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Genetic Diversity of White Pine (Pinus strobus) in the Beaver Island Archipelago

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GENETIC DIVERSITY OF WHITE PINE (*Pinus strobus*) IN THE BEAVER ISLAND ARCHIPELAGO

By

Eric R. Myers

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ABSTRACT

GENETIC DIVERSITY OF WHITE PINE (*Pinus strobus*) IN THE BEAVER ISLAND ARCHIPELAGO

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Geographical isolation is thought to permit divergence among populations by disrupting gene flow. This study examined the long-term effects of fragmentation on variable sized, insular populations of white pine (*Pinus strobus*). The four study sites were connected to mainland Michigan roughly 10,000 y.b.p. and have been isolated from the mainland and each other since approximately 6,000 y.b.p. Results determined that white pine harbors high amounts of genetic diversity within populations, and little diversity among fragmented populations. This study found slight divergence with allozymes. The confidence interval for Fst among islands was 0.00 to 0.03 and among sample plots was 0.01 to 0.07. Diversity measures based on microsatellite markers also indicated some divergence. The Rst value among islands was 0.01, and 0.03 among sample plots. Estimates of inbreeding within islands were significantly different form zero. The confidence interval for Fis among islands ranged from 0.02 to 0.12, but was not significantly different from zero among sample plots. In addition, significant positive spatial autocorrelation was found in distance classes less than 5 km and 8.5 km among islands. Negative spatial autocorrelation was found at distances greater than 13.5 km among islands. Within the largest island, Beaver Island, significant positive spatial autocorrelation was found in distance classes less than 3.6 km, and a negative

autocorrelation was found at distance classes greater than 13.5 km. These results suggest that the fragmentation and subsequent isolation has resulted in some divergence and inbreeding within islands, but that a limited amount of migration has prevented more severe divergence.

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Introduction

Habitat Fragmentation

Habitat fragmentation, resulting in the formation of isolated ecological islands is one of the most important issues facing wild species today (Saunders et al., 1991). In almost every nation and continent, the previously continuous landscape is becoming a fragmented patchwork of wilderness and human development (Chiras et al., 2002). Habitats are being fragmented by agriculture, housing, commerce and other human development. Habitat fragmentation can lead to a reduction in biodiversity (the number of species in a given area) as useful or unique parts of the habitat are lost (Saunders et al., 1991; Blake 1991). Along with the obvious loss of habitat due to human development, fragmentation can also lead to a decrease in genetic diversity of the species in the fragmented habitat. A loss of genetic diversity has been theorized to reduce the fitness of a population and can lead to species extinction (Ledig, 1992)

Several factors can alter the genetic diversity of individuals in fragmented areas. When a population's numbers are small, it can be considered to be in a population bottleneck. A population bottleneck can be described as a drastic reduction in the size of the population. The individuals that survive the bottleneck will be the basis of the genetic diversity for future generations in the population. The smaller the number of individuals that survive the population bottleneck, the smaller the gene pool will be in future subsequent generations. With a small gene pool, there will potentially be a loss of variation in the genetic make up of individuals in the population. A bottleneck usually results in a loss of heterozygosity and polymorphism as low frequency alleles are lost from the gene pool. A potential indicator of past population bottlenecks is when the

number of alleles is lower compared to unaffected populations (Cornuet and Luikart, 1996).

Fragmented and small populations are influenced by other factors that can affect their genetic diversity. For instance, factors such as genetic drift and inbreeding, which reduce genetic diversity in populations, are usually problematic in small populations. Genetic drift is a random process where sampling between generations can ultimately fix or lose specific genes in a population. Inbreeding results when individuals have consanguineous matings. Conversely, other factors can increase genetic diversity in a population such as mutation and migration. Mutation is a change in the genetic sequence of an individual's DNA and is the ultimate cause of genetic differences. Migration in this text is the movement of seeds and/or pollen between populations. The overall genetic diversity in a population will depend on the strength and intensity of the varying factors as well as stochastic events.

The scope of this study will examine factors acting on variable sized island populations of white pine (*Pinus strobus*) and the genetic diversity which results. This study will use allozymes and DNA sequence variations (DNA length polymorphisms) in the chloroplast genome to estimate genetic diversity among and within insular white pine populations. The variable sized populations are located in northern Lake Michigan at different distances from adjacent populations. The group of islands is collectively known as the Beaver Island Archipelago (BIA). Perhaps the greatest utility of this study site is that the BIA is a running model. It is a conglomeration of discrete, variable sized populations that have been naturally fragmented for thousands of years. To explain, the islands were exposed from the Laurentide ice sheet around 15,000 thousand years ago.

Figure 1 The Beaver Island Archipelago

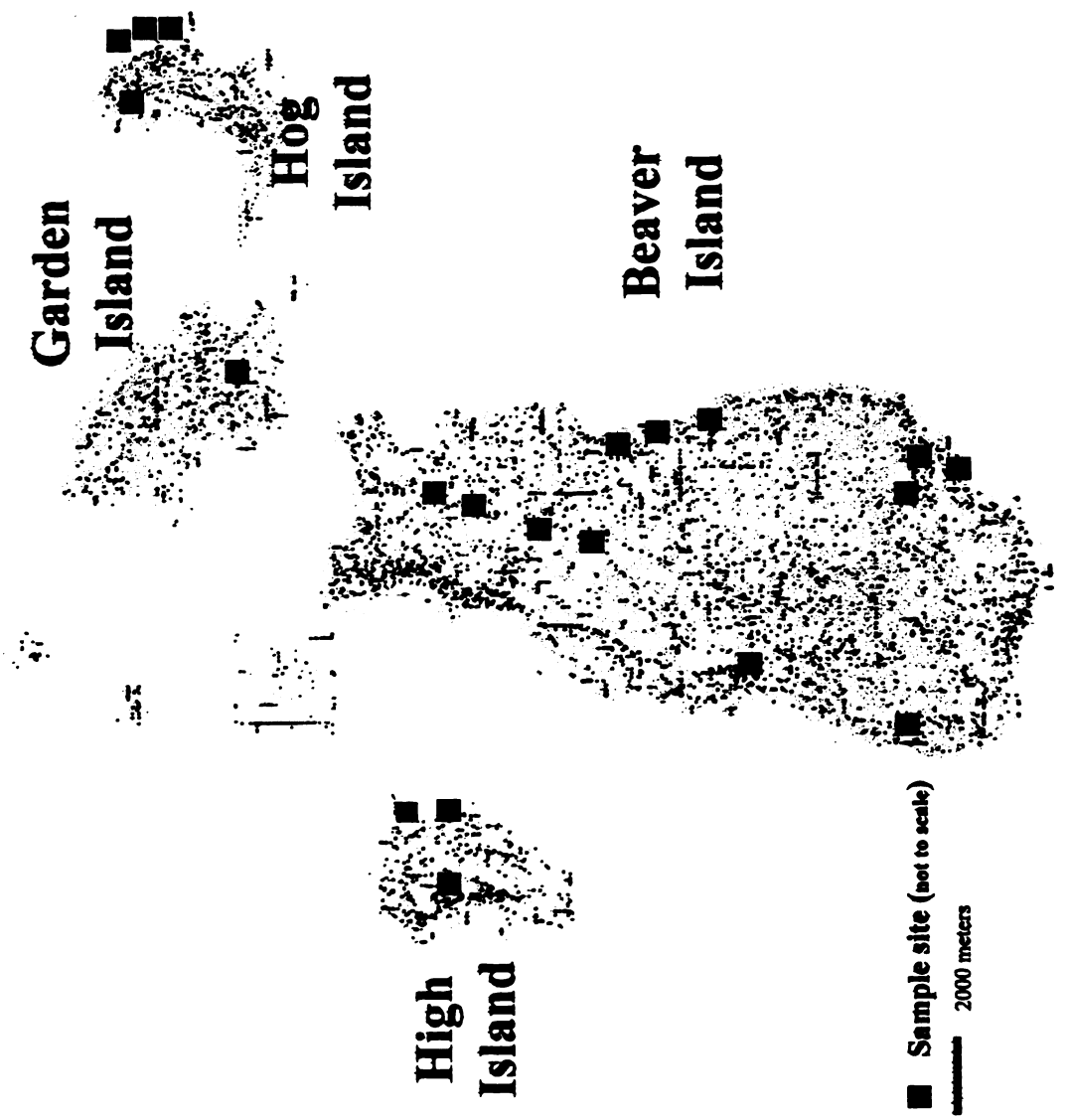
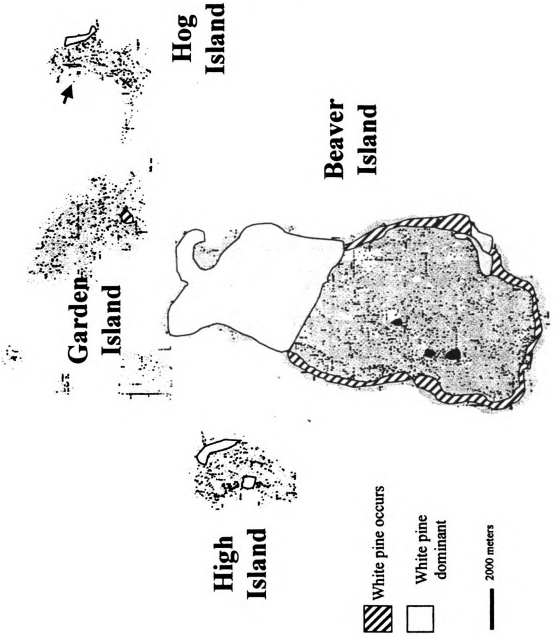


Figure 2 White Pine Distribution on the Archipelago



Then, around 10,000 years ago, the lake levels were dramatically reduced, (Hansel, et al., 1985) and the islands would have been connected to each other and also to mainland Michigan via a land bridge. The islands would have been part of mainland Michigan intermittently for 4,000 years until around 6,000 years ago when the lake levels reached a height similar to current lake levels. The Great Lakes reached a peak in lake levels around 4,000 years ago during the Nipissing period, when the lake levels were roughly 7m above present day levels (Hansel, et al., 1985). The lake levels remained high enough so that the islands would have been isolated before the Nipissing period. The lake levels have been at present levels for around 2,500 years. Based on the geological history, it is hypothesized that there will be a loss of genetic diversity among the archipelago. Moreover, there should also be a greater loss of diversity within the small islands which have smaller populations next to the Lake Michigan shoreline. Populations next to the shoreline would have been severely affected by the fluctuating lake levels.

Time scales are of importance when studying and /or managing a species. Here, we have a chance to study the effects of long-term fragmentation and possible isolation under natural conditions with a relatively well known start to ecological succession. It is uncommon to have a situation where the known ecological events can be compared with the genetic diversity which results (Comps et al., 2001). This study will use two types of assays to measure genetic diversity. Allozymes will be used to measure genetic diversity of BIA, which will add to the vast array of studies that have used allozymes to measure genetic diversity (See Hamrick and Godt, 1990). In addition, this study will measure genetic diversity with microsatellites, a relatively new tool, and will be one of the first studies to use chloroplast markers to examine white pine genetic diversity in fragmented

populations. This study can also be useful in that it will allow a direct comparison between the traditional allozyme measures and the more recent microsatellite techniques. Lastly, Moran's I statistic will be used to examine spatial autocorrelation trends in genetic markers in space among the archipelago, as well as within Beaver Island.

Forces Affecting a Population's Genetic Diversity

Selection: One mechanism hypothesized by Darwin to affect differences among individuals is termed "Natural Selection". Selection was one of the first mechanisms invoked to explain how the vast diversity of life found on this planet evolved. Basically, natural selection is effective when genetic differences result in different phenotypes. Different phenotypes will provide different abilities to organisms to survive; this is termed absolute fitness, whether or not an individual survives. Selection can also act to increase or decrease the number of surviving offspring. When survivorship is compared to other members in the population, it is termed relative fitness.

Detection of selection acting specifically on genes or gene products can be difficult. First, the effect of selection on a specific gene is usually weak so that the consequences of selection often takes many generations to make noticeable changes in a population. This problem can be compounded by the fact that the strength of selection can fluctuate from season to season and year to year. Therefore, without long-term observations it can be difficult to prove that selection is responsible for genetic differences among individuals or populations. Furthermore, linkage disequilibrium can obscure the effects of selection. Linkage disequilibrium is defined as two genes that are not in random association (Hartl and Jones, 1998). If genes that are neutral in benefit or even mildly deleterious are linked to beneficial genes, all linked genes increase in

frequency. Conversely, even beneficial genes can decrease in frequency if they are linked to extremely harmful or lethal genes. This is one of the problems with studying factors that affect individual loci. Selection is not viewed as a major force affecting the genetic diversity in this study. First, the huge amount of variability in white pine combined with the sampling scheme will not allow analysis of the effects due to selection. Second, some of the genetic markers used in this study are from regions of the genome that are non-coding, and therefore will not be directly affected by selection.

Mutation: The ultimate cause of genetic differences is mutation. Essentially, a mutation results in a new or unique DNA sequence. Any change that occurs in the DNA of germ line cells will be passed on to consecutive offspring. Most of the work on mutations have been done on humans. Most of the germ line mutations in human genes potentially represent errors of endogenous error-prone processes involving either physical, chemical, or enzymatic mechanisms (Cooper and Krawczak, 1993). There are many causes of mutations in the genetic material of an organism; ultraviolet light and cosmic radiation are natural mutagens and there are numerous anthropogenic chemical and organic compounds that are considered mutagens. Mutations also occur with some frequency as errors during DNA replication. During replication, the polymerase mechanism can slip, adding to or subtracting from the template sequence. A loss of nucleotides from the original sequence is called a deletion, when one or more continuous nucleotides are excised from the template sequence (Hale and Marghan, 1991). The addition of one or more nucleotides between two adjacent nucleotides in the existing DNA sequence is termed an insertion (Hale and Marghan, 1991). Mutations that result from a single nucleotide change are often called point mutations. The impact of point

mutations may seem trivial, but the addition or subtraction of a single nucleotide in an active gene can drastically change the structure/function of the resulting gene product. Fragments of DNA can also be inserted or deleted from areas of an organism's genome. These fragments are often called indels, a hybrid term put together to describe sequence length change when the direction of the change is unspecified. Moreover, it is possible for DNA sequences to be altered by transposition, where DNA sequences excise and insert autonomously. These sequences often leave behind DNA fragments in the "host" genome. Sequences of DNA can also be changed when viruses insert and later excise, leaving fragments of DNA behind.

Another form of DNA alteration occurs when pieces of a chromosome are moved or rearranged. Chromosomal rearrangement is not uncommon during meiosis and is termed chromosomal crossover. In addition, the structure and number of one or more chromosomes can change due to improper segregation of gametes. With unequal segregation and/or crossing over, perhaps more important than creating unique DNA sequences is the possibility of creating multiple copies of genes, freeing the second gene from the effects of selection. Multiple gene copies allows mutations to alter the second gene/gene product while the metabolic need is still met by the unmutated gene.

Mutations constantly create new alleles or DNA sequences, however, the initial frequency of new mutations (alleles) is low (1/2N with N= the number of the diploids in the population). Therefore, the effects of mutations on changing genetic diversity are weak in large populations. In small populations, the effects of mutation can be quite noticeable and effective in changing the genetic diversity. In the field of population genetics the cause of DNA alteration is usually not the primary interest, rather,

population genetic studies are concerned with how these genetic differences change in frequency.

Recombination: The act of recombination often occurs during chromosomal crossing over. Recombination rarely occurs in the genomes of mitochondria and chloroplasts and consequently should have no effect on the chloroplast markers used in this study. The allozyme markers could be subject to recombination and a recombination event would be detected in the laboratory.

Genetic Drift: A factor that can affect genome-wide genetic diversity in fragmented population is termed genetic drift. Genetic drift can be the most important factor controlling genetic diversity in small populations (less than 50 individuals) (Hartl and Clark, 1997). Genetic drift can occur between seed establishment and reproductive maturity. If many individuals are unable to reach reproductive maturity, then the possibility exists for some genes to be lost or fixed from the population. Genetic drift also occurs between generations due to random fluctuations in gametic allele frequencies. In other words, an almost infinite number of gametes are produced each generation, but only a specific number of organisms result from a sample of the gametes. With fewer individuals, the possibility of allele frequencies changing between generations increases. For example, one individual plant can produce millions of pollen spores in a single season but often produces a smaller number of recipient female gametes. This dissimilar ratio of gametes results in a greater possibility of a sampling phenomenon dramatically changing the allele frequencies. With small populations, consisting of a few individuals, some alleles could be lost or fixed in every member in the population, due to the

sampling of gametes. Genetic drift tends to decrease genetic diversity found within populations, resulting in a loss of rare alleles and an increase in homozygosity.

Genetic drift changes the genetic diversity within populations but can also alter the genetic diversity found among populations. Since drift is a random process, fragmented populations affected by drift can be fixed for different allele frequencies. The effect would be for drift to increase the genetic diversity found among populations as different populations loose or become fixed for different alleles.

