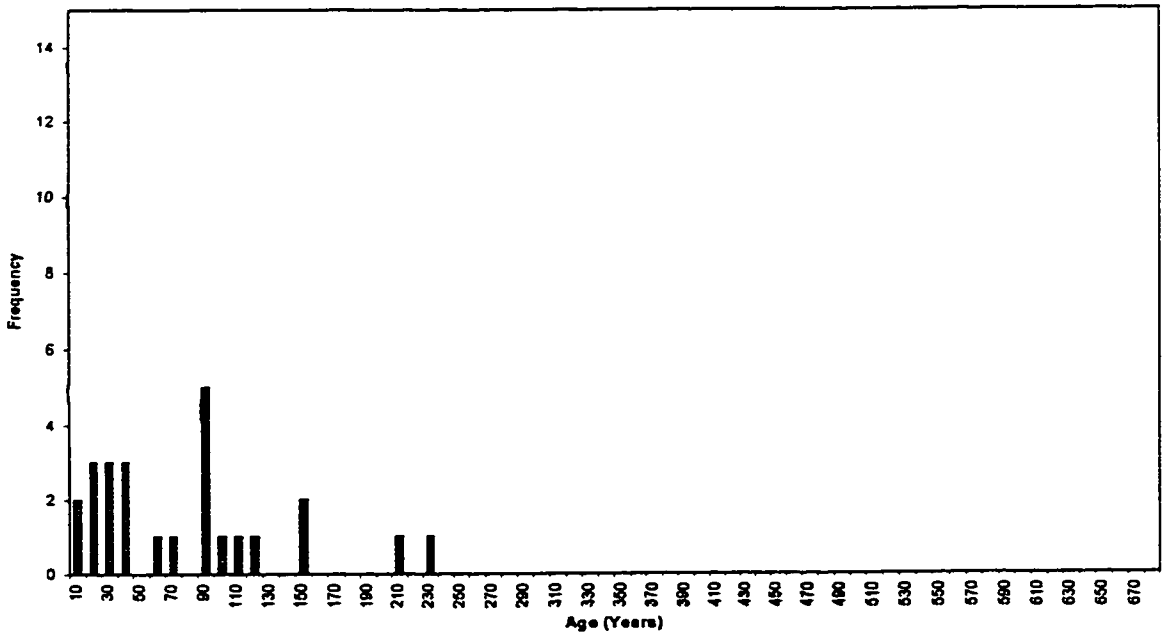

Figure 24 Age distribution at site 24, Wilcox, Jasper National Park, AB

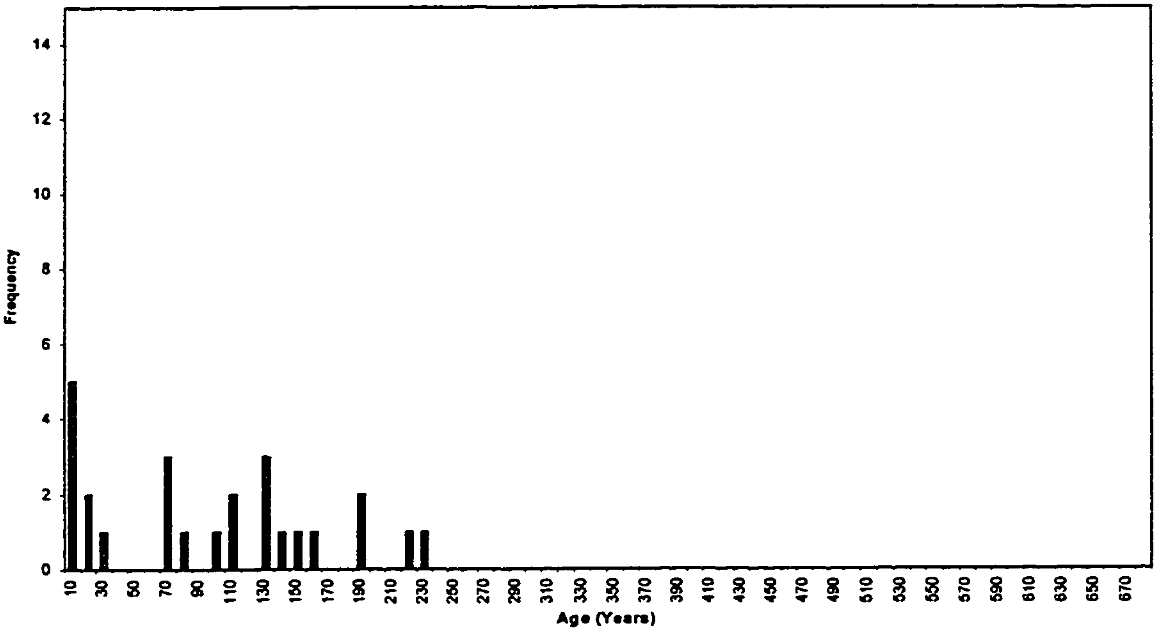

Figure 25 Age distribution at site 25, Geraldine, Jasper National Park, AB

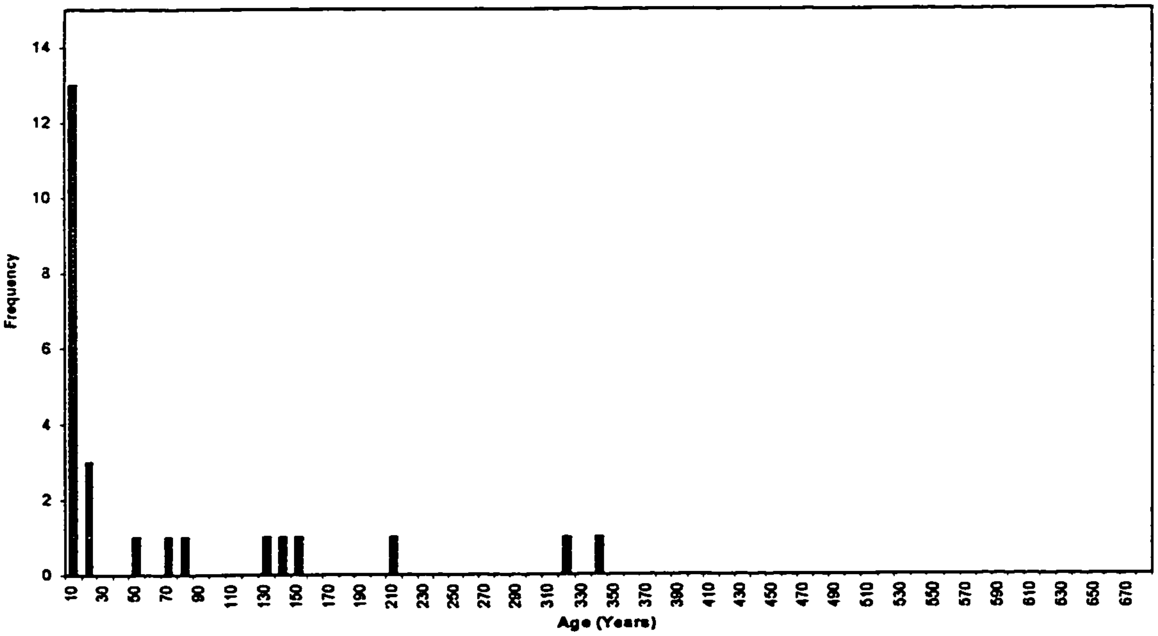

Figure 26 Age distribution at site 26, Astoria, Jasper National Park, AB

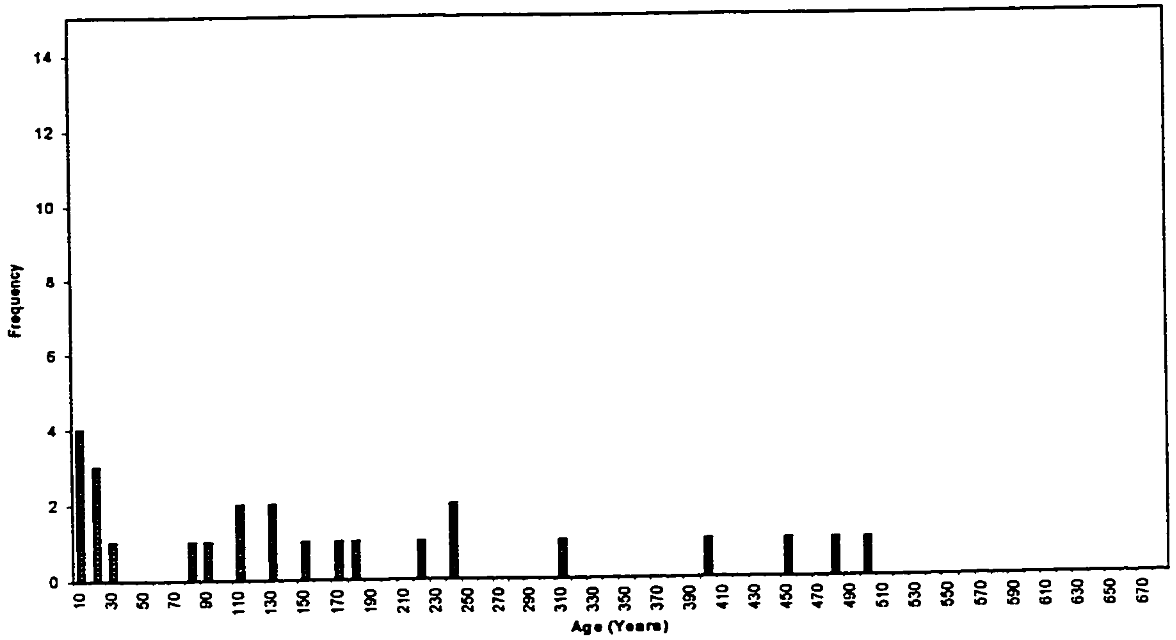