Isolation by Distance / Inbreeding: A phenomenon observed by Alfred Russel Wallace over a century ago was that over geographical distances differences among species' composition tend to occur. This observation helped lead to the theory of natural selection which is based on the fact that different phenotypes will have greater or lesser fitness (survival) values in different areas. This phenomenon of detectable differences occurring over space can be further advanced by a theory in population genetics; with increased geographic distance there will be an increase in genetic differences between populations. This theory is based on the fact that due to the limit of migration, individuals will tend to mate with their neighbors thereby increasing the frequency of local alleles. The phrase isolation by distance was described by Sewal Wright (1943; 1946) to explain the accumulation of local genetic used under geographically separated areas. The theory of isolation by distance has been developed by Wright (1946), Malecot (1948) and others. The most severe result of isolation by distance is inbreeding. When plants mate in close proximity there is the chance inbreeding will occur. Inbreeding is the gauge used to decide whether individuals are mating with relatives or more distant members of the population. Inbreeding can be especially dramatic in small populations with a restricted

range resulting from habitat fragmentation. With small fragmented populations there is an increased chance that individuals will have consanguineous matings. Along with consanguineous matings, selfing can greatly increase the effects of inbreeding.

With inbreeding, the probability that two recessive deleterious alleles will come together in an individual increases. However, in large populations the effects of the deleterious alleles will often be masked as heterozygotes. Inbreeding is usually not detectable or problematic in large populations. The effects of inbreeding can be more easily seen in small populations because the number of deleterious alleles will be relatively substantial compared to large populations. Populations with individuals homozygous for deleterious alleles often show a higher mortality rate and lower fecundity (Hartl and Clark, 1997; Chiras et al., 2002) .

The effect of inbreeding can be a major problem with fragmentation which can prevent immigration of individuals from other areas, especially when there is inhospitable habitat between populations. Limited migration results in mating with neighbors, who over generations will be more related. Continued inbreeding will result in a reduction of heterozygosity. This loss of heterozygosity can be measured within a population or among groups of populations using Wright's F statistics.

Biparental Inbreeding: In a species like white pine, where reproduction does not often include selfing, inbreeding can still be a problem. Inbreeding can be biparental. Biparental inbreeding can be important in the case of plants because individuals tend to grow in close proximity to their parents and siblings. Matings between progeny of the same two parents are full sibs while mating between individuals with the same maternal or paternal parents are half siblings. Mating with one's parents or siblings often results

in having two copies of the exact same alleles which would be identical by decent (autozygous). So, with biparental inbreeding there is an increased chance for individuals to be homozygous for deleterious alleles.

Any form of inbreeding tends to reduce genetic diversity within populations but it does not change the gene frequency within a population. In other words, the same number of alleles will still be found in the population as homozygotes, but there will be less of a potential for loss of any alleles in the populations as with genetic drift. Unlike genetic drift, the effects of biparental inbreeding can decrease genetic diversity and increases spatial structure even in populations that are large in size.

Migration: In the context of this study migration will mean gene migration which involves the movement of genes via pollen or seed. Migration can be viewed in terms of the influence of one population from another. The net effect of migration on the receiving population is determined by several factors: the initial gene frequency in the receiving population, the rate of immigration and the frequency of the allele in the donor population. In one very simple model, migration can be expressed mathematically (Hartle and Clark, 1997)

$$P`=(1-M)P+MP^*$$

Where P` is the gene frequency in the next generation, M is the migration rate, P is the original gene frequency in the receiving population and P* is the allele frequency in the donor population. Pollen tends to travel long distances, especially in wind pollinated (anemophilous) plants. Even plants that rely on animals to disperse pollen can have extensive pollen flow between individuals and populations. Pollen can contribute to changes within a population's genetic structure (Ledig, 1998). Seeds are usually larger,

and therefore usually travel shorter distances than pollen does in anemophilous species. When there is a high amount of gene flow, the genetic diversity will usually be high within populations, and there will be a low amount of divergence or diversity among populations. With low gene flow, individual populations will be subdivided into a mosaic of gene distributions or deme's and there will be an increase in the genetic diversity found among populations. Migration can decrease the genetic diversity found among populations. This can happen in two ways: first, if there is pollen from one population entering all populations, (one way migration) recipient populations will tend to have the same genotype; second, if populations are consistently exchanging pollen and seeds, there will tend to be a blending of genotypes among all populations.

Fragmented populations can avoid the loss of genetic diversity associated with isolation/inbreeding, bottlenecks, and genetic drift, if there is a high amount of gene flow. By introducing new alleles or DNA sequences, migration usually increases the genetic diversity within populations. Migration will be examined by looking at the occurrence of private alleles, spatial autocorrelation measures and the levels of heterozygosity.

The study of population genetics involves the genetic differences among individuals in one or more populations (a population being a group of breeding individuals in a discrete area). A population's genetic diversity is influenced by several factors such as population size, mating system, geographical distribution, habitat fragmentation and other factors such as selection, mutation, migration and genetic drift.

Table 1 Comparative effects on the genetic diversity of populations

	Diversity within populations	Diversity among populations
Migration	Increase	Decrease
Genetic drift	Decrease	Increase
Inbreeding	Decrease	Increase
Fragmentation	Decrease	Increase
Mutation	Increase	Increase

Table 2 Comparative effects on genetic measures of populations

	Fit	Fis	Fst
Migration	Decrease	Decrease	Decrease
Genetic drift	Increase	Increase	Increase
Inbreeding	Increase	Increase	Increase
Fragmentation	Increase	Increase	Increase
Mutation	Increase	Decrease	Increase

Levels of Genetic Diversity

Genetic diversity exists on three levels, within individuals, within populations and within a species. First, there can be genetic diversity within individuals, for example, diploid and polyploid individuals can have multiple alleles for a particular gene. An allele is a particular form of a gene; diploid organisms have two copies at every gene. Individuals with both alleles producing identical gene products or DNA length are considered homozygous, whereas individuals that have two different alleles are heterozygous for that locus. Individuals that are heterozygotic for a particular locus have more genetic diversity than individuals that are homozygotic.

The second level of genetic diversity is at the population level. Genetic differences at the population level can be measured at two levels, within, and among populations. Depending on the scientific question being tested, it may be better to focus on one type of diversity or the other. In populations, DNA markers (discussed below) can be used to estimate the frequency of alleles among individuals in the population(s).

Measurements of genetic diversity (descriptive statistics) such as the percentage of polymorphic loci (P), number of alleles at a loci (A), the number of polymorphic alleles (Ap), genetic diversity (He) and observed heterozygosity (Ho) can be used to estimate if the current diversity is due to particular forces acting on the population(s) now or in the past. Spatial autocorrelation of alleles in plant populations can also be informative in understanding the influences on a population.

The third level of genetic diversity is the species level. Genetic diversity at this level must often be addressed using measurements among populations because of the vast number of individuals in a species. Descriptive statistics can also be used to measure diversity at the species level. The diversity measures are compared among populations over the range of a particular species to look for patterns or correlations that could suggest selection, migration, isolation or recent speciation events.

Genetic Markers Used in This Study

The first markers used to differentiate between organisms and varieties were observable differences such as color and shape. One of the first molecular or genetic markers used to test for diversity was allozymes (For example, Soltis et al., 1983). Today, allozymes are perhaps one of the least expensive and effective markers used to estimate population genetic diversity. The use of allozymes is also beneficial because the results can be compared to many other organisms (summarized in Hamrick and Godt, 1990). However, allozyme loci may not be able to display the true diversity of an organism's genome. It is possible for allozymes to be identical by state, meaning that the allozymes are the same allele (migrate the same on an electrophoretic medium) but they could have different DNA sequences. The difference in alleles that are identical by state

and identical by descent (replica's of a single gene from a common ancestor) cannot be fully distinguished using allozymes as genetic markers.

In order to more specifically characterize differences in the genetic makeup of individuals, assays of the actual DNA of organisms were devised. With the advent of the Polymerase Chain Reaction (PCR), (Mullis and Faloona, 1987; Saiki et al., 1987) it became possible to amplify specific areas of an organisms genome. Today, there are numerous types of genetic markers that use PCR to estimate genetic diversity. One such type is termed microsatellites or simple sequence repeats (SSR's). Microsatellites are areas of an organism's genome, usually in non-coding regions (Hancock, 1985), where there are repeats of a specific nucleic acids motif. For example, (ACACACACAC) would be described as a five repeat of an adenosine and cytosine motif. The number of nucleotides in a repeat can vary from one to many. Microsatellites have been used in numerous other studies (Hearne, et al., 1992; Morgante and Olivieri, 1993; Queller et al., 1993. Echt et al., 1996; Rajora et al., 2000; Walter and Epperson, 2001). When the DNA sequence of flanking regions (both upstream and down stream) of an SSR are determined, specific primers can be made to amplify the microsatellite sequence. The use of SSR's in molecular studies is useful because the specific length of the SSR can be consistently amplified and there are usually high levels of polymorphisms (Powell, et al., 1996).

Genetic diversity can be measured using nuclear DNA or using the DNA of organelles such as mitochondria and chloroplasts. The different genomes (organism or organelle) have their potential usefulness. Nuclear DNA is inherited from both the mother and father, (biparentally) while mitochondria and chloroplast DNA can be inherited (uniparentally) either maternally, as mitochondria are in many organisms, or

paternally as is the case of chloroplasts in the genus *Pinus*. Organelle DNA is obviously in greater abundance since a single cell has but one pair of genes at a nuclear locus, and potentially hundreds of copies of organelle's DNA. This fact allows for more consistent amplification of organelle DNA under *in vitro* conditions compared to nuclear DNA.

Mutation could be a major cause of variation in SSR diversity examined in this study. In vertebrates it has been suggested that regions with repeats have a tendency to increase in the number of repeats over time (Weber and Wong, 1993). A potential drawback to SSR's is that a mutation may occur at the primer binding site, which results in no amplification or a null allele. With nuclear DNA this could result in heterozygotes being scored as homozygotes. With organelle DNA the result would be an inability to amplify SSR's in an individual. If a mutation occurs in the primer binding region it will in effect result in a underestimate in the genetic diversity.

Natural History of White Pine

Eastern white pine can live up to 450 years and can reach heights of 90-180 feet, has cones usually around five inches in length (4-8 inches) and needles which persist for 2 years, rarely 3 (Preston, 1989). Mature white pine has a shape that perhaps can best be described as irregular, with occasional branches protruding out in seemingly random directions. Mature trees are usually broad crowned with a single straight trunk. White pine is also one of the tallest trees in northeastern North America, making it conspicuous as mature trees emerge above the forest forming a super canopy.

The current distribution of white pine is from Minnesota to Maine through the Great Lakes region and from Newfoundland to Georgia through the Appalachians (Preston, 1989). There are also isolated populations of white pine south of the Great

Lakes region but these are usually in isolated habitats such as lake dunes (Daniel and Sullivan, 1981). One can also observe white pine on almost every lake shore throughout BIA. Historical evidence suggests that white pine survived the last glacial age in what is now the southeastern United States (Davis, 1981; Pieliou, 1991). Pollen records indicate that white pine has been in the Great Lakes region for several thousand years (Jacobson, 1979; Macdonale et al., 1998)

White pine is found consistently in two forest types in the Great Lakes region, boreal and northern hardwoods. It is also occasionally found in bog and northern white-cedar communities as well. White pine's strategy for surviving in boreal forests in the Great Lakes region is for individuals to survive near water, or on rock outcrops where fire intensity is low (Frelich and Reich 1995). During prehistoric times, fires would kill invading white cedar, spruce and fir trees but would only scar the large thick barked pines (Frelich and Reich 1995). White pine could then establish in areas where fire destroyed the existing vegetation, producing even aged stands of white pine. Further inland, depending on the time of year of the fire, aspen and birch could be the first trees to succeed in after a burn. Since white pine is moderately shade tolerant it could gradually stock the under story of adjacent aspen or paper birch stands with seedlings for decades after the fire. This type of establishment would result in uneven aged stands of white pine. It was also shown in Wisconsin (Swain 1978) that white pine increased in frequency during wetter climactic times when the more fire adapted species such as paper birch, oaks and aspens began to decline. Pollen analysis suggests that white pine was able to succeed the early successional fire adapted trees. (Swain 1978)

Many forests described as northern-hardwoods contain white pine. Some areas have soil with nutrients and permeability that are unfavorable for northern hardwood trees but would support white pine. White pine has a generally high tolerance for soil type and nutrient availability. (Smith and Hinkley, 1995; Whitney, 1986). This range of soil tolerance allows white pine to persist in habitats that are widely distributed across the landscape of the northern Great Lake states. This would also allow for white pine stands to act as a seed and pollen source to invade any micro-disturbance such as wind throw areas. Furthermore, white pine's soil tolerance would allow for it to persist in other areas that are not optimum for northern hardwood species such as glacial outwashes (Daniel and Sullivan, 1981).

Reproductive Mechanisms

The mating system is an important characteristic because it has an effect on the distribution of genotypes of populations. White pine, like most conifers has a high amount of genetic diversity (Hamrick and Godt, 1990). Reproduction in white pine, as in most pines, is sexual. There is no reported coppice reproduction in white pine, no layering of branches to form new trees has been reported and no documentation of apomixis is in the current literature. White pine is an outcrossing, monecious, anemophilous gymnosperm. In white pine populations, the pollen is released in the spring from male strobili. The pollen is captured in the female strobili and adheres to a sticky fluid about the micropyle. (Raven et al., 1992). As the fluid evaporates, the pollen is drawn into the micropyle and at this time a pollen tube forms and slowly digests its way through tissues toward the megaspores (Raven et al., 1992). Fertilization occurs the following year. Then after several more months, winged seeds form that will be released

in autumn almost 1.5 years after the pollen was released. At maturity cone scales separate and the winged seeds are released and can be carried considerable distances by wind.

White pine's reproductive mechanism helps to maintain high levels of genetic diversity. First, mechanisms that increase genetic diversity can be found at the gametic interface: female cones secrete a pollination droplet between the cone scales, and pollen lodges on the droplet and will be drawn in (reabsorbed) and eventually will be deposited in the pollen chamber. If pollen is plentiful, two to four pollen grains can be lodged in the pollen chamber (Ledig, 1998). One to many archegonia can be formed in a egg (Ledig, 1998). When extra pollen adheres to the female cone, this allows sampling (randomization) so that not all seeds, even in a single cone will be as likely to have the same father. White pine is a primarily out crossing anemophilous species with pollen that has the capacity to travel long distances; pollen grains have two small air bladders that aid in the pollen remaining air borne for long periods of time. An increase in diversity results from the fact that millions to billions of pollen grains are released in a white pine population, thereby increasing the sampling of gametes via pollen flow. White pine has an out crossing rate that approaches 1.0 (Beaulieu and Simon, 1994); with low amounts of selfing there will be a low amount of inbreeding, which should increase/maintain genetic diversity.

Beaver Island Archipelago

The study site, BIA provides a model system for studying variable sized populations of white pine and the genetic diversity that results from isolation and variable population size. Not only does this system contain variable sized and spaced populations

of white pine, but also the relative starting point of ecological succession can be estimated. During the last ice age BIA, as was most of the Great Lakes region, was covered by the Laurentide ice sheet. As the ice sheet melted, proglacial lakes were formed. The proglacial Lake Algonquin began to form around 16,000 years ago (Pielou, 1991); today, the remnants of proglacial Lake Algonquin consists of Lake Michigan and Lake Huron. Eventually, the Algonquin lake levels were dramatically lowered as the retreating lobes of the glacier revealed new outlets to the ocean for the glacial lakes. It is at this time of low water, around 10,000 years ago, that the BIA was connected to what is now the northern lower peninsula of Michigan. It was also at this time that white pine was migrating through the Great Lakes area (Pielou, 1991; Macdonale, 1998). The BIA was then connected as a peninsula or a chain of islands to mainland Michigan intermittently for around 3,500 years. The BIA has been isolated for roughly the last six thousand years. The lakes reached a maximum height about 4,000 years ago during the Nipissing period, about seven meters above current levels. During the Nipissing period the islands would have been much smaller in size. After the Nipissing period, the lake levels decreased for about two thousand years and have remained relatively stable for the last 2,500 years.

Native Americans frequented BIA perhaps as soon as it was revealed. The effect that Native Americans had on the ecosystem is unknown but assumed to be minimal. The first European's to visit BIA were French trappers in the17th century (1650's). It has been suggested (Hatt, 1950) that there was some colonization and farming by the French in the seventeenth century. There would have also been some Native American's on BIA at that time. The islands were reinhabited by lumbermen and some settlers during the

1800's. The BIA proved to be in a logistic place one-half way between Detroit and Chicago. 'The old steamers would stop to refuel in the deep harbors of the islands in the archipelago especially from 1840-1889'(Hatt, 1950). Logging was mostly for cordwood until fossil fuels replaced wood as an energy source in the late 1800's. Logging continued until today, where single tree selection is a common practice on Beaver Island.

Four islands in the archipelago harbor white pine populations: Garden Island, Hog Island, High Island, and Beaver Island (Figure 1, page 1)

Garden Island: 7.8 square miles, 20.7 miles of shore line. There were Native American residents on the island until 1839. Garden Island had yearlong European-Americans inhabitants during the 1800's. The island has been uninhabited since 1938 (Hatt, 1950). Garden Island had the smallest number of white pine trees of any of the four islands. White pine is found only in one bay, Northcut bay. This is a sandy bay with a large area for camping. White pine is also found among otherwise pure stands of northern white-cedar (*Thuja occidentalis*) by the lakeshore. White pine is in the inner dunal area with white-cedar, balsam fir (*Abies balsamea*) and other inner dunal plants. White pine is also found interspersed in the forest in the watershed of Northcut bay with red pine (*Pinus resinosa*) and quaking aspen (*Populous tremuloides*). White pine regeneration is limited to the dune areas, where there is a slow succession toward the lake from forest to dunal forbs, to sand, to the lake.

Hog Island: 3.9 square miles, 16.0 miles of shore line. Hog Island probably never had permanent settlers but was logged most likely during the late 19th century. White pine is prominent on the lakeshore on the northern half of the east side of Hog Island. The stand of white pines extends back into the island several dozen meters.

White pine is found growing in a near boreal forest of balsam fir, white spruce and white cedar. There are several white pine trees (around 13) on the northwest side of Hog Island. In both populations, white pine reproduction is limited to the dune areas, where there is a slow succession toward the lake from forest to dunal forbs, to sand.

High Island: 5.8 square miles, 12.5 miles of shore line. Hog Island was settled and inhabited by Mormon settlers in the 1800's. Evidence of their agriculture and settlement still remains. White pine is found on the east and west lakeshore on the northern part of the island. The east side white pine population ranges through two large bays and rounds the northeast corner until the tree composition changes from a near boreal forest to a deciduous forest. The white pine stand on the west side is found at the southern end of the large sand dunes. White pine is intermixed with red pine in a near boreal community. The population on the west side is much smaller in size. There is limited white pine reproduction on both populations in the inner dunal areas. White pine reproduction is limited to the dune areas, where there is a slow succession toward the lake from forest to dunal forbs, to sand.

Beaver Island: 58.4 square miles, 41.6 miles of shore line. Beaver Island had Native American residents when French explorers visited the island in the 1600's. A French colony did not survive but the island was visited by trappers and explores, and was recolonized in the 1800's by Mormons. The population of Mormons was estimated to be between 1300-2600 people in 1855. The island human population never again grew large (Hatt, 1950) until, today, where there are about 450 year round residents with thousands of seasonal visitors.

Beaver Island had the greatest number of white pine in the archipelago, growing in many bays at the edge of the Lake Michigan shore, along most inland lake shores, on old beach bluffs representing higher lake levels, and in almost pure stands in some meadows and abandoned agricultural areas. It is quite abundant in some old fields, and now grows on soils unsuitable for agriculture. White pine reproduction can be quite prolific in some areas of Beaver Island. The northern half of Beaver Island has a large contingent of white pine interspersed in almost every plant community, from bogs to fields, to northern white-cedar swamps to northern hardwood forests, but in the southern part of the island, white pine is limited to lakeshores and clearings.

Materials and Methods

Sampling: The BIA was surveyed using an airplane during the first week of April 2000. At this time no deciduous trees had leaves, and white pine stands were identified by vegetative patterns and by the emergent silhouette shape of individual white pine. White pine is found on four islands: Beaver, High, Hog and Garden Islands. The approximate size of white pine populations in area, number of trees, as well as location, were mapped.

The sampling procedure was to locate specific areas within the white pine populations, where 13-20 contiguous trees would be sampled. The sampled areas are hereafter referred to as plots. The exact area to be sampled was chosen so that roughly 20 trees would be included in the plot. Trees with a diameter less than 12 cm were not sampled. Plots were spaced throughout the archipelago to allow estimates of genetic diversity within and among islands (white pine populations). Most plots were located in areas where white pine was a dominant or codominant tree. From May until July 2000, 19 plots were sampled throughout the four islands containing white pine, except Garden Island. White pine on Garden Island was not a dominant tree, but was intermixed with red pine, and quaking aspen, in the upland areas and occurred sporadically with northern white-cedar, near the shoreline. All sampled trees on Garden Island are considered a single plot. Four hundred twenty eight trees were sampled from the four islands. Each tree was tagged with sequentially numbered aluminum tags and the plot was marked using a global positioning system (GPS) (Geotracker II). Needle and bud tissue were taken with a hand prunners, a pole prunner or with a shotgun depending on the height of the lowest living needle tissue. Several grams of needle and bud tissue were collected from each tree. The plant material was divided into roughly two halves with each half

being wrapped in a wet paper towel and kept on ice until returning to Michigan State University. Upon returning to Michigan State University, one sample was frozen, the other was refrigerated. The refrigerated samples were used for the allozyme analysis, the frozen samples were used for extracting chloroplast DNA.

The sampling scheme was constructed to cover the entire archipelago to give an estimate of the genetic diversity of the archipelago as a whole. The sampling scheme also should provide an estimate of the genetic diversity found within each island. There were 12 sampling plots on Beaver Island. High Island had 3 sample plots, one on the west side and two at the north and south ends of the eastern population. Hog Island had four sample sites. Almost every tree was sampled from the western population (this was the smallest plot with 13 trees), and three plots were sampled from the eastern population, one at the northern end of the eastern white pine population, one in the middle, and one plot from the southern most part of the eastern shore population. The Garden Island population was sampled along transects throughout the entire population because white pine was found to be too dispersed to group trees into a plot. It is estimated that 15%-25% of the entire Garden Island population was sampled.

Allozyme Extraction and Genotyping: The allozyme laboratory work was done in South Korea in the lab of Dr. Myong G. Chung. Collected needles were kept at 4 °C and then shipped to his laboratory, and stored at 4 °C until protein extraction. Of the twenty-one putative loci systems assayed by Dr. Chung, five were polymorphic: Pgm-1, Pgi-2, Mdh-2, Tpi-2 and Aat-3.

The following protocol was reported by Dr. Chung:

Leaf samples were cut and crushed with a mortar and pestle, and phosphate-polyvinylpyrrolidone extraction buffer was added (Mitton et al., 1979). Enzyme extracts were absorbed onto 4mm x 6mm wicks cut from Whatman 3MM chromatography paper, and stored at –70 °C until allozyme analysis. Starch gels (11.5 %) were stained for 13 enzyme systems, which produced 21 putative loci. A Poulik buffer system, a modification (Haufler, 1985) of Soltis et al. (1983) "system 6" was used to resolve alcohol dehydrogenase (*Adh*), fluorescencent esterase (*Fe*), glutamate dehydrogenase (*Gdh*), leucine aminopeptidase (*Lap-1, Lap-2*), phosphoglucomutase (*Pgm-1*), and triosephosphate isomerase (*Tpi-1, Tpi-2*). A histidine citrate buffer system, a modification (Chung and Kang, 1994) of Soltis et al. (1983) "system 11" was used to resolve isocitrate dehydrogenase (*Idh*), formate dehydrogenase (*Fdh*), malate dehydrogenase (*Mdh-1, Mdh-2, Mdh-3*), 6-phosphogluconate dehydrogenase (*Pgd*), and phosphoglucoisomerase (*Pgi-1, Pgi-2*). Soltis et al. (1983) "system 7" was used to resolve diaphorase (*Dia-1, Dia-2*) and aspartate aminotransferase (*Aat-1, Aat-2, Aat-3*). Stain recipes were taken from Soltis et al. (1983), except for diaphorase (Cheliak and Pitel, 1984). The genetic basis of allozyme banding patterns was inferred from segregation patterns with reference to typical subunit structure (Weeden and Wendel, 1989; Wendel and Weeden, 1989) and conceptual methods described in Gillet (1988). Putative loci were designated sequentially, with the most anodally migrating isozyme designated '*1*', the next '*2*', and so on. Similarly, alleles were designated sequentially with the most anodally migrating alleles designated with superscript 'a'. (M.G. Chung Pers. Comm)

DNA Extraction and Genotyping

Chloroplast Microsatellite Markers: Six different primer pairs (pt9383 cp2; pt15169 cp3; pt30204 cp5; pt36480 cp6; pt63718 cp11 and pt71936 cp12) designed from known sequences of black pine, *Pinus thunbergii* (Vendramin et al. 1996), were used to amplify specific regions of white pine's chloroplast genome. The forward strand of each primer pair was labeled with one of three phosphoramidites: 6-FAM, HEX and NED (ABI) for detection of alleles. This permitted multiplexing and simultaneous analysis of polymorphic primer pair loci.

DNA Isolation: Chloroplast DNA isolation was conducted in the forest genetics lab, Michigan State University. The protocol consisted of first weighing ~120 milligrams of needle tissue for each tree. The needle tissue was then cut finely with a

razor, and placed into a 2 ml screw cap centrifuge tube between one ceramic bead at the bottom of the tube and another bead on top of the needle tissue. Then, 750 ml of CTAB buffer (Tris/EDTA/NaCl/CTAB) was added to the sample. The amalgamate in the tube was placed into the FastPrep FP 120 (Savant Bio 101) cell disrupter for 2 cycles of 45 seconds on speed setting 4.5.

Afterwards, the samples were placed into a water bath (GP-200 Neslab) at 65 degrees centigrade for 20-30 minutes, to further aid in cell lyses. The samples were then taken to the Genomics Technology Support Facility at Michigan State University using the automatic DNA isolation system AutoGen 850 α(AutoGen, Inc.) for DNA isolation, applying the plant protocol "Plant tissue DNA (system 4)". The dry DNA was then placed into 100 ml of TE buffer solution, and the concentration was determined with a Hoefer DyNA Quant 200 flurometer. The DNA was then diluted with water to approximately 5ng per ul. The modal yield of DNA was around 500 ng/μl but sample DNA concentrations had a wide range from less than 10 ng/μl to over 3000 ng/μl.

PCR Amplification: Polymerase chain reaction amplification was conducted in a total volume of 15 μl containing 30ng of template DNA and 9 μl of PCR buffer. The PCR buffer was a PFU/SCR buffer consisting of 20mM Tris-HCl, pH 8.75, 10mM $(NH_4)_2SO_4$, 10mM KCl, 2mM $MgSO_4$, 0.1mM Cresol red, 0.1% Triton X-100, 10% bovine serum albumin, and 6% sucrose. The reaction tube contained 200 μl of each dNTP, 3.5 mM MgCl, 200 nM of each primer and 0.04 units of Amplitaq Gold (Perkin-Elmer). The PCR reaction was conducted using a ptc-100 Programmable Thermo Cycler. The protocol began with two cycles at 94°C for one minute (denaturing) 65°C for one minute (annealing) and 70°C for 35 seconds (extension) followed by 18 cycles of 93°C

for 45 seconds (den.), 64°C for 45 seconds (ann.) with 0.5°C decrease during the remaining cycles, and then 70°C for 45 seconds (ext.). This was then followed by 20 cycles at 92°C for 30 seconds (den.), 55°C for 30 seconds (ann.), and then 70°C for 20 minutes (ext.). At least 7% of all samples were checked for a PCR product using a 2.5% agarose gel.

Three PCR products with different fluorescent labels were then combined into a single capillary tube and run on a 3100 Genetic Analyzer (Applied Bio Systems) at the Genomics Technology Support Facility at Michigan State University.

Sample genotypes were viewed using an OS 9.2 Macintosh computer running Genescan ver. 2.1. The lab protocol and techniques allowed for one base pair differences to be resolved. At least 10% of the polymorphic primer pairs were repeated in order to check for consistency. Sample genotypes were recorded and polymorphic primer pairs were combined to form a haplotype for each tree.

Statistical Analysis of Allozymes: For the analysis of allozyme diversity, each locus was considered polymorphic if two or more alleles were detected. Of the 21 enzyme systems only five were polymorphic (Pgm-1, Pgi-2, Mdh-2, Tpi-2, Aat-3). Allozyme analysis was conducted using these five polymorphic systems. The program Fstat (Goudet, 2001) was used to measure allele frequencies among the islands and plots. Standard genetic measures (descriptive statistics), *F*-statistics (F, f and θ as in Weir, 1996), and Nei's genetic distance and genetic identity (Nei, 1978) were calculated using Lewis and Zaykin's (2000) GDA program both among islands and within each island. Descriptive statistics include the average sample size (n), proportion of loci polymorphic (P), average number of alleles per locus (A) and polymorphic locus (Ap), expected

proportion of heterozygotes (He), observed proportion of heterozygotes (Ho), and inbreeding coefficient (f). Wright's standard F statistics estimate the parameters f (corresponding to Fis), F (Fit), and Theta (Fst). For the allozymes analysis, F-statistics bootstrapping (1000 replicates) was done to obtain 95% bootstrap confidence intervals for the parameters f (=Fis), F (=Fit), and Theta (=Fst). The allozyme allele frequencies among islands were compared to Hardy-Weinberg (H-W) expected values using a chi-square tests in GENEPOP (Yeh and Boyle, 1997) to measure the relative excess of homozygotes or heterozygotes compared with panmictic expectations

Spatial autocorrelation was conducted for averaged allele frequencies for each polymorphic loci among plots using SAAP ver. 4.3 (D. Wartenberg, 1989). For diallelic loci, only one allele was considered because the second allele would contribute identical information. For multiallelic loci, all alleles at that locus were used for analysis. Spatial autocorrelation analysis was conducted on the average allele frequencies of the 20 plots and a separate analysis was done within Beaver Island. The analysis of the Beaver Islands plots was conducted because the number of joins in the smaller distance classes would be influenced by the results from within Beaver Island since most plots (12/20) were found within Beaver Island. The spacing of plots allowed for seven distance classes throughout the archipelago, and five distance classes within Beaver Island. Moran's I statistics (Sokal and Oden, 1978) were calculated for each of the distances classes by

$$I=N\Sigma\Sigma(w_{ij}z_iz_j) / (\Sigma\Sigma w_{ij}\Sigma zi^2)$$

Where N is the number of plots, Wij is a join in the weighting matrix, where wij is set equal to 1.0 if the ith and jth plots are in the distance class and zero otherwise. Zi= xi-x, Zj= xj-x and the variables xi and xj are the genotypic scores for the ith and jth plots

respectively and x is the mean score for all plots. Each I value was used to test for significant deviations from the expected values E(I) = -1/(N-1) (Cliff and Ord, 1981), under the random distribute null hypothesis. In addition, the I values for each allele in a distance class were combined and averaged to create a standard normal deviate (SND) to test for significance within distance classes. A significant positive SND indicates that plots in the distance class have similar gene frequencies. A significant negative value indicates that the plots in the distance class have gene frequencies more dissimilar than expected by chance.

Statistical analysis of Microsatellites: Statistical analyses were conducted using polymorphic locations of the chloroplast genome. Three primer pairs (pt15169; pt30204; pt63718) were polymorphic and all analyses were conducted using the polymorphic primer pairs. For each population and plot, standard diversity measures and *F*st (Wright, 1965) were computed using the Lewis and Zaykin (2000) GDA program both within and among islands as was done with the allozymes. Since the microsatellite data was treated as haplotypes, the following measures were estimated, the proportion of loci polymorphic (P), and polymorphic locus (Ap), observed proportion of heterozygotes (Ho), and inbreeding coefficient (f) were not conducted. For haplotype data, the average number of alleles per locus (A) is the average number of haplotypes and He is gene diversity. The program Fstat (Goudet, 2001) was used to estimate Rst and to give haplotype frequencies among the islands and plots.

Abbreviations for Islands and Plots

Island abbreviations:

Garden Island.....Ga
Beaver Islands.....Be
High Island.....Hi
Hog Island.....Ho

Plot Abbreviations:

Garden Island.....GI
Hog West.....Ho-W
Hog North East.....HN-E
Hog Middle East.....HM-E
Hog South East.....HS-E
High South East.....HiS-E
High N. East.....HiN-E
High West.....Hi-W
Powerline.....PL
Blue Trail.....BT
Foot Bridge.....FB
French Bay.....FBy
Green's Bay.....GB
Wujek.....WK
CMU.....CMU
DUDA.....DA
Font Lake.....FL
Barney's Lake.....BL
Sloptown.....SN
Airport.....AT

Results

Allozyme genetic diversity measures among islands, Tables 3-10

Genetic diversity measures of allozymes for all islands are found in Tables 3-10. Table 3 shows the number of alleles found on each island. The number of alleles is the same for all islands with the exception of Pgi-2 and Tpi-2. The locus Pgi-2 had a fourth private allele on Beaver Island, and Tpi-2 was monomorphic only on Hog Island, and dialleleic elsewhere. Table 4 shows the allele frequencies among islands. The locus Pgi-2 was the only one to show substantial variability in allele frequency between islands. The other loci had rather consistent frequencies among islands. Table 5 contains the results for the descriptive statistics. Descriptive measures were found to be consistent with the mean among islands for measures of the proportion of loci polymorphic (P mean = 0.95), average number of alleles per locus (A mean = 2.4) and polymorphic locus (Ap mean = 2.48), expected proportion of heterozygotes (He mean = 0.33) and the observed proportion of heterozygotes (Ho mean = 0.31). The measure of inbreeding coefficient (f mean = 0.07) was highest on Hog Island (0.10), which is somewhat higher than expected for an outcrossing plant. Table 6 demonstrates the results of testing for Hardy-Weinberg equilibrium. The locus Pgi-2 was found not to be in Hardy-Weinberg equilibrium on Beaver, High and Garden Islands, as was Aat-3 on Hog Island and Tpi-2 on Beaver Island.