Figure 27 Age distribution at site 27, Esplinade, Jasper National Park, AB

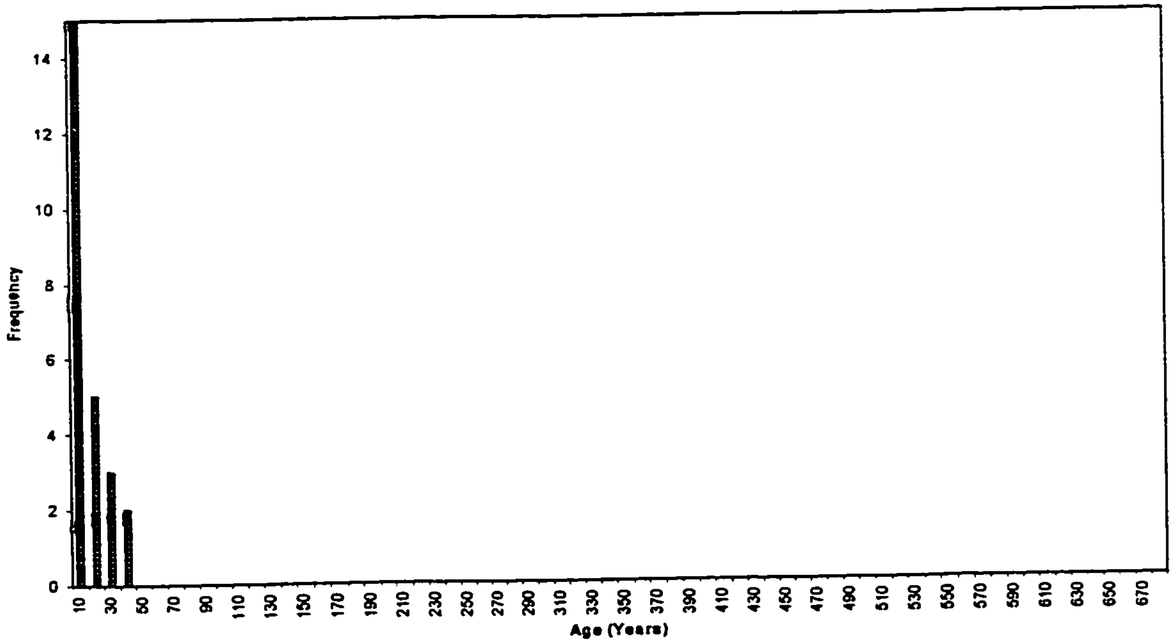

Figure 28 Age distribution at site 28, Mt. Robson, Mt. Robson Provincial Park, BC

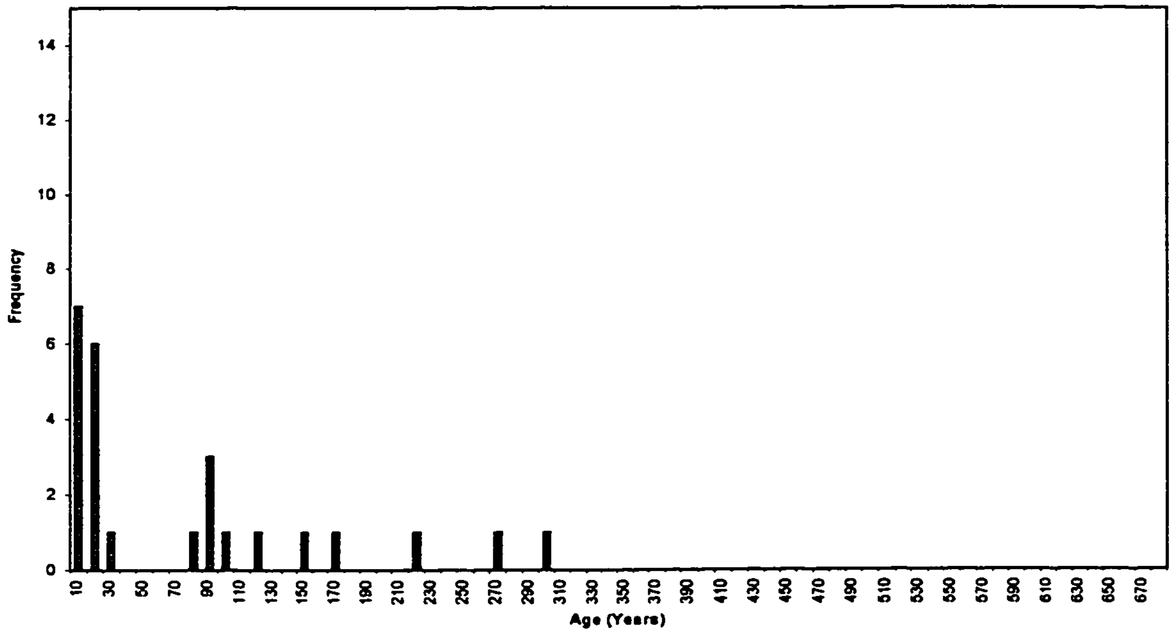

Figure 29 Age distribution at site 29, Adolphus, Jasper National Park, AB

Appendix B: A Review of Statistical Methods for the Analysis of Population Structure Using Data from Electrophoresis Studies

Simple measures of Genetic Diversity

Due to the simplicity of calculation, the most often reported statistics are the proportion of loci that are polymorphic, the average number of alleles per locus, and the effective number of alleles per locus. Because of this simplicity, the usefulness of these statistics is limited especially in determining significant differences between species or populations. However, they are useful as a relative measure of genetic diversity and for comparison purposes since they have been widely reported in the literature.

The proportion of polymorphic loci (P) is calculated by totalling the number of polymorphic loci and dividing by the total number of loci examined (Hartl and Clark 1989). Polymorphic loci are commonly defined in terms of an arbitrary maximum frequency. If the frequency of the most common allele is less than 0.95, such as was used in this study, the locus is considered polymorphic. Using a limit places more emphasis on the common allelic variation and avoids an inflated estimate if only one or two individuals in a population exhibit a rare allele (Liengsiri et al. 1990). However, there are problems with using the number of polymorphic loci as a measure of genetic diversity. Firstly, it does not take into account the proportion of each allele at a locus (Hartl and Clark 1989). Furthermore, because polymorphic loci are required for estimation of other indices of genetic diversity, most electrophoresis studies examine loci that are more likely to exhibit variation, (Liengsiri et al. 1990). The electrophoretic conditions has also been shown to affect the number of alleles detected (Bonhomme and Selander 1978, Coyne et al. 1978). There is no method to test the difference in the proportion of polymorphic loci estimated between populations or studies because the variance of this statistic has not been determined (Chakraborty and Rao 1991). However, studies that compare the proportion of polymorphic loci among groups of populations and species (for example Hamrick et al. 1992) have used the normal approximation in order to calculate variance estimates and for tests of significance. Because of the large number of loci examined, this approximation may be justified. Chakraborty and Rao (1991) suggest that this measure of genetic variation is, however, too crude to use in the examination of evolutionary processes.

The average number of alleles per locus, A, or allelic richness, is estimated on a population or species basis (Chakraborty and Rao 1991). This measure of diversity has the advantage over the proportion of polymorphic loci because it takes into account the variability in numbers of alleles at a locus. However, A does not take into account the variability of the allele frequencies at each locus (Liengsiri et al. 1990). Because the likelihood of finding unique alleles increases with the number of individuals sampled, A is also sensitive to sample size (Liengsiri et al. 1990). Often, the average number of alleles is calculated from only the polymorphic loci. This gives a more accurate representation of how much variation is present at loci that are variable. However, without knowledge of the number of monomorphic loci present, the average number of alleles at polymorphic loci is a misrepresentation and overestimation of the extent of variation. As for P, there is no simple method available to estimate variance for the average number of alleles. Comparative studies have assumed the normal approximation for the calculation of variances and simply used parametric tests of significance such as t-tests or ANOVAs (for example Hamrick et al. 1992).

For a locus with r alleles, where the frequency of the kth allele is p_k, the effective number of alleles is defined as the inverse of the proportion of homozygotes (Crow and Kimura 1970).

$$A_e = \frac{1}{\sum_{k=1}^{r} p_k^2} \qquad (1)$$

This statistic is equivalent to the number of alleles that would be found in the population if all alleles were in the same frequency (Crow and Kimura 1970). The allelic frequencies as well as the numbers of alleles are taken into account by this calculation. The effective number of alleles is usually estimated using the square of the sample allele frequency. However, as pointed out by Nei (1978), x_i^2 is a biased estimator of p_i^2. Although Nei (1978) has corrected for this bias in calculating heterozygosity estimates, authors calculating A_e have not accounted for error. Furthermore, since the effective number of alleles is simply the inverse of the homozygosity, it is proportional to the measures of heterozygosity and fixation indices and therefore does not seem to present any new information. The redundancy of the effective number of alleles index can be seen in the survey done by Hamrick et al. (1992); comparing A_e and H_e (homozygosity) between groups of plants shows similar results in almost every case. Differences may simply be an artifact of the number of tests performed (Lessios 1992) or due to the approximation of the normal distribution used to test these differences. Therefore, the effective number of alleles is reported here simply for comparison.

Fixation Indices and Heterozygosities

In a theoretical population with an infinite number of individuals, mating would be completely at random if none of the evolutionary forces such as selection, mutation, migration, drift, and assortative mating were present. To determine if any of these forces have been present, it must be determined whether or not the genetic structure of a population differs from what would be found under completely random mating (Guo and Thompson 1992). This model is referred to as the Hardy-Weinberg equilibrium: in an infinitely large randomly mating population, with the absence of all evolutionary forces, the allelic and genotypic frequencies can be determined by a simple relationship (Hartl and Clark 1989). For a locus with r alleles A_1, A_2... A_r, the frequency of each genotype in the population is given by the following formula where p_i is the frequency of the allele, A_i (Guo and Thompson 1992).

$$\sum_{i=1}^{r} p_i^2 A_i A_i + \sum_{i<j=1}^{r} 2 p_i\, p_j A_i A_j \qquad (2)$$

Hardy-Weinberg equilibrium is reached in a population when, for a specific locus, the genotypic frequencies are found in the above proportions. This law forms the basis for all possible hypotheses testing regarding the structure of populations (Guo and Thompson 1992).

How to appropriately determine whether a population is at Hardy-Weinberg equilibrium is not clear at present (Lessios 1992). There are many statistics available, some of which are functionally equivalent, although derived from different assumptions (thus philosophically different), which adds to the confusion (Swofford and Selander 1989, Guo and Thompson 1992, Lessios 1992). Many reviews have attempted to determine the most appropriate statistics to use (for example Nei 1975, 1987, Weir and Cockerham 1984, Weir 1990, Lessios 1992, Cockerham and Weir 1993). However, the lack of consistency and understanding in terms of statistical theory and notation along with complications due to differing assumptions has resulted in reviews which only further this confusion.