Table 7 lists the results for the standard F statistics. Significant deviations from zero were found for Fis, (95% confidence interval 0.02-0.12) and for Fit (95% confidence interval 0.04-0.12). The Fis and Fit values for individual loci were very high for Tpi-2 and Aat-3, which may have skewed the overall results among islands. Table 8 lists the

values of Fis among islands and loci. It is not uncommon for Fis to have a wide range of values in genetic studies, which is indeed the case here. The Fis values ranged from -0.244 to 0.318. The mean Fis over all loci for Garden was 0.04, Hog, 0.1o, High 0.07 and for Beaver Island was 0.06. These values suggest that some inbreeding is occurring in populations of white pine. Table 9 lists the gene diversity values among islands and loci. Gene diversity is used as a measure of the evenness of allele frequencies. High values for gene diversity suggest evenness in the allele frequencies, whereas low values suggest one or a few alleles are in very high frequency. The gene diversity measures were relatively high for all loci among islands, except Tpi-2 where a second allele occurs on three islands in a very low frequency. Table 10 shows the values for Nei's genetic distance and genetic identity. The values for Nei's genetic distance were quite low, ranging from 0.00 to 0.03. Values for genetic identity were high, ranging from 0.99 to 1.00.

Table 3 Number of alleles for each polymorphic locus found on each island

	Garden	Hog	High	Beaver
Pgm-1	2	2	2	2
Pgi-2	3	3	3	4
Mdh-2	3	3	3	3
Tpi-2	2	1	2	2
Aat-3	2	2	2	2

Table 4 Allele frequencies of allozyme loci among islands

	Island (sample size)			
Allele	Garden (57)	Hog (53)	High (34)	Beaver (196)
Locus: Pgm-1				
1	0.798	0.877	0.912	0.839
2	0.202	0.123	0.088	0.161
Locus: Pgi-2				
1	0.132	0.236	0.338	0.191
2	0.018	0.009	0.147	0.051
3	0.851	0.755	0.515	0.753
4	0.000	0.000	0.000	0.005
Locus: Mdh-2				
1	0.649	0.528	0.618	0.671
2	0.237	0.387	0.294	0.222
3	0.114	0.085	0.088	0.107
Locus: Tpi-2				
1	0.026	0.000	0.029	0.023
2	0.974	1.000	0.971	0.977
Locus: Aat-3				
1	0.684	0.670	0.632	0.671
2	0.316	0.330	0.368	0.329

Table 5 Descriptive statistics of polymorphic allozymes for the islands including measures of, sample size (n), proportion of loci polymorphic (P), average number of alleles per locus (A) average number of alleles per polymorphic locus (Ap), expected proportion of heterozygotes (He), observed proportion of heterozygotes (Ho), and inbreeding coefficient (f). Values are averaged over loci

a)

Island	n	P	A	Ap	He	Ho	f
Garden	57.00	1.00	2.40	2.40	0.32	0.31	0.04
Hog	53.00	0.80	2.20	2.50	0.32	0.29	0.10
High	34.00	1.00	2.40	2.40	0.37	0.34	0.07
Beaver	196.00	1.00	2.60	2.60	0.33	0.31	0.06
Mean	85.00	0.95	2.40	2.48	0.33	0.31	0.07

b) **Private alleles among islands**

Locus	Allele	Frequency	Found in
Pgi-2	4	0.005	Beaver

Table 6 Chi square test for Hardy-Weinberg equilibrium

Island	Locus	P-value
Garden	Pgm-1	0.062
Garden	Pgi-2	<0.000*
Garden	Mdh-2	0.323
Garden	Tpi-2	0.868
Garden	Aat-3	0.136
Hog	Pgm-1	0.099
Hog	Pgi-2	0.178
Hog	Mdh-2	0.543
Hog	Tpi-2	NA
Hog	Aat-3	0.03*
High	Pgm-1	0.070
High	Pgi-2	< 0.000*
High	Mdh-2	0.864
High	Tpi-2	0.901
High	Aat-3	0.062
Beaver	Pgm-1	0.592
Beaver	Pgi-2	<0.000*
Beaver	Mdh-2	0.160
Beaver	Tpi-2	0.002*
Beaver	Aat-3	0.350

* When the P value is less than 0.05 it indicates that the genotypes have frequencies significantly different from Hardy-Weinberg equilibrium

Table 7 F statistics for allozymes among islands, estimating f (=Fis), F (=Fit), and θ (=Fst)

Locus	Allele	f	F	θ
Pgm-1	All	0.02	0.02	0.01
	2	0.02	0.02	0.01
	1	0.02	0.02	0.01
Pgi-2	All	0.02	0.05	0.04
	4	0.00	0.00	0.00
	2	0.77	0.78	0.04
	1	-0.18	-0.15	0.02
	3	0.00	0.05	0.05
Mdh-2	All	0.05	0.06	0.01
	3	0.14	0.14	-0.01
	2	0.04	0.06	0.02
	1	0.02	0.03	0.01
Tpi-2	All	0.13	0.13	0.00
	1	0.13	0.13	0.00
	2	0.13	0.13	0.00
Aat-3	All	0.15	0.14	-0.01
	2	0.15	0.14	-0.01
	1	0.15	0.14	-0.01
Overall		0.07	0.08	0.01
Upper bound		0.12	0.12	0.03
Lower bound		0.02	0.04	0.00

The 95 % confidence interval is derived from boot strapping over loci

Table 8 Fis of allozymes for loci and islands:

Locus	Garden	Hog	High	Beaver
Pgm-1	-0.244	0.220	0.283	0.038
Pgi-2	-0.010	-0.149	-0.016	0.071
Mdh-2	0.114	0.073	-0.107	0.053
Tpi-2	-0.018	NA	-0.015	0.206
Aat-3	0.197	0.284	0.318	0.067
All	0.039	0.099	0.070	0.063

Table 9 Gene diversity of loci and islands

Locus	Garden	Hog	High	Beaver	Mean/loci
Pgm-1	0.324	0.218	0.164	0.270	0.244
Pgi-2	0.261	0.378	0.608	0.396	0.411
Mdh-2	0.515	0.570	0.531	0.490	0.527
Tpi-2	0.052	0.000	0.058	0.045	0.039
Aat-3	0.437	0.448	0.474	0.443	0.451
Mean/Island	0.318	0.324	0.367	0.329	

Table 10 Genetic distance (below) and genetic identity (above) of allozymes among islands

	Ga	Ho	Hi	Be
Garden		0.99	0.98	1.00
Hog	0.01		0.99	0.99
High	0.03	0.01		0.99
Beaver	0.00	0.01	0.01	

Allozyme genetic diversity measures among plots, Tables 11-13

Genetic diversity measures among all plots are found in Tables 11-13. Table 11 contains the results for the descriptive statistics. Descriptive measures were found to be similar for islands with the mean among plots for measures of the proportion of loci polymorphic (P mean = 0.83), average number of alleles per locus (A mean = 2.14) and polymorphic locus (Ap mean = 2.38), expected proportion of heterozygotes (He mean = 0.32) and the observed proportion of heterozygotes (Ho mean = 0.30). The only exceptions were in plots with small sample sizes (less than 10 trees). In contrast, the measure of inbreeding coefficient (f mean = 0.04) was high in plots Hog South East and High North East as well as on Beaver Island in plots Barney's lake, Sloptown, and Airport, on Beaver Island, all plots with high Fis values are located in close proximity to each other. The high Fis values in plots on High and Hog Islands could be an effect of sampling. Table12 lists the results for the standard F statistics. Significant deviations from zero were found for overall values of Fit, (95% confidence interval 0.04-0.12) and for Fst (95% confidence interval 0.01-0.17). The Fit values for individual loci were very high for Tpi-2 and Aat-3, which may have skewed the overall results among plots. The locus Pgi-2 had the highest overall Fst value of any loci (Fst = 0.09). Table 13 shows the values for Nei's genetic distance and genetic identity. The values for Nei's genetic distance were quite low, ranging from -0.01 for many plots to 0.11 between Green's bay and Hog West. Values for genetic identity were high, ranging from 0.99 to 1.00. with several measures equal or greater than 1.0, with the lowest measure between High North East and Hog West. Figure 3 shows the UPGMA phenogram of allozymes among plots. There was little consistency of plots with their geographical locations.

The allele frequencies among plots can be found in the appendix

Table 11 Descriptive statistics for allozymes among plots including measures of, sample size (n), proportion of loci polymorphic (P), average number of alleles per locus (A) and polymorphic locus (Ap), expected proportion of heterozygotes (He), observed proportion of heterozygotes (Ho), and inbreeding coefficient (f).

Population	n	P	A	Ap	He	Ho	f
Garden	57	1.00	2.40	2.40	0.32	0.31	0.04
Hog-West	9	0.60	1.80	2.33	0.18	0.18	0.00
HogN-east	16	0.80	2.00	2.25	0.34	0.35	-0.03
HogM-east	16	0.80	2.20	2.50	0.34	0.33	0.05
HogS-east	12	0.80	2.00	2.25	0.35	0.25	0.30
HighS-east	5	0.80	2.20	2.50	0.41	0.40	0.02
HighN-East	15	1.00	2.40	2.40	0.38	0.33	0.12
High West	14	1.00	2.40	2.40	0.32	0.33	-0.02
Powerline	15	0.80	2.00	2.25	0.25	0.23	0.09
Bluetrail	15	1.00	2.20	2.20	0.33	0.39	-0.19
FootBridge	11	0.80	2.00	2.25	0.27	0.25	0.05
Frenchbay	10	0.80	2.00	2.25	0.24	0.22	0.09
Greensbay	8	0.40	1.60	2.50	0.23	0.23	0.00
Wujek	17	0.80	2.20	2.50	0.30	0.27	0.09
CMU	18	0.80	2.20	2.50	0.29	0.29	0.00
DUDA	22	0.80	2.40	2.75	0.35	0.36	-0.05
Fontlake	21	1.00	2.20	2.20	0.33	0.40	-0.24
Barney'slake	20	0.80	2.20	2.50	0.39	0.34	0.12
Sloptown	18	0.80	2.40	2.75	0.37	0.33	0.11
Airport	21	1.00	2.00	2.00	0.34	0.26	0.26
Mean	17	0.83	2.14	2.38	0.32	0.30	0.04

Table 12 F statistics for allozymes among plots, F statistics estimating f (=Fis), F (=Fit), and Θ (=Fst)

Locus	Allele	f	F	Θ
Pgm-1	All	0.03	0.02	-0.01
	2	0.03	0.02	-0.01
	1	0.03	0.02	-0.01
Pgi-2	All	-0.05	0.05	0.09
	4	0.00	0.00	-0.01
	2	0.77	0.78	0.04
	1	-0.26	-0.16	0.08
	3	-0.09	0.04	0.12
Mdh-2	All	0.04	0.06	0.02
	3	0.12	0.14	0.02
	2	0.04	0.06	0.01
	1	0.01	0.03	0.02
Tpi-2	All	0.10	0.13	0.03
	1	0.10	0.13	0.03
	2	0.10	0.13	0.03
Aat-3	All	0.13	0.15	0.02
	2	0.13	0.15	0.02
	1	0.13	0.15	0.02
Overall		0.04	0.07	0.03
Upper bound		0.10	0.12	0.07
Lower bound		-0.02	0.04	0.01

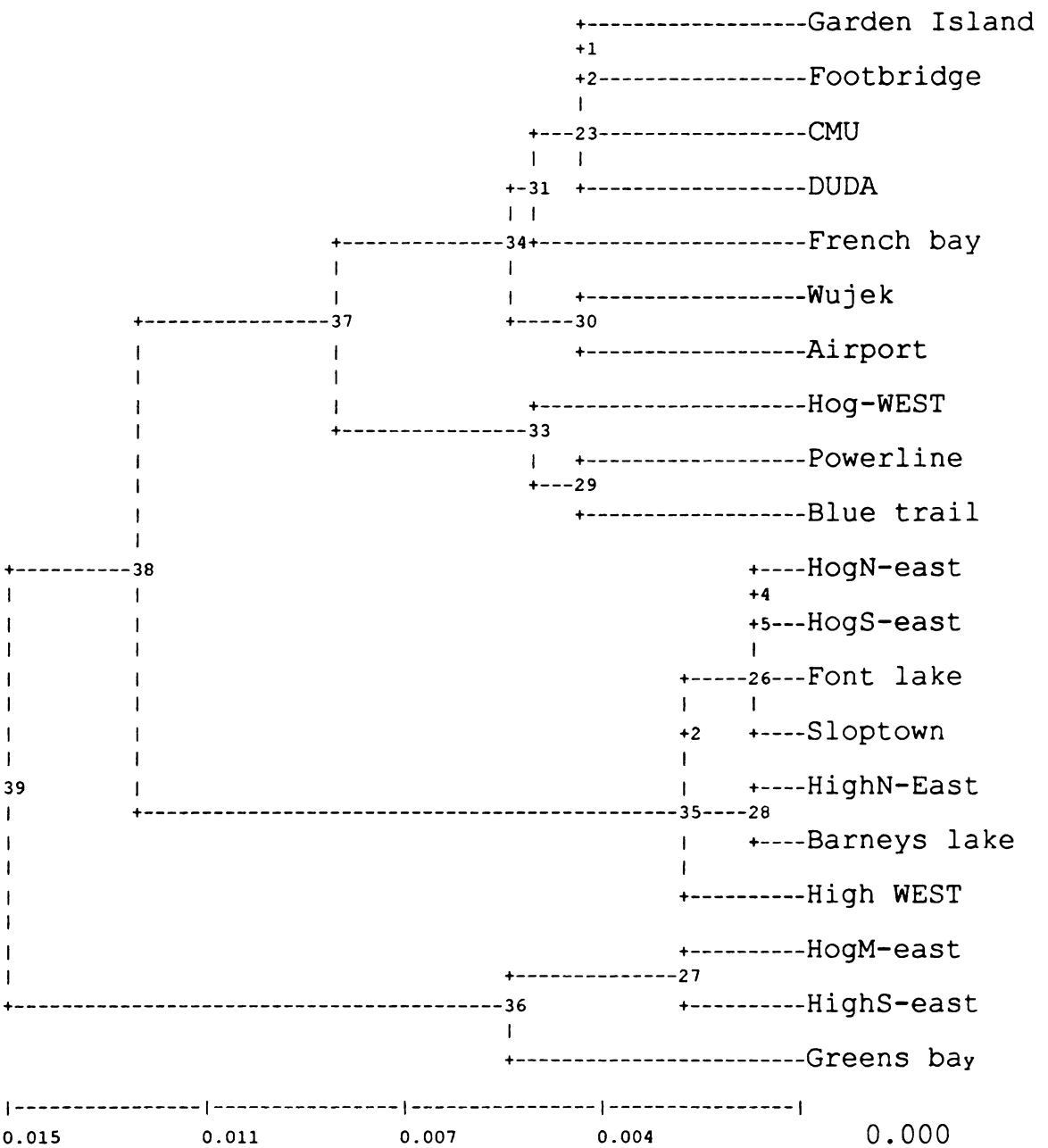
The 95 % confidence interval from boot strapping over loci

Table 13 Nei's Genetic distance (below) and genetic identity (above) of allozymes among plots (in two blocks)

	GI	Ho-W	HN-E	HM-E	HiS-E	HiS-E	HiN-E	Hi-W	PL	BT
Garden Island		0.99	0.99	0.99	0.99	0.98	0.97	0.96	0.99	1.00
Hog-West	0.01		0.96	0.98	0.97	0.95	0.93	0.95	1.00	0.99
HogN-east	0.01	0.04		1.00	1.01	1.00	1.01	0.99	0.97	0.96
HogM-east	0.01	0.02	0.00		1.00	1.02	0.98	0.96	0.98	0.99
HogS-east	0.01	0.03	-0.01	0.00		0.99	1.00	1.00	0.98	0.97
HighS-east	0.02	0.05	0.00	-0.02	0.01		0.99	0.94	0.94	0.96
HighN-East	0.03	0.07	-0.01	0.02	0.00	0.01		0.99	0.94	0.94
High West	0.04	0.05	0.01	0.04	0.00	0.07	0.01		0.97	0.94
Power line	0.01	0.00	0.03	0.02	0.02	0.06	0.06	0.03		1.00
Blue trail	0.00	0.01	0.04	0.01	0.03	0.04	0.07	0.07	0.00	
FootBridge	-0.01	0.01	0.00	0.00	0.02	0.01	0.02	0.04	0.01	0.01
French bay	0.00	0.03	0.03	0.03	0.04	0.04	0.04	0.06	0.01	0.01
Greens bay	0.03	0.06	0.04	0.02	0.06	0.00	0.05	0.11	0.06	0.03
Wujek	0.01	0.05	0.01	0.03	0.03	0.03	0.01	0.04	0.03	0.04
CMU	-0.01	0.01	0.01	0.02	0.02	0.04	0.03	0.04	0.00	0.01
DUDA	0.00	0.02	0.00	0.01	0.00	0.01	0.01	0.02	0.01	0.02
Font lake	0.01	0.02	0.00	0.01	-0.01	0.04	0.01	0.00	0.01	0.03
Barney's lake	0.04	0.08	0.00	0.02	0.00	0.01	-0.01	0.01	0.07	0.08
Sloptown	0.00	0.03	-0.01	0.00	-0.01	-0.01	0.00	0.01	0.02	0.03
Airport	0.01	0.05	0.00	0.03	0.01	0.04	0.00	0.02	0.04	0.05