Statistics derived for the examination of population structure can be used to test the underlying hypothesis of Hardy-Weinberg equilibrium (Chakraborty and Rao 1991). Tests of whether or not Hardy-Weinberg equilibrium holds at various hierarchical levels allows the determination of divisions among groups of subpopulations (Weir 1990). Conclusion of non-equilibrium can lead to speculation as to evolutionary forces that may have acted or are acting on a population (Weir 1990). The various statistics available may suggest, for example, that several subpopulations make up a study population. However, it is the allelic and genotypic frequencies, tested against Hardy-Weinberg equilibrium that are the underlying basis of these statistics (Chakraborty and Rao 1991). Although these statistics are necessary in order to obtain an understanding of the genetic structure of a population or species, the underlying assumptions and the fact that summary statistics are based on the observed genotypic frequencies must be kept in mind.

Wright's fixation indices or *F* statistics are among the oldest and most widely reported genetic diversity statistics (see Wright 1978 for a review). Originally defined as the correlation between two uniting gametes, the derivation of *F* statistics rests on the assumption of a hypothetical ancestral population from which the present populations are derived. The present populations are assumed identical, in size as well as lack of evolutionary influence, over their evolutionary time. If, for example, a population is made up of s subpopulations, these measures of relative correlation satisfy the well-known equation

$$(1-F_{IT})=(1-F_{IS})(1-F_{ST}) \qquad (3)$$

where

F_{IT}= correlation between 2 uniting gametes to produce individuals relative to the total population

F_{IS}= the correlation between 2 uniting gametes to produce individuals relative to the subpopulation

F_{ST}= the correlation between 2 randomly chosen gametes from 2 subpopulations

Wright (1965) originally derived these statistics for the case of two alleles at a locus but they can be extended to the case of multiple alleles. However, there are conceptual as well as statistical problems in applying these measures to real populations due to the numerous assumptions on which they are based such as the absence of selection (Nei 1973). These problems have lead to two main lines of thought, resulting in two philosophically different approaches to the study of population structure (Chakraborty and Danker-Hopfe 1991). The first approach to solving these difficulties was the development of Nei's gene diversity statistics (Nei 1972). His approach was to decompose the total heterozygosity in gene diversity into the diversity within subpopulations and among these subpopulations. Later, Nei (1977, 1978) used this approach to redefine the concept of fixation indices in terms of heterozygosities and their Hardy-Weinberg expectations. Weir and Cockerham (1984) criticized Nei's approach because they said parameter estimates of fixation indices could not be defined in terms of heterozygosities. The second approach to extending Wright's statistics to real populations was to redefined the fixation indices through an extension of the principles of analysis of variance (Cockerham 1969, 1973, Weir and Cockerham 1984, Long 1986, Weir 1990). This approach has an inherent deficiency since it can produce variance components that are negative (when $F_{ST} > F_{IT}$, which occurs whenever F_{IS} is negative). Chakraborty and Danker-Hopfe (1991) point out that this integral feature of the variance approach is "particularly uncomfortable".

Despite problems in philosophical approach to defining *F* statistics, the parameter estimates from real data using these two approaches are nearly identical (Nei 1986, Chakraborty and Danker-Hopfe 1991). The gene diversity decomposition approach of Nei (1973) was preferred by Chakraborty and Danker-Hopfe (1991) because it avoids the inconsistency of the variance approach of Weir and Cockerham (1984). To avoid the problem concerning the weighting of subpopulations in Nei's approach, Chakraborty and Danker-Hopfe (1991) derived fixation indices in terms of a specific allele. These fixation indices will hold for any general mating system since they are derived without assumptions about evolutionary relationship among subpopulations (Chakraborty and Danker-Hopfe 1991). For a population made up of s subpopulations with r alleles at a locus, the kth allele in the ith subpopulation,

$$F_{IT(ik)} \quad = \quad \frac{P_{ik} - p_{ik}^2}{p_{ik}(1 - p_{ik})}$$

The fixation indices for the entire population can be stated similarly,

$$F_{IT(k)} \quad = \quad \frac{P_{.k} - \bar{p}_{.k}^2}{\bar{p}_{.k}(1 - \bar{p}_{.k})} \tag{4}$$

$$F_{IS(k)} = \frac{P_{k} - \overline{p^{2}_{.k}}}{\bar{p}_{.k} - \overline{p^{2}_{.k}}} \qquad (5)$$

$$F_{ST(k)} = \frac{\overline{p^{2}_{.k}} - \bar{p}^{2}_{.k}}{\bar{p}_{.k} - \bar{p}^{2}_{.k}} \qquad (6)$$

Where $P_{ik} = \frac{\sum_{k=1}^{r} A_k A_k}{N_i}$ = frequency of homozygotes $A_k A_k$

$$p_{ik} = \frac{2A_k A_k + \sum_{k \neq j=1}^{r} A_k A_j}{N_i} = \text{frequency of the } A_k \text{allele}$$

$$P_{k} = \frac{\sum_{i=1}^{s} P_{ik}}{s} = \text{average frequency of } A_kA_k \text{ homozygotes over all subpopulations} \quad (7)$$

$$\bar{p}_{k} = \frac{\sum_{i=1}^{s} p_{ik}}{s} = \text{average frequency of } A_k \text{ overall subpopulations} \qquad (8)$$

$$\overline{p^{2}_{.k}} = \frac{\sum_{i=1}^{s} p^{2}_{ik}}{s} = \text{expected average frequency of } A_kA_k \text{ over all subpopulations} \qquad (9)$$

Although these formulas can be derived so that the relative size of each subpopulation is taken into account, this information is rarely available for real populations (Chakraborty and Danker-Hopfe 1991). By assuming all subpopulations are equal in size and can be weighted equally relative to the overall population, the averages can be calculated over the entire population. Even though the assumption of equal population size may not reflect the present relative population sizes, Nei (1986) suggest that this assumption is valid because populations sizes are known to vary widely over evolutionary time (However, see Weir and Cockerham 1984).

Summing the numerators and denominators over all alleles at a locus (Chakraborty and Danker-Hopfe 1991), the fixation indices at a locus can be defined as

$$
\begin{aligned}
\mathrm{F_{IS}} &= \frac{\sum_{k=1}^{r}\left(P_{.k}-\overline{p_{.k}^{2}}\right)}{\sum_{k=1}^{r}\left(\bar{p}_{.k}-\overline{p_{.k}^{2}}\right)} \\
&= \frac{\frac{1}{s}\sum_{i=1}^{s}\left(\sum_{k=1}^{r}P_{ik}-\sum_{k=1}^{r}p_{ik}^{2}\right)}{\frac{1}{s}\sum_{i=1}^{s}\left(\sum_{k=1}^{r}p_{ik}-\sum_{k=1}^{r}p_{ik}^{2}\right)} \\
&= \frac{\left(1-\frac{\sum_{i=1}^{s}\sum_{k=1}^{r}p_{ik}^{2}}{s}\right)-\left(1-\frac{\sum_{i=1}^{s}\sum_{k=1}^{r}P_{ik}}{s}\right)}{1-\frac{\sum_{i=1}^{s}\sum_{k=1}^{r}p_{ik}^{2}}{s}} \qquad (10)
\end{aligned}
$$

Which, if we use Nei's concepts of gene diversity, are equivalent to

$$\mathrm{F_{IS}} = \frac{H_S - H_O}{H_S} \qquad (11)$$

Where

$$\mathrm{H_S} = 1-\frac{\sum_{i=1}^{s}\sum_{k=1}^{r}p_{ik}^{2}}{s} = \frac{\sum_{i=1}^{s}\left(1-\sum_{k=1}^{r}p_{ik}^{2}\right)}{s} = \frac{\sum_{i=1}^{s}H_{Si}}{s} \qquad (12)$$

which is the average within population heterozygosity expected under Hardy-Weinberg equilibrium.

Also

$$\mathrm{H_O} = 1-\frac{\sum_{i=1}^{s}\sum_{k=1}^{r}P_{ik}}{s} \qquad (13)$$

which is the actual proportion of heterozygotes in the total population. Similarly, by summing equation 5 over s,

$$F_{IT} = \frac{\left(1-\sum_{k=1}^{r}\bar{p}_{.k}^{2}\right)-\left(1-\sum_{i=1}^{s}\sum_{k=1}^{r}P_{ik}\right)}{1-\sum_{k=1}^{r}\bar{p}_{.k}^{2}} \qquad (14)$$

$$F_{IT} = \frac{H_T - H_O}{H_T} \qquad (15)$$

where

$$H_T = 1-\sum_{k=1}^{r}\bar{p}_{k}^{2} \qquad (16)$$

is the total heterozygosity expected under Hardy-Weinberg equilibrium. Similarly again,

$$F_{ST} = \frac{H_T - H_S}{H_T} \qquad (17)$$

which satisfy Wright's identity (equation 3) simply as a "mathematical consequence" (Chakraborty and Danker-Hopfe 1991). These statistics are defined on a per locus basis so the average over all loci is the population statistic for the entire genome.

Nei (1973) defined the inter-populational gene differences D_{ST}, as

$$D_{ST} = H_T - H_S \qquad (18)$$

and the absolute magnitude of gene diversity, G_{ST}, as the ratio of the interpopulational gene differences D_{ST}, to H_T, the total population heterozygosity expected under Hardy-Weinberg equilibrium.

$$G_{ST} = \frac{D_{ST}}{H_T} \qquad (19)$$

When the concept of fixation indices is extended from Wright's original assumptions for one locus with two alleles, G_{ST} becomes equivalent to F_{ST} as derived above (Nei 1977). Therefore, the estimation of fixation indices, applied to more than one allele at a locus, is equivalent to the estimation of gene diversity (or heterozygosity) parameters (Nei 1977).

A deficiency in Nei's approach, however, is that his G_{ST} as originally defined is dependent on the number of subpopulations sampled and is therefore not comparable across different sets of population estimates (Weir and Cockerham 1984). In order to solve this problem, Nei (1978) suggested that D_{ST}, the measure of interpopulational gene differences, be redefined to remove the effect of the number of populations. However, if this correction is made, H_T must also be redefined and can no longer be considered conceptually as the total heterozygosity (Nei 1986). Chakraborty and Danker-Hopfe (1991) examined this problem and concluded that unless the number of populations is small (they suggest less than 10) this change in fixation indices has a negligible effect. Because 29 sites were used in this study, the correction was not made in calculating G_{ST}.

Parameter Estimates of Heterozygosities and Fixation Indices

Confusion has arisen in the use of heterozygosity estimates due to the difference between a statistic and an estimate of that statistic (Nei 1978, Chakraborty and Danker-Hopfe 1991). Population statistics, such as the fixation indices, must be estimated from sample populations. Often, the best, i.e. unbiased, estimates of these population parameters are those derived from the sampled individuals (Chakraborty and Danker-Hopfe 1991). For example, an estimate of the frequency of a specific allele in a subpopulation can be produced by calculating the frequency of that allele from the samples collected. The expected value (expressed as *E(x)* in statistical notation) of this estimate of the population allele frequency is unbiased (Nei and Roychoudhury 1974). Stated in mathematical terms, where N_{ikk} is the number of A_kA_k homozygotes, N_{ikj} is the number of heterozygotes for A_k and N_i is the number of individuals in population i,

$$E(x_{ik}) = E\left(\frac{2N_{ikk} + \sum_{j>k=1}^{r} N_{ikj}}{2N_i} \right) = p_{ik}$$

Similarly, an unbiased estimate of the number of homozygotes in a subpopulation with allele *k* is

$$E(X_{ik}) = E\left(\frac{N_{ikk}}{N_i}\right) = P_{ik}$$

However, a problem arises when we try to obtain an unbiased estimate of the square of the allelic frequency, p_{ik}^2. Using the estimate

$$\hat{p}_{ik}'^2 = x_{ik}^2$$

introduces a bias since the expected value of this estimate is

$$E\left(\hat{p}_{ik}'^2\right) = p_{ik}^2\left(1 - \frac{1}{2N_i}\right) + \frac{p_{ik}}{2N_i} \qquad \text{(Nei and Roychoudhury 1974)} \qquad (20)$$

When only a few loci or a few individuals sampled are examined, this bias can affect the resulting estimates of parameters (Nei 1986, Chakraborty and Danker-Hopfe 1991). Nei and Roychoudhury (1974) addressed this problem by using the unbiased estimates of p_{ik}^2 to produce the following formulas that are now widely used to estimate heterozygosity within and among populations.

$$\hat{H}_O = 1 - \frac{\sum_{i=1}^{s}\sum_{k=1}^{r} X_{ik}}{s} \qquad (21)$$

$$\hat{H}_S = \frac{1}{s}\sum_{i=1}^{s}\hat{H}_{Si} \quad \text{where } \hat{H}_{Si} = \frac{2N_i}{(2N_i - 1)}\left(1 - \sum_{k=1}^{r} x_{ik}^2\right) \qquad (22)$$

$$\hat{H}_T = 1 - \sum_{k=1}^{r}\left(\frac{\sum_{i=1}^{s} x_{ik}}{s}\right)^2 + \frac{1}{s^2}\sum_{i=1}^{s}\left(\frac{1 - \sum_{k=1}^{r} x^2{}_{ik}}{2N_i - 1}\right) \qquad (23)$$

These estimates are then summed over all loci to obtain an estimate for these statistics for the entire genome. By deriving these formulas in terms of a specific allele, Chakraborty and Danker-Hopfe (1991) avoid the approximations involved in the formulas derived by Nei and Chesser (1983). (However, see Nei (1986) for observations on the use of the sample size correction). These unbiased heterozygosity estimates can be used to calculate estimates of fixation indices simply by replacing the population statistics in equations 11, 15 and 17 by the above unbiased estimates.

$$\hat{F}_{IS} = 1 - \hat{H}_O / \hat{H}_S \qquad (24)$$

$$\hat{F}_{IT} = 1 - \hat{H}_O / \hat{H}_T \qquad (25)$$

$$\hat{F}_{ST} = 1 - \hat{H}_S / \hat{H}_T \qquad (26)$$

However, as Chakraborty and Danker-Hopfe (1991) point out, the resulting estimates are not unbiased. This is because the estimate of a ratio is not equal to the ratio of these estimates. For example,

$$E\left(\frac{\hat{H}_O}{\hat{H}_S}\right) \neq \frac{E\left(\hat{H}_O\right)}{E\left(\hat{H}_S\right)}$$

However, they are considered consistent estimators since they approach the true value as sample size increases. A condition referred to as asymptotically unbiased (Chakraborty and Danker-Hopfe 1991).