	FB	FBy	GB	WK	CMU	DA	FL	BL	SN	AT
Garden Island	1.01	1.00	0.97	0.99	1.01	1.00	0.99	0.96	1.00	0.99
Hog-West	0.99	0.97	0.94	0.95	0.99	0.98	0.98	0.92	0.97	0.95
HogN-east	1.00	0.97	0.96	0.99	0.99	1.00	1.00	1.00	1.01	1.00
HogM-east	1.00	0.97	0.98	0.97	0.98	0.99	0.99	0.98	1.00	0.97
HogS-east	0.98	0.96	0.94	0.98	0.98	1.00	1.01	1.00	1.01	0.99
HighS-east	0.99	0.96	1.00	0.97	0.97	0.99	0.96	0.99	1.01	0.96
HighN-East	0.98	0.96	0.95	0.99	0.97	0.99	0.99	1.01	1.00	1.00
High West	0.96	0.94	0.90	0.96	0.97	0.98	1.00	0.99	0.99	0.98
Power line	0.99	0.99	0.94	0.97	1.00	0.99	0.99	0.94	0.98	0.96
Blue trail	0.99	0.99	0.97	0.97	0.99	0.98	0.97	0.93	0.97	0.95
Foot Bridge		1.01	0.98	1.00	1.01	1.01	0.99	0.96	1.00	0.99
French bay	-0.01		0.98	1.00	1.00	1.00	0.97	0.93	0.98	0.98
Greens bay	0.02	0.02		0.96	0.95	0.96	0.93	0.93	0.96	0.94
Wujek	0.00	0.00	0.04		1.00	1.00	0.98	0.96	1.00	1.00
CMU	-0.01	0.00	0.05	0.00		1.01	0.99	0.95	1.00	0.99
DUDA	-0.01	0.00	0.04	0.00	-0.01		1.00	0.98	1.01	1.00
Font lake	0.01	0.03	0.08	0.02	0.01	0.00		0.98	1.00	0.99
Barneys lake	0.04	0.07	0.07	0.04	0.05	0.02	0.02		1.00	0.98
Sloptown	0.00	0.02	0.04	0.00	0.00	-0.01	0.00	0.00		0.99
Airport	0.01	0.02	0.06	0.00	0.01	0.00	0.01	0.02	0.01	

Figure 3 UPGMA phenogram of allozymes among plots



Microsatellite genetic diversity measures for islands, Tables 14-17

The microsatellites genetic diversity measures for all islands are found in Tables 14-17. Table 14 shows the haplotype frequencies. The most common haplotypes (numbers 1, 2 and 3) were found on all four islands. All haplotypes were found on Beaver Island, those with a frequency of 0.005 were found in a single tree. The small islands had 4-6 rare alleles (frequencies less than 0.1), and only one rare haplotype, number 6, was found on all four islands. Table 15 contains the results for the descriptive statistics. Descriptive measures were found to be consistent with the mean among islands for measures of average number of haplotypes per locus (A mean = 10.5) and gene diversity (He mean = 0.80). Garden Island has the smallest population of white pine, and had the lowest number of haplotypes (7). Both Hog and High Islands had nine haplotypes, but not all of these were the same. Beaver Island had the largest number of white pine trees of any island, and the largest number of haplotypes (17). There were four private haplotypes found on Beaver Island (haplotype numbers 11, 13, 16, 17). Each of these occurred in a single tree. Table 16 lists the results for Fst and Rst. The Rst value among islands was 0.04. The overall Fst value was 0.01, but haplotype number 14 had an Fst value of 0.08. Haplotype 14 had a relatively high frequency of 0.098, on High Island, and a singe tree had haplotype 14 on Beaver Island. All other haplotypes had very low Fst values. Table 17 shows the values for Nei's genetic distance and genetic identity. The values for Nei's genetic distance were higher for microsatellites than for allozymes, ranging from 0.02 to 0.13. Values for genetic identity were high, ranging from 0.88 to 098.

Table 14 Haplotype frequencies among islands

Haplotype	Garden	Hog	High	Beaver	Mean
N	(49)	(37)	(41)	(185)	
1	0.245	0.324	0.146	0.200	0.229
2	0.367	0.270	0.390	0.232	0.315
3	0.143	0.189	0.146	0.227	0.176
4	0.061	0.054	0.000	0.059	0.044
5	0.082	0.000	0.024	0.081	0.047
6	0.082	0.054	0.049	0.038	0.056
7	0.020	0.000	0.000	0.011	0.008
8	0.000	0.027	0.073	0.022	0.031
9	0.000	0.027	0.049	0.070	0.037
10	0.000	0.027	0.000	0.005	0.032
11	0.000	0.000	0.000	0.005	0.001
12	0.000	0.027	0.000	0.016	0.011
13	0.000	0.000	0.000	0.005	0.001
14	0.000	0.000	0.098	0.005	0.026
15	0.000	0.000	0.024	0.011	0.008
16	0.000	0.000	0.000	0.005	0.001
17	0.000	0.000	0.000	0.005	0.001

The most common haplotypes 1,2 and 3 were found on all four islands and over 72% of the sampled trees consisted of one of the three most common haplotypes.

Table 15 Descriptive statistics for haplotypes among islands including measures of, sample size (n), number of haplotypes (A), gene diversity (He)

a)

Island	n	A	He
Garden	49	7	0.78
Hog	40	9	0.79
High	42	9	0.78
Beaver	181	17	0.84
Mean	78.00	10.50	0.80

b) **Private haplotypes among islands**

Haplotype	Frequency	Found in
17	0.001	Beaver
16	0.001	Beaver
13	0.001	Beaver
11	0.001	Beaver

Table 16 Fst of haplotypes among islands

Haplotype	Fst
17	-0.01
16	-0.01
13	-0.01
11	-0.01
15	-0.01
14	0.08
8	0.01
10	0.00
12	-0.01
9	0.01
6	-0.01
5	0.01
4	0.00
7	-0.01
3	0.00
2	0.02
1	0.01
Overall	0.01

Table 17 Nei's Genetic distance (below) and genetic identity (above) of haplotypes among islands

	Ga	Ho	Hi	Be
Garden		0.98	0.95	0.95
Hog	0.02		0.88	0.95
High	0.05	0.13		0.89
Beaver	0.05	0.05	0.11	

Microsatellite genetic diversity measures among plots, Tables 18-20

Microsatellite genetic diversity measures among all plots are found in Tables 18-20. Table 18 contains the results for the descriptive statistics. Descriptive measures were found to be consistent with the mean among plots for measures of average number of haplotypes per locus (A mean = 6.1) and gene diversity (He mean = 0.76). The only exception was plot Hog East North which had only two haplotypes and a gene diversity measure of 0.48, and this is most likely due to the low sample size. Four haplotypes were found in a single plot, in a single tree. Haplotype 18 was found at the Airport plot, haplotype 16 was found in the Duda plot, haplotype 13 was found only in the Wujek plot, and haplotype eleven was found only in the Footbridge plot. Table19 lists the results for Fst. The Rst value was 0.01. The overall Fst value was 0.03, but the haplotype number 14 had an Fst value of 0.08. Haplotype 14 had a high frequency (for a rare haplotype) on High Island, and a singe tree had haplotype 14 on Beaver Island. Interestingly, the most common haplotypes (number 1, 2 and 3) also had relatively high Fst values (0.04, 0.04 and 0.07 respectively). All other haplotypes had very low Fst values. Table 20 shows the values for Nei's genetic distance and genetic identity. The values for Nei's genetic distance were variable, ranging from -0.05 to 1.91. Hog East Middle had the highest value between Powerline with a value of 1.91. Hog East Middle also had several other values greater than 1.0 between other plots on the archipelago. Hog East Middle had several rare haplotypes which would have increased the measure of genetic distance. Values for genetic identity were also variable, ranging from 0.11 to 1.02. Hog East Middle had the lowest values, suggesting it had the least number of haplotypes in common with other plots. Interestingly, the highest genetic identity value was between

Garden Island and the tiny population Hog West. This may suggest that the prevailing wind allowed Garden Island trees to pollinate the small number of trees in the Hog West plot. Figure 4 shows the UPGMA phenogram of haplotypes among plots. There was little consistency with grouping of plots with their geographical locations.

The Haplotype frequencies among plots can be found in the Appendix.

Table 18 Descriptive statistics for haplotypes among plots including measures of, sample size (n), number of haplotypes (A), gene diversity (He)

a)

Population	n	A	He
Garden	49	7	0.78
Hog-West	10	4	0.76
Hog-East North	6	2	0.48
Hog-Midd	14	7	0.67
Hog-East South	10	4	0.74
High-East South	13	6	0.74
High-West	15	6	0.74
High-East North	14	7	0.83
Power line	12	6	0.83
Blue trail	19	6	0.66
Footbridge	16	7	0.77
French bay	17	8	0.79
Greens bay	18	7	0.82
Wujek	14	6	0.81
CMU	15	6	0.81
Duda	13	8	0.86
Font LK	17	7	0.83
Barney's LK	13	5	0.78
Sloptown	17	7	0.82
Airport	11	6	0.76
Mean	15.7	6.1	0.76

b) **Private haplotypes among plots**

Haplotype	Frequency	Found in
17	0.01	Airport (Beaver)
16	0.01	Duda (Beaver)
13	0.01	Wujek (Beaver)
11	0.01	Footbridge (Beaver)

Table 19 Fst of haplotypes among plots

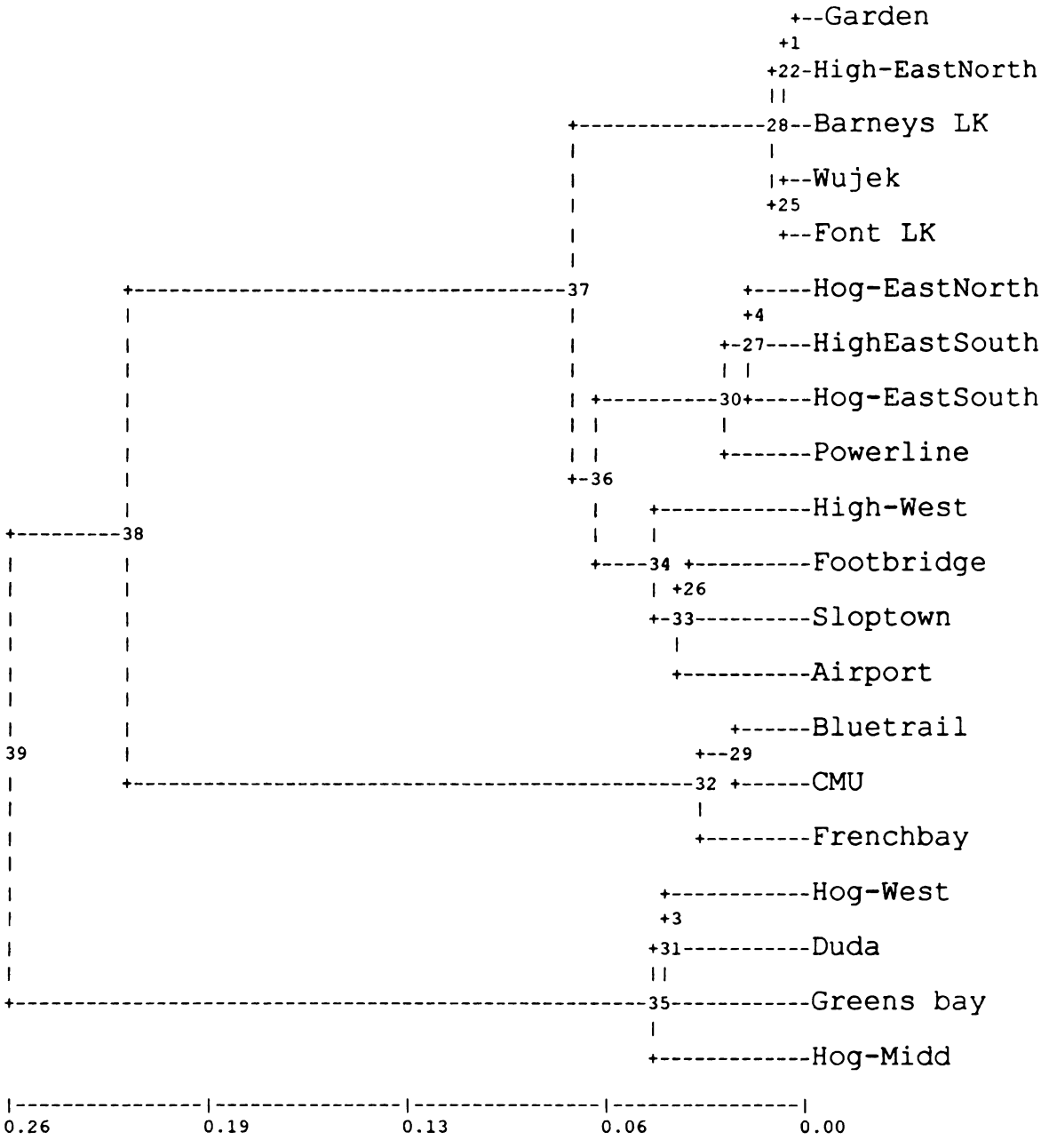
Haplotype	Fst
17	0.03
16	0.01
13	0.01
11	0.00
15	0.00
14	0.08
8	-0.02
10	0.00
12	0.02
9	0.01
6	0.00
5	0.00
4	0.01
7	-0.02
3	0.07
2	0.04
1	0.04
Overall	0.03

Table 20 Nei's genetic distance (below) and genetic identity (above) of haplotypes among plots (in two blocks)

	GI	Hi-W	HN-E	HM-E	HoS-E	HoS-E	HoN-E	Hi-W	PL	BT
Garden		0.93	0.86	0.62	0.93	0.86	0.90	1.02	0.90	0.61
Hog-West	0.08		0.57	0.85	0.71	0.61	0.69	0.97	0.65	0.77
Hog-EastNorth	0.15	0.57		0.11	1.00	1.05	0.90	0.79	0.93	0.50
Hog-Midd	0.47	0.16	2.16		0.34	0.15	0.38	0.80	0.15	0.47
Hog-EastSouth	0.07	0.34	0.00	1.09		0.97	0.83	0.87	0.93	0.72
High-EastSouth	0.15	0.49	-0.05	1.90	0.03		0.98	0.82	0.99	0.49
High-West	0.11	0.38	0.10	0.95	0.18	0.03		0.86	0.86	0.32
High-EastNorth	-0.02	0.03	0.23	0.22	0.14	0.20	0.15		0.79	0.69
Powerline	0.10	0.43	0.07	1.91	0.07	0.01	0.16	0.24		0.49
Bluetrail	0.50	0.26	0.69	0.76	0.33	0.72	1.15	0.36	0.72	
Footbridge	0.06	0.46	0.11	0.92	0.18	0.11	0.10	0.07	0.07	1.05
Frenchbay	0.35	0.31	0.25	1.50	0.16	0.18	0.60	0.32	0.16	0.08
Greendsbay	0.20	0.10	0.86	0.06	0.37	0.67	0.49	0.06	0.65	0.61
Wujek	0.12	0.21	0.08	0.79	0.03	0.05	0.26	0.01	0.23	0.21
CMU	0.25	0.22	0.45	0.67	0.26	0.44	0.70	0.09	0.31	0.04
Duda	0.18	-0.01	0.96	0.08	0.32	0.83	0.73	0.08	0.55	0.20
FontLK	0.01	0.00	0.22	0.36	0.04	0.17	0.26	-0.06	0.18	0.15
BarneysLK	-0.01	0.01	0.19	0.30	0.09	0.19	0.17	-0.10	0.21	0.22
Sloptown	0.02	0.31	0.18	0.54	0.08	0.18	0.07	0.05	0.12	0.83
Airport	0.16	0.63	0.14	1.00	0.18	0.11	0.11	0.19	0.25	1.60

	FB	FBy	GB	WK	CMU	DA	FL	BL	SN	AT
Garden	0.94	0.70	0.82	0.89	0.78	0.84	0.99	1.01	0.98	0.85
Hog-West	0.63	0.73	0.90	0.81	0.81	1.01	1.00	0.99	0.73	0.53
Hog-EastNorth	0.90	0.78	0.42	0.93	0.64	0.38	0.81	0.83	0.84	0.87
Hog-Midd	0.40	0.22	0.94	0.45	0.51	0.92	0.70	0.74	0.58	0.37
Hog-EastSouth	0.83	0.85	0.69	0.97	0.77	0.73	0.96	0.92	0.92	0.84
High-EastSouth	0.89	0.83	0.51	0.95	0.64	0.44	0.85	0.83	0.83	0.90
High-West	0.90	0.55	0.61	0.77	0.49	0.48	0.77	0.84	0.93	0.89
High-EastNorth	0.93	0.73	0.94	0.99	0.91	0.92	1.06	1.11	0.95	0.83
Powerline	0.93	0.85	0.52	0.80	0.73	0.58	0.84	0.81	0.89	0.78
Bluetrail	0.35	0.92	0.54	0.81	0.96	0.82	0.86	0.81	0.44	0.20
Footbridge		0.57	0.62	0.82	0.69	0.56	0.84	0.88	0.99	0.94
Frenchbay	0.57		0.47	0.94	0.94	0.67	0.91	0.82	0.57	0.43
Greens bay	0.47	0.75		0.74	0.64	1.05	0.94	0.85	0.78	0.73
Wujek	0.20	0.06	0.31		0.94	0.75	1.06	0.97	0.78	0.80
CMU	0.37	0.06	0.45	0.06		0.88	0.99	0.94	0.68	0.43
Duda	0.58	0.41	-0.05	0.28	0.12		1.02	0.91	0.75	0.50
FontLK	0.17	0.09	0.06	-0.05	0.01	-0.02		1.06	0.88	0.78
BarneysLK	0.13	0.20	0.16	0.03	0.06	0.10	-0.05		0.92	0.73
Sloptown	0.01	0.56	0.25	0.25	0.39	0.29	0.13	0.08		0.90
Airport	0.06	0.84	0.31	0.22	0.85	0.69	0.25	0.32	0.10	

Figure 4 UPGMA phenogram of haplotypes among plots



Allozyme genetic diversity measures within islands, Tables 21-30

Allozyme genetic diversity measures within Garden Island, Tables 21-22

Table 21 contains the results of the descriptive statistics for Garden Island. Descriptive measures for all allozyme loci were found to be consistent with the mean within Garden Island for measures of the average number of alleles per locus (A mean = 2.40), expected proportion of heterozygotes (He mean = 0.32) and the observed proportion of heterozygotes (Ho mean = 0.31) The locus Tpi-2 had values much lower than the mean He (Tpi-2, He = 0.05) and Ho (Tpi-2, Ho = 0.05) This could be due to the low frequency of the second Tpi-2 allele. The loci Mdh-2 had values much higher than the mean He and Ho. The value for He for Mdh-2 was 0.53 and for Ho was 0.46. The measure of inbreeding coefficient (f) was highest for Aat-3 (0.2) and the lowest for locus Pgm-1 (-0.24). Since there were no individual plots within Garden Island, the only F statistic that could be estimated was Fis, found in Table 22. There was no significant deviation from zero (95% confidence interval -0.16 to 0.15).

Table 21 Descriptive statistics measured with allozymes within Garden Island including measures of, sample size (n), proportion of loci polymorphic (P), average number of alleles per locus (A), expected proportion of heterozygotes (He), observed proportion of heterozygotes (Ho), and inbreeding coefficient (f)

Locus	n	A	He	Ho	f
Pgm-1	57.00	2.00	0.32	0.40	-0.24
Pgi-2	57.00	3.00	0.26	0.26	-0.01
Mdh-2	57.00	3.00	0.51	0.46	0.11
Tpi-2	57.00	2.00	0.05	0.05	-0.02
Aat-3	57.00	2.00	0.44	0.35	0.20
All	57.00	2.40	0.32	0.31	0.04

Table 22 Fis measured with allozymes within Garden Island

Locus	Allele	f
Pgm-1	All	-0.24
	2	-0.24
	1	-0.24
Pgi-2	All	-0.01
	2	1.00
	1	-0.14
	3	-0.03
Mdh-2	All	0.11
	3	0.23
	2	0.09
	1	0.08
Tpi-2	All	-0.02
	1	-0.02
	2	-0.02
Aat-3	All	0.20
	2	0.20
	1	0.20

Overall	0.04
Upper bound	0.15
Lower bound	-0.16

The 95 % confidence interval from boot strapping over loci

Allozyme genetic diversity measures within Hog Island, Tables 23-25

Table 23 contains the results for the descriptive statistics for Hog Island. Descriptive measures were found to be consistent with the mean within Hog Island for measures of the proportion of loci polymorphic (P mean = 0.75), average number of alleles per locus (A mean = 2.0) and polymorphic locus (Ap mean = 2.3), expected proportion of heterozygotes (He mean = 0.3) and the observed proportion of heterozygotes (Ho mean = 0.28) The only exception was the plot Hog West which only had nine samples. Hog West expected proportion of heterozygotes (He)was only 0.18 and the Ho value was only 0.18. Hog East Middle was the only plot to have a single copy of the allele Pgi-2 allele number 2. The measure of inbreeding coefficient (f) was highest for Hog South East (0.30) which may have skewed the overall mean which was (0.09). Table 24 shows the values for standard F statistics. There is no significant divergence from zero for Fis, Fit or Fst. Table 25 shows the values for Nei's genetic distance and genetic identity. The values for Nei's genetic distance were quite low, ranging from -0.01 to 0.03. Values for genetic identity were high, ranging from 0.96 to 1.01.

Table 23 Descriptive statistic measures of genetic diversity within Hog Island including measures of, sample size (n), proportion of loci polymorphic (P), average number of alleles per locus (A) and polymorphic locus (Ap), expected proportion of heterozygotes (He), observed proportion of heterozygotes (Ho), and inbreeding coefficient (f)

a)

Population	n	P	A	Ap	He	Ho	f
Hog-West	9.00	0.60	1.80	2.33	0.18	0.18	0.00
HogN-East	16.00	0.80	2.00	2.25	0.34	0.35	-0.03
HogM-East	16.00	0.80	2.20	2.50	0.34	0.32	0.05
HogS-East	12.00	0.80	2.00	2.25	0.35	0.25	0.30
Mean	13.25	0.75	2.00	2.33	0.30	0.28	0.09

b) **Private alleles within Hog Island**

Locus	Allele	Frequency	Found in
Pgi-2	2	0.03	Hog East-Middle

Table 24 F statistics measured using allozymes within Hog Island estimating the parameters f (=Fis), F (=Fit), and Θ (=Fst)

Locus	Allele	f	F	Θ
Pgm-1	All	0.24	0.21	-0.04
	2	0.24	0.21	-0.04
	1	0.24	0.21	-0.04
Pgi-2	All	-0.25	-0.12	0.11
	2	0.01	0.00	-0.01
	1	-0.31	-0.16	0.12
	3	-0.20	-0.08	0.10
Mdh-2	All	0.09	0.07	-0.02
	3	0.17	0.16	-0.01
	2	0.12	0.09	-0.04
	1	0.03	0.02	-0.01
Tpi-2	All	***	***	***
	2	***	***	***
Aat-3	All	0.27	0.29	0.02
	2	0.27	0.29	0.02
	1	0.27	0.29	0.02
Overall		0.09	0.10	0.02
Upper bound		0.26	0.27	0.08
Lower bound		-0.13	-0.05	-0.03

The 95 % confidence interval from boot strapping over loci

Table 25 Genetic distance (below) and genetic identity (above) of allozymes within Hog Island

	Ho-W	HN-E	HM-E	HS-E
Hog-WEST		0.96	0.98	0.97
HogN-east	0.04		1.00	1.01
HogM-east	0.02	0.00		1.00
HogS-east	0.03	-0.01	0.00	

Allozyme genetic diversity measures within High Island, Tables 26-28

Table 26 contains the results for the descriptive statistics for High Island. Descriptive measures were found to be consistent with the mean within plots for measures of the proportion of loci polymorphic (P mean = 0.93), average number of alleles per locus (A mean = 2.33) and polymorphic locus (Ap mean = 2.43), expected proportion of heterozygotes (He mean = 0.37) and the observed proportion of heterozygotes (Ho mean = 0.35). The measure of inbreeding coefficient (f mean = 0.04) was highest for High North East (f = 0.12) which may have skewed the overall mean. Table 276 shows the values for standard F statistics. There is no significant divergence from zero for Fis, Fit or Fst. Table 28 shows the values for Nei's genetic distance and genetic identity. The values for Nei's genetic distance were quite low, ranging from 0.01 to 0.07. Values for genetic identity were high, ranging from 0.94 to 0.99.

Table 26 Descriptive statistics measures within High Island including measures of, sample size (n), proportion of loci polymorphic (P), average number of alleles per locus (A) and polymorphic locus (Ap), expected proportion of heterozygotes (He), observed proportion of heterozygotes (Ho), and inbreeding coefficient (f)

Population	n	P	A	Ap	He	Ho	f
HighS-East	5.00	0.80	2.20	2.50	0.41	0.40	0.02
HighN-East	15.00	1.00	2.40	2.40	0.38	0.33	0.12
High West	14.00	1.00	2.40	2.40	0.32	0.33	-0.02
Mean	11.33	0.93	2.33	2.43	0.37	0.35	0.04

Table 27 F statistics measured with allozymes within High Island F statistics estimates the parameters f (=Fis), F (=Fit), and Θ (=Fst)

Locus	Allele	f	F	Θ
Pgm-1	All	0.28	0.28	0.00
	2	0.28	0.28	0.00
	1	0.28	0.28	0.00
Pgi-2	All	-0.01	-0.02	0.00
	1	-0.41	-0.35	0.04
	2	1.00	1.00	-0.10
	3	-0.22	-0.22	0.00
Mdh-2	All	-0.11	-0.10	0.01
	3	-0.06	-0.10	-0.04
	2	-0.13	-0.11	0.02
	1	-0.12	-0.10	0.01
Tpi-2	All	0.01	-0.03	-0.04
	1	0.01	-0.03	-0.04
	2	0.01	-0.03	-0.04
Aat-3	All	0.26	0.35	0.12
	1	0.26	0.35	0.12
	2	0.26	0.35	0.12
Overall		0.05	0.08	0.03
Upper bound		0.25	0.32	0.09
Lower bound		-0.07	-0.07	-0.01

The 95 % confidence interval from boot strapping over loci

Table 28 Nei's genetic distance (below) and genetic identity (above) of allozymes within High Island

	HiS-E	HiN-E	Hi-W
High S-east		0.99	0.94
High N-East	0.01		0.99
High West	0.07	0.01	

Allozyme genetic diversity measures within Beaver Island, Tables 29-31

Table 29 contains the results for the descriptive statistics for Beaver Island. Descriptive measures were found to be consistent with the mean within plots for measures of the proportion of loci polymorphic (P mean = 0.82), average number of alleles per locus (A mean = 2.12) and polymorphic locus (Ap mean = 2.39), expected proportion of heterozygotes (He mean = 0.31) and the observed proportion of heterozygotes (Ho mean = 0.3). The overall mean for Fis was 0.03. The measure of Fis was highest for Barney's lake, Sloptown and Airport (Fis= 0.12, 0.11 and 0.26 respectively). While the measure of Fis was lowest in the Blue trail and Duda plots (-0.19 and -0.24 respectively). Table 30 shows the values for standard F statistics. There was significant divergence from zero for Fit, (confidence internal was 0.05 to 0.09) and for Fst (confidence interval was 0.01 to 0.08). Table 31 shows the values for Nei's genetic distance and genetic identity. The values for Nei's genetic distance were quite low, ranging from -0.01 to 0.07. Values for genetic identity were high, ranging from 0.93 to 1.01. Figure 5 shows the UPGMA phenogram of allozymes among plots. There was some consistency of grouping of plots with there geographical locations. Plots, Font Lake, Sloptown and Airport are located in close proximity geographically and were grouped together in a branch. Plots Wujek, CMU and Duda are located in close proximity geographically and were grouped together, although French Bay and Foot bridge plots are distant plots in geographical area but were also included in this branch.

Table 29 Descriptive statistics measured within Beaver Island including measures of, sample size (n), proportion of loci polymorphic (P), average number of alleles per locus (A) and polymorphic locus (Ap), expected proportion of heterozygotes (He), observed proportion of heterozygotes (Ho), and inbreeding coefficient (f)

Population	n	P	A	Ap	He	Ho	f
Power line	15.00	0.80	2.00	2.25	0.25	0.23	0.09
Blue trail	15.00	1.00	2.20	2.20	0.33	0.39	-0.19
FootBridge	11.00	0.80	2.00	2.25	0.27	0.25	0.05
French bay	10.00	0.80	2.00	2.25	0.24	0.22	0.09
Greens bay	8.00	0.40	1.60	2.50	0.22	0.22	0.00
Wujek	17.00	0.80	2.20	2.50	0.30	0.27	0.09
CMU	18.00	0.80	2.20	2.50	0.29	0.29	0.00
DUDA	22.00	0.80	2.40	2.75	0.35	0.36	-0.05
Font lake	21.00	1.00	2.20	2.20	0.33	0.40	-0.24
Barney's lake	20.00	0.80	2.20	2.50	0.39	0.34	0.12
Sloptown	18.00	0.80	2.40	2.75	0.37	0.33	0.11
Airport	21.00	1.00	2.00	2.00	0.34	0.26	0.26
Mean	16.33	0.82	2.12	2.39	0.31	0.30	0.03

Table 30 F statistics measured within Beaver Island F statistics estimates the parameters f (=Fis), F (=Fit), and Θ (=Fst)

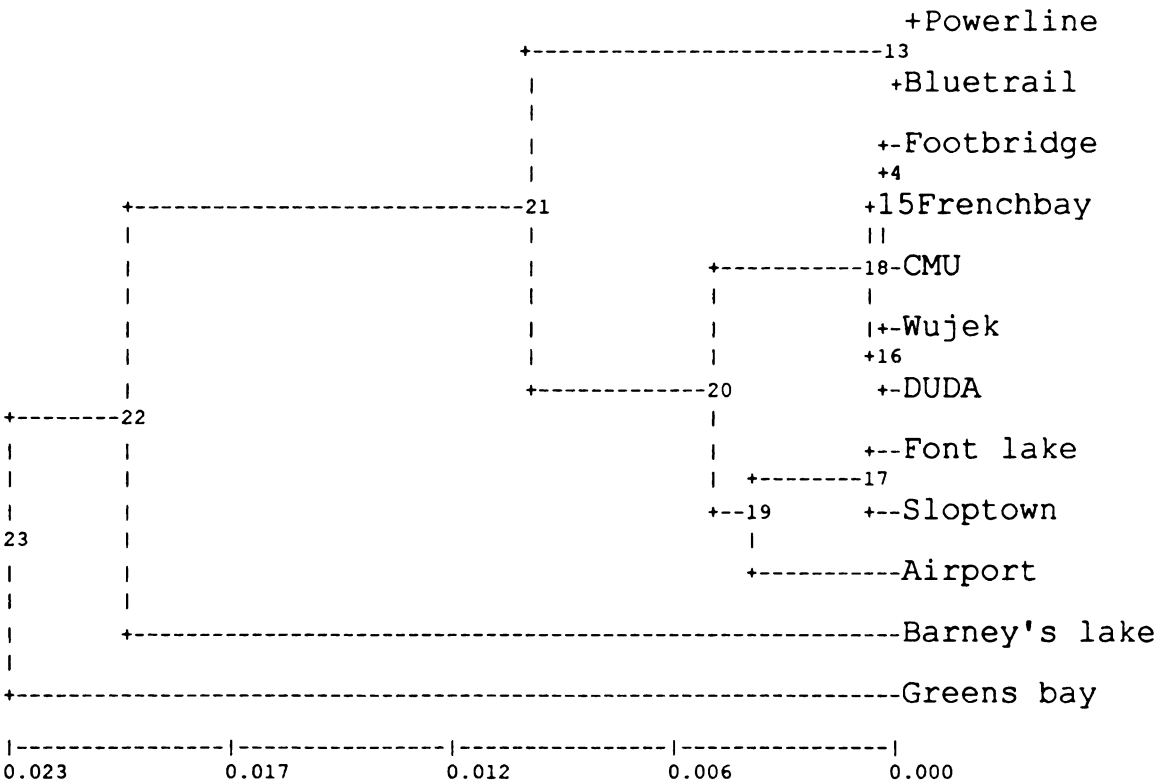
Locus	Allele	f	F	Θ
Pgm-1	All	0.05	0.04	-0.01
	2	0.05	0.04	-0.01
	1	0.05	0.04	-0.01
Pgi-2	All	-0.02	0.08	0.10
	4	0.01	0.00	-0.01
	2	0.67	0.69	0.05
	1	-0.23	-0.13	0.08
	3	-0.04	0.09	0.13
Mdh-2	All	0.03	0.06	0.02
	3	0.11	0.15	0.05
	1	0.00	0.02	0.03
	2	0.04	0.04	0.01
Tpi-2	All	0.16	0.21	0.05
	1	0.16	0.21	0.05
	2	0.16	0.21	0.05
Aat-3	All	0.05	0.07	0.02
	2	0.05	0.07	0.02
	1	0.05	0.07	0.02
Overall		0.03	0.07	0.04
Upper bound		0.06	0.09	0.08
Lower bound		0.00	0.05	0.01

The 95 % confidence interval from boot strapping over loci

Table 31 Genetic distance (below) and genetic identity (above) of allozymes within Beaver Island

	PL	BT	FB	FBy	GB	WK	CMU	DA	FL	BL	SN	AT
Power line		1.00	0.99	0.99	0.94	0.97	1.00	0.99	0.99	0.94	0.98	0.96
Blue trail	0.00		0.99	0.99	0.97	0.97	0.99	0.98	0.97	0.93	0.97	0.95
Footbridge	0.01	0.01		1.01	0.98	1.00	1.01	1.01	0.99	0.96	1.00	0.99
French bay	0.01	0.01	-0.01		0.98	1.00	1.00	1.00	0.97	0.93	0.98	0.98
Greens bay	0.06	0.03	0.02	0.02		0.96	0.95	0.96	0.93	0.93	0.96	0.94
Wujek	0.03	0.04	0.00	0.00	0.04		1.00	1.00	0.98	0.96	1.00	1.00
CMU	0.00	0.01	-0.01	0.00	0.05	0.00		1.01	0.99	0.95	1.00	0.99
DUDA	0.01	0.02	-0.01	0.00	0.04	0.00	-0.01		1.00	0.98	1.01	1.00
Font lake	0.01	0.03	0.01	0.03	0.08	0.02	0.01	0.00		0.98	1.00	0.99
Barney's	0.07	0.08	0.04	0.07	0.07	0.04	0.05	0.02	0.02		1.00	0.98
Sloptown	0.02	0.03	0.00	0.02	0.04	0.00	0.00	-0.01	0.00	0.00		0.99
Airport	0.04	0.05	0.01	0.02	0.06	0.00	0.01	0.00	0.01	0.02	0.01	

Figure 5 UPGMA phenogram of plots within Beaver Island



Genetic diversity measured with haplotypes within islands Tables 32-41

Table 32 contains the results for the descriptive statistics for Garden Island. There was only one plot on Garden Island so there is no mean value, and no estimation of F statistics.