Some authors calculate the estimate for F_{ST} from polymorphic loci only. However, since H_S and H_T are both divided by the number of loci (equation 26), the estimate of F_{ST} becomes independent of the number of loci sampled. It is therefore valid to compare the estimates of F_{ST} among studies that have examined polymorphic loci only and those that have used both monomorphic and polymorphic loc.

Testing deviation from Hardy-Weinberg Equilibrium

At present, there is no method to test for significance between different estimates of fixation indices (Chakraborty and Danker-Hopfe 1991). The most commonly used approach is to test the fixation indices against the null hypothesis of no genetic variation by using a chi-squared approximation (Li and Horvitz 1953, Workman and Niswander 1970, Sokal and Rolf 1995). However, a more appropriate test can be constructed by making use of Fisher's exact test (Haldane 1954, Louise and Dempster 1987). In order to calculate the probability of the observed distribution of genotypes, all possible tables with the same allelic frequencies are enumerated. The probability of the observed distribution is the sum of the probabilities of all distributions with probabilities the same or smaller than the observed distribution (Rousset and Raymond 1997). In some instances, the possible number of distributions, especially in the cases with many samples per population (more than 50) and more than five alleles at a locus, makes enumeration unfeasible. In order to address this problem, the most common method has been to pool rare alleles and perform the test on only two alleles per locus (Swofford and Selander 1989). Recently, Monte Carlo techniques have been developed which consider only a random subset of all the possible distributions, reducing the computational effort (Guo and Thompson 1992). From this subset, an estimate of the probability of the observed distribution can be calculated. Algorithms, such as the Markov Chain method, have been programmed to perform this random subsample (Raymond and Rousset 1995). This algorithm is particularly useful in situations where there are many alleles (more than five at a locus). However, because this algorithm is only estimate of the exact probability, for loci with only a few alleles (four or less) the exact test is preferred (Guo and Thompson 1992).

Population Differentiation

Population differentiation can be tested using an exact test (Raymond and Rousset 1995). The distribution of alleles among populations at a locus can be tested in order to determine the probability of the observed distribution. Since the computational requirements for this test become cumbersome even with only a few populations, the Markov Chain algorithm is the most suitable approach to determining the significance of this test (Raymond and Rousset 1995). This test can also be used to determine if genotypes, not just allelic types, differ in their distribution among populations. A contingency table can be arranged with the genotypic counts at a specific locus in each population that can be tested for significance using the Markov Chain algorithm (Goudet et al. 1996). The significance of the F_{ST} is traditionally tested using a chi-squared approximation. However, Workman and Niswander (1970) showed that this test is equivalent to a contingency table analysis. Therefore, using the exact test to test for population differentiation is also equivalent to testing for the significance of F_{ST}.

Lessios (1992) suggests that the degrees of freedom for multiple tests should be adjusted to reflect the number of tests of significance performed. The significance level should be divided by the number of tests preformed (referred to as the Bonferroni technique) in order to predetermine the significance level of multiple tests. Although Lessios (1992) was specifically referring to the contingency table analysis (such as in Workman and Niswander 1970), this consideration could theoretically apply to testing the significance of estimates over all loci.

Gene flow among populations was estimated using the indirect technique developed by Wright (1931). When populations are at equilibrium, an approximate estimate of gene flow among populations, *Nm*, can be calculated from F_{ST} as follows

$$Nm = \frac{1}{4}\left(\frac{1}{F_{ST}} - 1\right) \tag{27}$$

Genetic Distances

The most commonly used method to estimate genetic distance was developed by Nei (1972). Following the notation used in Nei (1972), the standard genetic distance between two populations at a specific locus is defined as

$$D_S = -\ln\left[\frac{J_{Sij}}{\sqrt{J_{Si}J_{Sj}}}\right] = -\ln\left[I_{ij}\right] \tag{28}$$

Where the statistic J, the probability of identity, is equivalent to the "homozygosity".

$$J_{Si} = 1 - H_{Si} = \sum_{k=1}^{r} p_{ik}^2 \tag{29}$$

J_{Sij}, the probability of identity between two populations, is the probability that individuals chosen from each of the two populations have the identical alleles.

$$J_{Sij} = \sum_{k=1}^{r} p_{ik} p_{jk} \tag{30}$$

In order to avoid the problem of bias in estimating genetic distances, J_S can be estimated using the unbiased estimate of H_S (Nei 1978).

$$\hat{J}_{Si} = 1 - \hat{H}_{Si} = \frac{2N_i \sum_{k=1}^{r} x_{ik}^2 - 1}{2N_i - 1} \tag{31}$$

An unbiased estimate of J_{Sij} can be calculated from the sample allele frequencies for each of k alleles at each locus, x_{ik} and x_{jk} (Nei 1978).

$$\hat{J}_{Sij} = \sum_{k=1}^{r} x_{ik} x_{jk} \tag{32}$$

Therefore, an asymptotically unbiased estimation of the standard genetic distance is

$$\hat{D}_S = -\ln\left[\frac{\hat{J}_{sij}}{\sqrt{\hat{J}_{si}\hat{J}_{sj}}}\right] \tag{33}$$

To calculate the average genetic distance over all loci in a study, J_{Sij}, J_{Si}, and J_{Sj} must be averaged over all loci before they are logarithmically transformed in the distance calculation (Chakraborty and Rao 1991).

Because the geographic distances are not independent from one another, a Mantel test was performed using the GENEPOP program to test for a significant relationship between the genetic and geographic distances (Raymond and Rousset 1995). This program uses a rank correlation (Spearman's rank correlation coefficient) instead of the Z statistic and its approximate distribution described by Mantel (1967).

Tree Construction

Many methods have also been proposed in order to arrange genetic distances in a visual presentation (Saitou 1991). This type of presentation allows for the comparison of genetic distance relationships with actual geographic patterns and may indicate patterns of evolutionary development. The two most commonly used methods for tree construction using isozyme data are the UPGMA, Unweighted Pair Group Method by Average (Sneath and Sokal 1973) and the Neighbor-Joining (NJ) method (Saitou and Nei 1987). The NJ method has been shown to produce the most consistent trees compared to the UPGMA and other methods (Saitou 1991). As Saitou (1991) points out, the algorithm of the NJ method and the UPMGA method are quite similar. For the UPMGA, the smallest distance is chosen and for the NJ method, the smallest branch sum is chosen. The UPGMA method assumes homogeneity of evolutionary rates whereas the NJ method does not (DeBry 1992). The jackknifing procedure can be used to test the 'stability' of the tree produced using the NJ method. The Phylip package (version3.572c Felsenstein 1995) was used to construct a consensus tree from the jackknifed resampled allele frequencies. Jackknifing was performed by randomly choosing half of the 16 loci and using only these 8 loci to calculate a genetic distance matrix. The number of resampled data sets created was 10 000. The NJ procedure was then used to construct a tree for each of the 10 000 resampled genetic matrices. Finally, a consensus tree was constructed using the Ml (M-sub-L) method (Felsenstein 1995). This method maximizes the number of times the same species occur to the right of a given branch location. The proportion of the trees that had the same populations to the right of a given node is printed at the branch point in the consensus tree. How well the populations group together is indicated by the number of trees that have the same groups present to the right of a given branch point.

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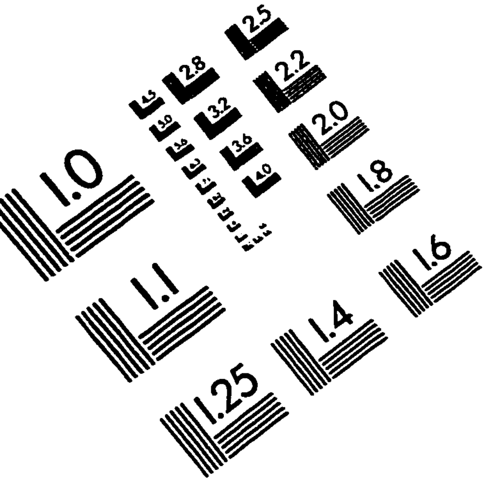
Appendix C: Allele Frequencies at 9 Polymorphic Loci in Whitebark Pine from 29 Locations throughout the Canadian Rockies

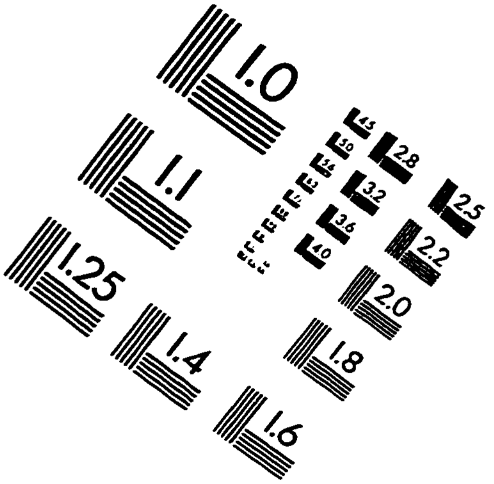
	Allele							
Site	*Mdh-2-1*	*Mdh-2-2*	*Fle-3-1*	*Fle-3-2*	*Fle-3-3*	*Mnr-1*	*Mnr-2*	*Mnr-3*
1	0.340	0.660	0.065	0.543	0.391	0.440	0.520	0.040
2	0.180	0.820	0.130	0.500	0.370	0.460	0.540	0.000
3	0.250	0.750	0.280	0.360	0.360	0.440	0.560	0.000
4	0.280	0.720	0.060	0.580	0.360	0.380	0.620	0.000
5	0.420	0.580	0.140	0.520	0.340	0.423	0.577	0.000
6	0.320	0.680	0.080	0.440	0.480	0.460	0.540	0.000
7	0.220	0.780	0.167	0.625	0.208	0.462	0.538	0.000
8	0.188	0.813	0.125	0.583	0.292	0.423	0.577	0.000
9	0.320	0.680	0.120	0.580	0.300	0.420	0.580	0.000
10	0.280	0.720	0.114	0.500	0.386	0.400	0.600	0.000
11	0.326	0.674	0.114	0.477	0.409	0.440	0.540	0.020
12	0.280	0.720	0.000	0.680	0.320	0.420	0.580	0.000
13	0.280	0.720	0.125	0.438	0.438	0.460	0.540	0.000
14	0.275	0.725	0.075	0.550	0.375	0.425	0.575	0.000
15	0.188	0.813	0.063	0.667	0.271	0.460	0.540	0.000
16	0.220	0.780	0.000	0.640	0.360	0.480	0.520	0.000
17	0.167	0.833	0.120	0.620	0.260	0.440	0.560	0.000
18	0.458	0.542	0.100	0.540	0.360	0.540	0.460	0.000
19	0.229	0.771	0.000	0.300	0.700	0.460	0.540	0.000
20	0.180	0.820	0.146	0.417	0.438	0.440	0.560	0.000
21	0.280	0.720	0.000	0.771	0.229	0.480	0.520	0.000
22	0.239	0.761	0.023	0.659	0.318	0.480	0.520	0.000
23	0.240	0.760	0.160	0.620	0.220	0.420	0.580	0.000
24	0.125	0.875	0.271	0.354	0.375	0.460	0.540	0.000
25	0.280	0.720	0.040	0.460	0.500	0.460	0.540	0.000
26	0.313	0.688	0.100	0.680	0.220	0.480	0.520	0.000
27	0.400	0.600	0.300	0.420	0.280	0.400	0.600	0.000
28	0.174	0.826	0.087	0.652	0.261	0.435	0.565	0.000
29	0.340	0.660	0.196	0.630	0.174	0.460	0.540	0.000

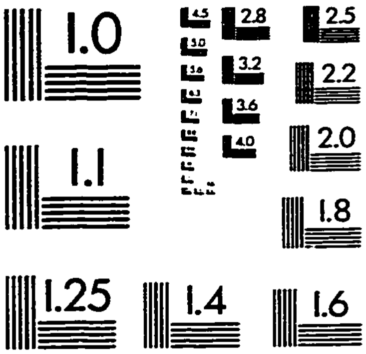
	Allele									
Site	*Pgm-2-1*	*Pgm-2-2*	*Pgm-2-3*	*Idh-1*	*Idh-2*	*Idh-3*	*Pgi-1-1*	*Pgi-1-2*	*Pgi-2-1*	*Pgi-2-2*
1	0.000	0.833	0.167	0.000	1.000	0.000	0.458	0.542	0.325	0.675
2	0.021	0.792	0.188	0.020	0.980	0.000	0.563	0.438	0.273	0.727
3	0.000	0.900	0.100	0.020	0.980	0.000	0.520	0.480	0.480	0.520
4	0.000	0.688	0.313	0.020	0.980	0.000	0.405	0.595	0.500	0.500
5	0.000	0.750	0.250	0.038	0.962	0.000	0.460	0.540	0.560	0.440
6	0.087	0.565	0.348	0.060	0.940	0.000	0.520	0.480	0.440	0.560
7	0.000	0.729	0.271	0.040	0.960	0.000	0.480	0.520	0.357	0.643
8	0.000	0.833	0.167	0.096	0.904	0.000	0.340	0.660	0.688	0.313
9	0.000	0.674	0.326	0.160	0.840	0.000	0.380	0.620	0.542	0.458
10	0.000	0.545	0.455	0.109	0.891	0.000	0.396	0.604	0.696	0.304
11	0.000	0.705	0.295	0.000	1.000	0.000	0.542	0.458	0.717	0.283
12	0.000	0.660	0.340	0.000	0.980	0.020	0.563	0.438	0.478	0.522
13	0.000	0.813	0.188	0.000	1.000	0.000	0.540	0.460	0.292	0.708
14	0.000	0.781	0.219	0.050	0.950	0.000	0.350	0.650	0.800	0.200
15	0.000	0.848	0.152	0.120	0.860	0.020	0.521	0.479	0.646	0.354
16	0.000	0.680	0.320	0.083	0.896	0.021	0.420	0.580	0.761	0.239
17	0.000	0.571	0.429	0.140	0.860	0.000	0.271	0.729	0.771	0.229
18	0.000	0.720	0.280	0.060	0.940	0.000	0.260	0.740	0.854	0.146
19	0.000	0.820	0.180	0.020	0.980	0.000	0.060	0.940	0.960	0.040
20	0.000	0.804	0.196	0.040	0.960	0.000	0.240	0.760	0.920	0.080
21	0.000	0.720	0.280	0.020	0.980	0.000	0.400	0.600	0.740	0.260
22	0.000	0.727	0.273	0.068	0.932	0.000	0.348	0.652	0.761	0.239
23	0.000	0.620	0.380	0.120	0.880	0.000	0.480	0.520	0.460	0.540
24	0.000	0.792	0.208	0.083	0.896	0.021	0.479	0.521	0.500	0.500
25	0.000	0.583	0.417	0.120	0.880	0.000	0.200	0.800	0.820	0.180
26	0.000	0.737	0.263	0.000	1.000	0.000	0.160	0.840	1.000	0.000
27	0.000	0.440	0.560	0.080	0.920	0.000	0.160	0.840	0.900	0.100
28	0.000	0.667	0.333	0.196	0.804	0.000	0.500	0.500	0.348	0.652
29	0.000	0.688	0.313	0.020	0.980	0.000	0.500	0.500	0.660	0.340