Table 32 Descriptive statistics measured within Garden Island including measures of, sample size (n), number of haplotypes (A), gene diversity (He)

Island	n	A	He
Garden	49	7	0.78

Genetic diversity measured with haplotypes within Hog Island
Tables 33-35

Table 33 contains the results for the descriptive statistics for Hog Island. Descriptive measures were found to be consistent with the mean within plots for measures of average number of haplotypes per locus (A) and gene diversity (He). The exception being Hog East North which had a small sample size (6). The mean He was 0.66 and the He within Hog East North was 0.48 also there were only two haplotypes in Hog East North when the average number of haplotypes per plot was 4.25. There were several private haplotypes within Hog Island, haplotype numbers 8, 10, 12, and 9 were found in single trees in Hog East Middle, and haplotype number 6 was found in 2 of the 10 trees in Hog West. Table 34 shows that the overall measure of Fst was quite high 0.13. Haplotype 1 had an Fst value of 0.21 haplotype 2 had an overall value of 0.19 and haplotype 6 had an overall value of 0.11. The four private haplotypes found in Hog East Middle had negative Fst values, possibly because they were found in single copies in one sample plot. Table 35 shows the values for Nei's genetic distance and genetic identity. The values for Nei's genetic distance were variable, ranging from 0.00 to 2.16. The high value (2.16) was between Hog East Middle and Hog East North. While the low value (0.00) was between Hog East North and Hog East South. Nei's genetic identity had a low between Hog East Middle and Hog East North (0.11) and a high of 1.0 between. Hog East North and Hog East South.

Table 33 Descriptive statistics measured within Hog Island including measures of, sample size (n), number of haplotypes (A), gene diversity (He)

a)

Population	n	A	He
Hog-West	10	4	0.76
Hog-East North	6	2	0.48
Hog-Midd	14	7	0.67
Hog-East South	10	4	0.74
Mean	10.00	4.25	0.66

b) **Private haplotypes within Hog Island**

Haplotypes	Frequency	Found in
8	0.07	Hog-Midd
10	0.07	Hog-Midd
12	0.07	Hog-Midd
9	0.07	Hog-Midd
6	0.20	Hog-West

Table 34 Fst measured within Hog Island

Haplotype	Fst
All	0.13
4	0.02
8	-0.04
10	-0.04
12	-0.04
9	-0.04
3	0.07
2	0.19
6	0.11
1	0.21
Overall	0.13

Table 35 Genetic distance (below) and genetic identity (above) of haplotypes within Hog Island

	Ho-W	HN-E	HM-E	HS-E
Hog-West		0.57	0.85	0.71
Hog-East North	0.57		0.11	1.00
Hog-Midd	0.16	2.16		0.34
Hog-East South	0.34	0.00	1.09	

Genetic diversity measured with haplotypes within High Island
Tables 36-38

Table 36 contains the results for the descriptive statistics for haplotypes within High Island. Descriptive measures were found to be consistent with the mean among plots for measures of average number of haplotypes per locus (A mean = 6.33) and gene diversity (He Mean = 0.77). Table 37 shows that the overall measure of Fst was 0.00. Table 38 shows the values for Nei's genetic distance and genetic identity. The values for Nei's genetic distance were variable but low, ranging from 0.03 to 0.2. Nei's genetic identity was relatively high ranging from 0.82 to 0.98.

Table 36 Descriptive statistics measured within high island including measures of, sample size (n), number of haplotypes (A), gene diversity (He)

Population	n	A	He
High-East South	13	6	0.74
High-West	15	6	0.74
High-East North	14	7	0.83
Mean	14.0	6.3	0.77

Table 37 Fst measured within High Island

Haplotype	Fst
All	0.00
5	0.00
15	0.00
1	0.09
14	0.05
9	-0.03
6	-0.04
8	-0.08
2	-0.03
3	-0.02
Overall	0.00

Table 38 Genetic distance (below) and genetic identity (above) of haplotypes within High Island

	HE-S	Hi-W	HE-N
High East South		0.98	0.82
High-West	0.03		0.86
High East North	0.20	0.15	

Genetic diversity measured with haplotypes within Beaver Island
Tables 39-41

Table 39 contains the results for the descriptive statistics for haplotypes within Beaver Island. Descriptive measures were found to be consistent with the mean among plots for measures of average number of haplotypes per locus (A mean = 6.33) and gene diversity (He mean = 0.8). The exceptions being Blue Trail, CMU and Barney's lake which had values for the number of haplotypes (5 for all three) lower than the mean of 6.3. The He value was lowest in Blue Trail, 0.63 compared to the overall mean of 0.80. There were four plots which had private haplotypes, Airport had a single copy of haplotype 17, Duda had a single copy of haplotype 16, Wujek had had a since copy of haplotype 13 and Footbridge had a single copy of haplotype 11. Table 40 shows that the overall measure of Fst was 0.03. Haplotype number 3 had the highest individual Fst value of 0.11. Table 41 shows the values for Nei's genetic distance and genetic identity. The values for Nei's genetic distance were variable ranging from 0.07 to 1.60. The high value for Nei's genetic distance was between Blue Trail and Airport. Nei's genetic identity was variable with a low value of 0.35 to a high of 1.06. The low value (0.34) was between Blue Trail and Footbridge which is interesting since the two plots are located in relatively close proximity

Table 39 Descriptive statistics measured within Beaver Island including measures of, sample size (n), number of haplotypes (A), gene diversity (He)

a)

Population	n	A	He
Power line	11	6	0.81
Blue trail	16	5	0.63
Footbridge	13	7	0.76
French bay	15	7	0.75
Greens bay	16	7	0.85
Wujek	10	6	0.86
CMU	14	5	0.78
Duda	11	8	0.88
Font LK	17	7	0.83
Barney's	13	5	0.78
Sloptown	17	7	0.82
Airport	9	6	0.84
Mean	13.50	6.33	0.80

b) **Private haplotypes within Beaver Island**

Haplotype	Frequency	Found in
17	0.09	Airport (Beaver)
16	0.08	Duda (Beaver)
13	0.07	Wujek (Beaver)
11	0.06	Footbridge (Beaver

Table 40 Fst measured within Beaver Island

Haplotype	Fst
All	0.03
17	0.04
14	-0.02
16	0.02
15	0.01
13	0.03
12	0.02
10	0.00
11	0.00
9	0.02
1	-0.02
7	0.01
5	-0.02
4	-0.01
2	0.03
8	-0.01
3	0.11
6	0.01
Overall	0.03

Table 41 Genetic distance (below) and genetic identity (above) of haplotypes within Beaver Island

	PL	BL	FE	FBy	GY	WK	CMU	DA	FL	BL	SN	AT
Power line		0.49	0.93	0.85	0.52	0.80	0.73	0.58	0.84	0.81	0.89	0.78
Blue trail	0.72		0.35	0.92	0.54	0.81	0.96	0.82	0.86	0.81	0.44	0.20
Footbridge	0.07	1.05		0.57	0.62	0.82	0.69	0.56	0.84	0.88	0.99	0.94
French bay	0.16	0.08	0.57		0.47	0.94	0.94	0.67	0.91	0.82	0.57	0.43
Greens bay	0.65	0.61	0.47	0.75		0.74	0.64	1.05	0.94	0.85	0.78	0.73
Wujek	0.23	0.21	0.20	0.06	0.31		0.94	0.75	1.06	0.97	0.78	0.80
CMU	0.31	0.04	0.37	0.06	0.45	0.06		0.88	0.99	0.94	0.68	0.43
Duda	0.55	0.20	0.58	0.41	-0.05	0.28	0.12		1.02	0.91	0.75	0.50
Font LK	0.18	0.15	0.17	0.09	0.06	-0.05	0.01	-0.02		1.06	0.88	0.78
Barney's	0.21	0.22	0.13	0.20	0.16	0.03	0.06	0.10	-0.05		0.92	0.73
Sloptown	0.12	0.83	0.01	0.56	0.25	0.25	0.39	0.29	0.13	0.08		0.90
Airport	0.25	1.60	0.06	0.84	0.31	0.22	0.85	0.69	0.25	0.32	0.10	

Spatial autocorrelation analysis Tables 42 and 43

Table 42 contains the results from Moran's I statistic among all plots in the archipelago. Alleles Pgm-1, Pgi-2b, Pgi-2c and Mdh-2b had significant overall correlograms. The SND showed significant positive spatial autocorrelations in distance class one (0-5127 meters) and in distance class 2 (5128m-8454m) Also, negative spatial autocorrelation was detected in distance class 4 (10907m-13517m). The analysis was repeated without Pgi-2b, which was a relatively low frequency allele. There was significant positive spatial autocorrelation at the first distance class when excluding Pgi-2b and significant negative spatial autocorrelation at the fifth distance class (13518m -18308m)

Table 42 Moran's I statistic for the archipelago

No. Pairs	27	27	27	27	27	27	28		
Distance class[a]	5127	8454	10906	13517	18308	22944	30776	p[b]	Mean allele frequency among plots
PGM-1	0.16	-0.35	0.10	0.24	-0.60	-0.11	0.19	0.01	0.862
PGI-2a	0.06	0.20	0.00	-0.18	-0.18	-0.20	-0.07	0.39	0.190
PGI-2b	0.33	0.32	-0.02	-0.54	-0.22	-0.20	-0.03	0.01	0.053
PGI-2c	0.28	0.36	0.10	-0.48	-0.33	-0.29	0.00	0.04	0.754
MDH-2a	0.17	-0.14	0.13	-0.11	-0.38	-0.15	0.11	0.18	0.629
MDH-2b	0.21	0.26	0.21	-0.28	-0.48	0.06	-0.34	0.04	0.267
MDH-2c	0.29	-0.21	-0.22	-0.08	-0.01	0.01	-0.14	0.12	0.104
AAT-2	-0.22	-0.01	0.08	-0.13	-0.14	0.08	-0.02	1.00	0.659
Average I	0.16	0.05	0.05	-0.2	-0.29	-0.1	-0.04		
Average S.E.	0.16	0.16	0.17	0.17	0.17	0.17	0.14		
SND[c]	3.56	1.89	1.68	-2.44	-4.01	-0.08	0.25		
p[c]	0.0002	0.03	0.056	0.007	0.0000	0.468	0.404		
SND[c]	2.95	1.14	1.72	-1.50	-3.09	-0.50	0.20		
P[d]	0.002	0.13	0.06	0.07	0.001	0.31	0.2		
a Upper bound for distance class in meters									
b Overall correlogram significance									
c including PGI-2b									
d excluding PGI-2b									

Table 43 contains the results from Moran's I statistic among all plots on Beaver Island. Alleles Pgi-2a, Pgi-2b, Pgi-2c and Mdh-2a had significant overall correlograms. The SND showed significant positive spatial autocorrelations in distance class one (0-3612 meters). In addition, negative spatial autocorrelation was detected in distance class 5 (9924 m-13394 m). The analysis was repeated without Pgi-2b, which was a relatively low frequency allele. Similar results were found, there was significant positive spatial autocorrelation at the first distance class excluding Pgi-2b and significant negative spatial autocorrelation at the 5th distance class.

Table 43 Moran's I statistic for Beaver Island

No. Pairs	13	13	13	13	14		
Distance class[a]	3612	6049	8056	9923	13394	p[b]	Mean allele frequency among plots
PGM-1	0.14	0.04	-0.42	-0.22	0.00	0.27	0.848
PGI-2a	0.47	0.04	-0.05	-0.17	-0.70	0.01	0.162
PGI-2b	0.18	-0.35	0.23	0.02	-0.50	0.08	0.044
PGI-2c	0.51	0.03	0.01	-0.04	-0.90	0.00	0.789
MDH-2a	-0.22	-0.10	-0.01	-0.50	0.35	0.08	0.667
MDH-2b	-0.03	0.01	-0.07	-0.03	-0.33	0.61	0.211
MDH-2c	0.23	0.11	-0.28	-0.20	-0.30	0.44	0.111
AAT-2	-0.01	-0.21	0.13	-0.20	-0.16	0.74	0.667
Average I	0.16	-0.05	-0.06	-0.17	-0.32		
Average S.E.	0.23	0.23	0.22	0.22	0.20		
SND[c]	3.05	0.73	0.44	-0.10	-3.77		
p[c]<	0.00	0.77	0.67	0.46	0.00		
SND[d]	2.08	1.26	-0.08	-1.23	-2.61		
p[d]<	0.00	0.11	0.47	0.10	0.01		
a Upper bound for distance class in meters							
b Overall correlogram significance							
c including PGI-2b							
d excluding PGI-2b							

Discussion

According to Hamrick and Godt, (1990) gymnosperms have a high number of alleles per locus and high levels of genetic diversity. Hamrick and Godt, suggest this is due to the relationship between genetic diversity and the characteristic of a species; long lived, outcrossing, wind pollinated species have higher levels of allozyme variation within populations and less variation among populations than species with other combinations of traits. This study finds similar measures even among fragmented populations (Fst confidence interval among island 0.00 to 0.03; Rst among islands 0.04; Nei's genetic distance among islands 0.00 to 0.03; Nei's genetic identity 0.98 to 1.0).

Furthermore, Hamrick and Godt (1990) suggest that the gene flow potential of a species had the predominant influence on the partitioning of allozyme variation among populations. Wide spread, long lived species with potential for long range gene movement should have the highest levels of variation within populations. Again, this study is consistent with this prediction.

It was hypothesized that there would be a loss of genetic diversity based on information in the geological history of the area. The islands were most likely attached to mainland Michigan around 10,000 y.b.p. The lake levels fluctuated greatly between eight and six thousand years ago, but the levels would have maintained a height equal to or greater than currently since 6,000 y.b.p. (Hansel, et al., 1985). The lake levels continued to rise until around 4,000 y.b.p. during the Nipissing period, when lake levels were estimated to have been about 7 meters higher than currently. After the Nipissing period, the lake levels fluctuated but there was a trend toward lower lake levels until about 2,500 years ago, when the lake levels reached a height similar to today's, which has been

maintained with minor fluctuations, less than 1m (Hansel, et al., 1985). The islands of BIA have been fragmented for a relatively short time considering white pine has the potential to be such a long lived species. Assuming it takes 20 years for a tree to reach reproductive maturity, there have only been roughly 300 generations since isolation. For a tree that can live centuries, this might not be a long enough time for isolation and drift to alter the genetic diversity.