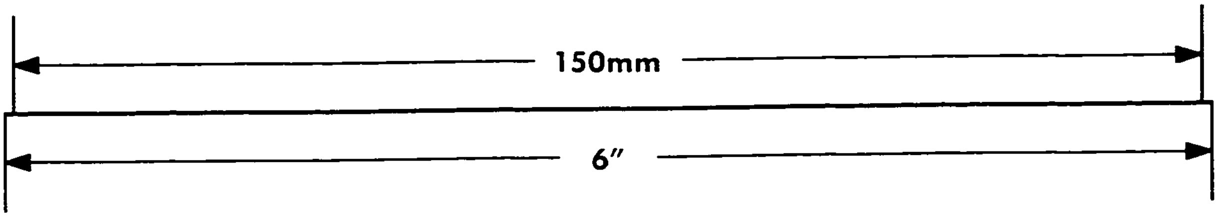
	Allele							
Site	*6Pg-1*	*6Pg-2*	*6Pg-3*	*6Pg-4*	*Udp-1*	*Udp-2*	*Udp-3*	*Udp-4*
1	0.000	1.000	0.000	0.000	0.000	0.900	0.100	0.000
2	0.000	0.957	0.043	0.000	0.000	0.180	0.820	0.000
3	0.000	1.000	0.000	0.000	0.000	0.273	0.727	0.000
4	0.000	0.860	0.140	0.000	0.000	0.650	0.275	0.075
5	0.000	0.840	0.160	0.000	0.000	0.231	0.769	0.000
6	0.040	0.960	0.000	0.000	0.000	0.333	0.667	0.000
7	0.000	0.957	0.043	0.000	0.040	0.920	0.040	0.000
8	0.000	0.960	0.040	0.000	0.000	0.109	0.804	0.087
9	0.000	0.900	0.100	0.000	0.020	0.760	0.220	0.000
10	0.000	0.920	0.080	0.000	0.000	0.125	0.875	0.000
11	0.000	1.000	0.000	0.000	0.000	0.636	0.364	0.000
12	0.000	0.841	0.159	0.000	0.000	0.420	0.580	0.000
13	0.000	1.000	0.000	0.000	0.000	0.580	0.420	0.000
14	0.000	0.925	0.075	0.000	0.000	0.700	0.300	0.000
15	0.000	0.938	0.063	0.000	0.000	0.479	0.521	0.000
16	0.020	0.940	0.040	0.000	0.000	0.500	0.500	0.000
17	0.000	0.900	0.100	0.000	0.000	0.520	0.480	0.000
18	0.000	0.900	0.100	0.000	0.000	0.560	0.440	0.000
19	0.000	0.900	0.100	0.000	0.000	0.540	0.460	0.000
20	0.000	0.833	0.167	0.000	0.042	0.833	0.125	0.000
21	0.024	0.952	0.024	0.000	0.000	0.560	0.440	0.000
22	0.000	0.957	0.043	0.000	0.000	0.630	0.370	0.000
23	0.000	0.940	0.060	0.000	0.000	0.680	0.320	0.000
24	0.000	0.795	0.182	0.023	0.000	0.130	0.826	0.043
25	0.000	0.860	0.100	0.040	0.000	0.500	0.417	0.083
26	0.000	0.980	0.020	0.000	0.000	0.674	0.326	0.000
27	0.000	0.860	0.100	0.040	0.040	0.360	0.600	0.000
28	0.000	0.977	0.023	0.000	0.000	0.565	0.435	0.000
29	0.040	0.940	0.020	0.000	0.000	0.540	0.460	0.000

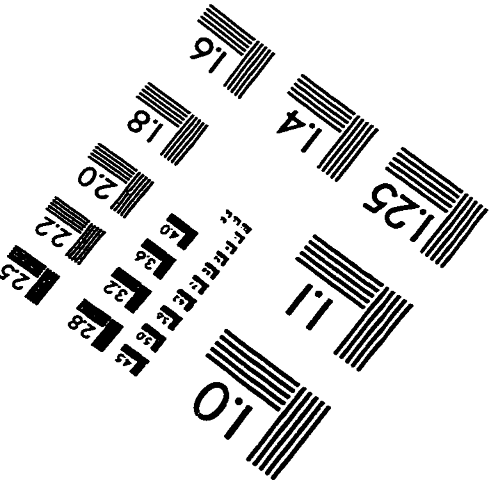
IMAGE EVALUATION
TEST TARGET (QA-3)

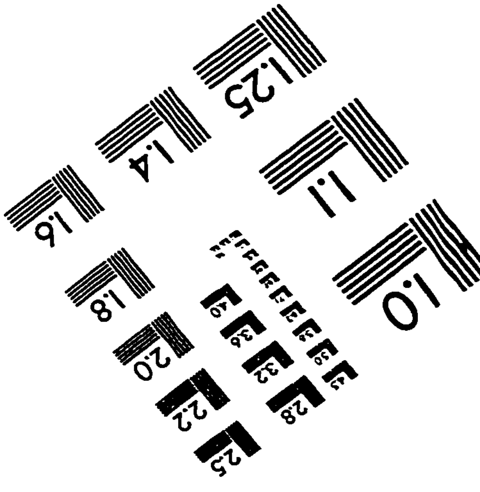












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