The hypothesis was formed however, because white pine populations on Garden, Hog and High Islands are found growing along the shores of Lake Michigan. White pine is also quite commonly found growing along the Lake Michigan shore on Beaver Island. White pine is only found growing in the sandy bays of the three smaller islands, and is seldom found growing in rocky bays, or in upland areas. The high water level during the Nipissing period would have greatly reduced the area available for white pine reproduction on all islands, but the lower elevation above lake levels of Garden and Hog islands should have resulted in a more extreme decrease in island size compared to Beaver and High islands. The smaller islands size resulting from fluctuations lake levels potentially reduced genetic diversity in a number of ways. First, there would be a loss of genetic diversity if the populations had fallen below a size (in numbers) that could support the diversity found before the population bottleneck. Second, if the populations were large enough in number to support the original genetic diversity, the size in area could have been small enough to lead to inbreeding, which would also reduce the genetic diversity of the populations. The loss of diversity should have been especially high on Hog and Garden Islands since both have a low elevation (less than 40 feet) above the current lake level (Divins, 2002). Beaver and High Islands both have areas over 200 feet

above the current lake level, but large portions of Beaver and High Islands are less than 30 feet above current lake levels. In brief, the effects of the high water during the Nipissing period should have caused a population bottleneck reducing the genetic diversity of all organisms on the islands, even white pine which is long lived with overlapping generations.

The results of the genetic diversity found within the BIA do not support the hypothesis of a loss of genetic diversity. Both the allozyme and SSR markers displayed high amounts of genetic diversity among the archipelago as well as relatively low but significant levels of divergence. Similar studies have found high diversity within populations, and low diversity among populations of conifers (Hiebert, and Hamrick, 1983; Hamrick and Godt, 1990; Vendramin et al., 1999; Thomas et al., 1999; Mariette, et al., 2001; Ribeiro, et al., 2002 and others). High levels of genetic diversity within BIA could result from several possibilities. First, perhaps the lake level estimates are wrong and there was not a bottleneck. This is doubtful since the Lake Michigan basin has been mapped (Divins, 2002) and basin topography agrees with the theory that the islands would have been a part of mainland Michigan during low lake levels. Furthermore, there are bluffs on the islands and throughout the Great Lakes region to support the high water theory. Additionally, with isolation and fragmentation the remnant areas would decrease in the number of species based on the theory of island biogeography. Garden and High Islands supposedly have no American beech (*Fagus Americana*) or eastern hemlock (*Tsuga Canadensis*) which are both found in similar plant communities on Beaver Island, suggesting that there was indeed a reduction in island size in the past.

Another possible cause for the high genetic diversity is that the populations of white pine may have been larger or more widespread in the past than currently found on BIA. If this is the case, it is possible there has been a loss of diversity, but relatively high diversity still persists. The diversity measures for allozymes overlap those found in other conifers (Hamrick and Godt, 1990). Moreover, the results in this study compare in number of haplotypes with a continuous populations of white pine in Wisconsin (Craig Echt. pers. comm.). Furthermore, allozyme allelic richness measured with the same laboratory protocol is similar to that found in an old growth white pine forest on mainland Michigan (Epperson and Chung, 2001). The similarity with Wisconsin and mainland Michigan populations would suggest that these islands maintain genetic diversity levels comparable with non-fragmented populations, not that they have high but reduced genetic diversity relative to other conifers. One more possibility is that migration has or is occurring to increase/maintain the genetic diversity in the populations. Using microsatellites Ledig (1998) found that pollen mediated gene flow is effective in preventing population differentiation. Prevention of differentiation could be accomplished by gene flow among islands, which would also create high levels of genetic diversity within populations.

Although the hypotheses of reduced genetic diversity was rejected, there are some interesting results found in this study. One such result is the variation in allele and haplotype frequencies among islands. The loci Tpi-2 was monomorphic only on Hog Island. When looking at the different haplotype frequencies found among the islands, the three most common haplotypes (numbers one two and three) are found on all islands. However, some plots did not contain a single copy of one or two of the most common

haplotypes. High East West and Powerline had no haplotype number one. High East Middle had no haplotype number two or number three. The Airport plot also had no number three haplotype. The lack of the common haplotypes in some plots explains the high Fst values within Hog Island. With a bottleneck it is expected that the low frequency alleles would be lost first since the probability of being lost is related to the frequency of the alleles (Hatle and Clark, 1997). The fact that Tpi-2 was monomorphic on one island, that the most common haplotypes are not found in all plots and rare haplotypes are not found on all islands, suggests that there was a bottleneck and 1) that occasional migration has occurred to reintroduce rare haplotypes 2) that mutations have occurred to create new rare haplotypes 3) it is an artifact of sampling. Based on the results of the spatial autocorrelation analysis (Tables 42 and 43), there does seem to be a limit to migration, since distances greater than 8 km had no spatial autocorrelation, and distances grater than 13.5 km had a negative spatial autocorrelation. However, the fact that rare microsatellite markers are found scattered throughout the BIA suggests that there has been low amounts of migration. It is unlikely that mutation is the cause of the genetic diversity, since mutations should results in private haplotypes found among the islands. One potential exception could be haplotype number 14. This haplotype had a relatively high frequency on High Island, and was found in a single tree on Beaver Island. It is possible this haplotype arose on High Island, and has since spread to Beaver Island. If haplotype number 14 did arise on High Island and spread to Beaver Island, it would be evidence for migration maintaining the genetic diversity within the archipelago. The fact that there are low frequency alleles missing from some of the smaller islands and common alleles missing from some plots could be a result of a past bottleneck.

Nei et al. (1975) suggested that heterozygosity could be maintained after a population bottleneck but that the number of alleles would be reduced. If migration occurs after the bottleneck this would increase the number of alleles in the population. It is hypothesized that the effect of admixture via migration has resulted in the high levels of genetic diversity. The genetic data analysis seems to be consistent with the geological data, which suggests the islands were smaller in area during high lake levels resulting in a population bottleneck for white pine, but the islands have since gotten larger and the populations of white pine have grown in size (number) as the islands grew in area.

Wright's F statistics among islands: The measure of Fis and Fit suggest that some inbreeding is occurring within the islands. This could be due to sampling trees that are homozygotes for rare alleles. When low frequency alleles are found in trees as homozygotes the Fis and Fit values will be high.

The relatively large values for Fis and Fst could be due to a number of factors such as variable migration, establishment history or sampling error. High values for Fis and Fst would suggest that there are low amounts of migration, while low values of Fis and Fst would suggest that there are high amounts of migration. The overall Fst values suggest there is some divergence, since there was a small Fst value among islands. With intermittent migration over distances, some plots may exchange pollen, and some may not. Plots that have exchanged pollen will have low Fis and Fst values, while plots that have not exchanged pollen over large distance would have high Fis and Fst values. The local establishment history could also cause variable values to be attained for Fst and Fis.

To explain, if a number of trees in a sample plot reach ecesis over a long period of time, the possibility of long distance migration occurring at some point would be higher.

Consequently, there would be a low Fis and Fst values. If however, it was possible for numerous individual trees to establish in a single year, then the possibility for siblings to be grouped in an area would be high. If a sample plot contained many siblings then it would appear that there is a high amount of inbreeding, and could also suggest limited gene flow among plots. Finally, as with any study, there are limited resources available, which is why sampling rather than a census was conducted. When sampling a population there is always the possibility that the sample is not a true representative of the populations.

Nei's genetic distance: The measures found with Nei's genetic distance measures the accumulated allele differences per locus between populations. Little differences were found between islands. Low differentiation is most likely due to the effect of migration. Nei's genetic identity describes the proportion of alleles that are alike between populations. This measure was high between populations suggesting little divergence, which could be accomplished by high gene flow. Nei's genetic distance measured between plots did show some variability in the genetic distance. The highest value for Nei's genetic distance was found with haplotype measures between Hog Middle East and several other plots. High Middle East plot had several rare alleles, and had no trees with the common haplotypes number one and two (Sample size in Hog East Middle is 14 trees). The sample plots with the exception of Garden Island are relatively small in number of trees, so it is not unexpected to find differences between areas with a low number of samples.

Spatial autocorrelation: Moran's I values found significant spatial autocorrelation within 3 km. on Beaver Island, and within 5 km. among all islands. There was a negative spatial autocorrelation at distances greater than 13.5 km. This would suggest that there is a limit to the effect of pollen and seed migration. When populations are separated by more than 13.5 kilometers genetic drift, mutations and inbreeding could alter the genetic diversity more than the effects of gene flow. Geographical separation with limited migration should result in population differentiation and there is slight differentiation between islands and a slight but significant differentiation among plots. When one looks at the occurrence of the common and private alleles within and among islands, a low migration and or mutation rate is suggested. It is possible that migration occurs enough to prevent severe divergence, but not frequently enough to bring the frequencies of alleles to a point where there is positive or no spatial autocorrelation at all distance class.

Conclusion

The genetic diversity measures are consistent with the geological history. It appears that the islands were much smaller in the past, which resulted in a population bottleneck. Since the bottleneck, the islands have grown in size, allowing for white pine populations to increase in numbers and allow for admixture via pollen migration to increase the genetic diversity within each island. The fact that the allozyme Tpi-2 was monomorpic on one island, that rare haplotypes are missing from some islands, and that the most common haplotypes are missing from some plots could be a result of genetic drift and infrequent migration.

The islands as a whole maintain high levels of genetic diversity due to the effects of mutation or migration. Mutation as the primary cause of the genetic diversity is unlikely, since no private haplotypes were found on any of the smaller islands. Most likely occasional long distance migration has occurred to maintain genetic diversity within each population, and counter the effect of drift and inbreeding.

Appendix

The Diameter at Breat Heigt (D.B.H.) of Sampled trees

Garden Island	Tree #	D.B.H. (cm)		Tree #	D.B.H. (cm)
	1	41.80		44	28.10
	2	48.60		45	48.30
	3	44.90		46	46.60
	4	29.40		47	47.10
	5	49.10		48	37.10
	6	44.90		49	52.70
	7	51.40		50	36.40
	8	38.20		51	41.00
	10	36.00		52	48.70
	11	35.60		53	25.10
	12	33.40		54	23.80
	13	28.70		55	18.90
	14	30.60		56	35.30
	15	37.70		57	21.50
	16	52.90		58	47.50
	17	66.50		59	55.00
	18	63.50		60	54.20
	19	58.80			
	20	59.50			
	21	52.70			
	22	41.50			
	23	43.30			
	24	43.10			
	25	27.20			
	26	39.80			
	27	51.80			
	28	65.90			
	29	24.10			
	30	15.10			
	31	29.00			
	32*	12.00			
	33	19.70			
	34	23.90			
	35	17.50			
	36	19.20			
	37	26.80			
	38	40.60			
	39	71.50			
	40	39.80			
	41	37.90			
	42	50.90			
	43	29.90			

Hog Island	Tree #	D.B.H. (cm)		Tree #	D.B.H. (cm)
	61	29.00	Hog	90	30.90
Hog	62	23.40	East	91	31.00
West	63	56.00	Middle	92	22.50
	64	33.20		93	12.30
	65	42.50		94	21.90
	66	29.20		95	28.50
	67	76.70		96	19.60
	68	26.80		97	30.30
	69	27.60		98	33.20
	70	60.00		99	33.90
	71	68.60		100	10.00
	72	32.30		101	38.70
	73	74.10		102	34.50
				103	33.20
Hog	74	30.20		104	25.00
East	75	31.90		105	38.00
North	76	23.70			
	77	21.10	Hog	106	22.90
	78	21.90	East	107	19.80
	79	34.40	South	108	12.50
	80	31.40		109	15.40
	81	28.90		110	21.90
	82	24.70		111	28.00
	83	21.90		112	24.90
	84	20.30		113	33.60
	85	28.20		114	38.40
	86	21.50		115	18.10
	87	18.90		116	23.40
	88	18.50		117	28.50
	89	18.00		118	35.20
				119	28.70
				120	35.10

High Island	Tree #	D.B.H. (cm)		Tree #	D.B.H. (cm)
High	121	45.30	**High**	153	12.60
East	122	32.00	**East**	154	26.50
South	123	24.70	**North**	155	20.50
	124	27.80		156	17.00
	125	7.10		157	10.40
	126	39.00		158	13.60
	127	32.90		159	20.00
	128	27.00		160	9.80
	129	18.90		161	26.80
	130	38.40		162	27.80
	131	40.70		163	17.50
	132	36.10		164	24.20
	133	21.90		165	11.30
	134	27.30		166	13.30
	135	23.40		167	22.40
	136	30.50			
High	137	40.80			
West	138	33.40			
	139	32.30			
	140	42.20			
	141	33.00			
	142	36.90			
	143	20.90			
	144	26.00			
	145	39.10			
	146	35.50			
	147	30.20			
	148	14.50			
	149	18.30			
	150	42.00			
	151	32.50			
	152	34.50			

Beaver Island	Tree #	D.B.H. (cm)		Tree #	D.B.H. (cm)
Powerline	200	55.50	Foot bridge	248	36.90
	201	37.30		249	32.80
	202	31.80		250	36.80
	203	31.90		251	26.80
	204	29.70		252	43.70
	205	47.50		253	36.80
	205	32.40		254	65.90
	207	41.90		255	43.00
	208	48.50		256	56.50
	209	47.60		257	38.30
	210	60.00		258	38.30
	211	33.00		259	40.50
	212	38.40		260	35.10
	213	40.10		261	49.60
	214	36.30		262	34.40
	215	51.80		263	45.30
	216	33.20		264	52.00
	217	32.10		265	23.80
	218	31.50		266	23.80
	219	39.00		267	33.20
				268	52.10
				269	57.60
Blue trail	225	37.40	French Bay	270	44.20
	226	37.80		271	44.30
	227	42.50		272	30.20
	228	62.60		273	48.70
	229	42.30		274	27.50
	230	31.00		275	39.50
	231	40.10		276	37.20
	232	46.30		277	31.00
	233	45.80		278	35.40
	234	40.20		279	45.40
	235	17.90		280	38.90
	236	26.10		281	54.30
	237	19.60		282	36.20
	238	21.30		283	23.70
	239	24.80		284	33.70
	240	32.10		285	56.50
	241	36.70		286	22.80
	242	60.00		287	35.40
	243	43.20		288	52.80
	244	38.30		289	37.20
	245	36.90		290	45.10
	246	57.60		291	38.30
	247	48.70			

	Tree #	D.B.H. (cm)		Tree #	D.B.H. (cm)
Green's bay	292	35.60	**CMU**	336	54.30
	293	31.50		337	36.20
	294	42.00		338	34.20
	295	24.20		339	40.30
	296	21.90		340	75.40
	297	26.10		341	51.60
	298	28.10		342	52.80
	299	43.00		343	69.00
	300	43.60		344	30.30
	301	20.40		345	97.70
	302	40.60		346	49.90
	303	22.60		347	52.90
	304	26.50		348	72.70
	305	41.00		349	42.00
	306	31.70		350	32.00
	307	32.30		351	41.00
	308	51.30		352	48.90
	309	27.20		353	51.30
	310	45.40		354	54.60
	311	34.90		355	21.30
	312	28.40		356	33.50
	313	52.50		357	33.60
Wujeck	314	52.40	**DUDA's**	358	32.00
	315	28.10		359	24.50
	316	52.10		360	18.70
	317	42.10		361	28.50
	318	31.60		362	43.10
	319	40.60		363	31.20
	320	42.70		364	30.80
	321	42.40		365	34.80
	322	15.00		366	25.10
	323	53.50		367	31.60
	324	40.30		368	22.80
	325	20.80		369	31.40
	326	59.00		370	34.70
	327	22.40		371	28.40
	328	28.20		372	18.50
	329	39.20		373	12.50
	330	25.00		374	40.40
	331	27.30		375	36.90
	332	21.80		376	25.40
	333	61.70		377	90.20
	334	34.80		378	61.30
	335	36.90		379	37.40

	Tree #	D.B.H. (cm)		Tree #	D.B.H. (cm)
Font Lake	380	32.80	**Sloptown Rd.**	424	31.10
	381	38.10		425	14.90
	382	17.30		426	47.40
	383	23.00		427	26.10
	384	22.00		428	19.50
	385	16.30		429	49.30
	386	29.80		430	20.10
	387	30.30		431	52.70
	388	18.30		432	14.30
	389	45.00		433	31.20
	390	18.40		434	20.30
	391	35.50		435	55.60
	392	36.30		436	28.40
	393	39.20		437	30.10
	394	42.00		438	39.60
	395	27.00		439	48.40
	396	19.30		440	49.90
	397	15.50		441	39.00
	398	14.30		442	34.00
	399	20.70		443	38.50
	400	30.00		444	22.30
	401	22.70		445	83.20
Barney's Lk	402	18.40	**Airport**	446	28.70
	403	31.00		447	16.20
	404	19.30		448	25.00
	405	29.30		449	34.50
	406	38.00		450	25.00
	407	29.50		451	26.20
	408	37.30		452	30.20
	409	35.60		453	94.30
	410	22.30		454	23.10
	411	27.70		455	27.10
	412	33.50		456	18.80
	413	35.50		457	18.60
	414	27.60		458	18.30
	415	26.40		459	27.20
	416	24.80		460	15.60
	417	35.20		461	33.60
	418	20.70		462	22.80
	419	25.90		463	18.50
	420	31.10		464	25.00
	421	29.00		465	26.00
	422	19.40		466	26.30
	423	26.30			

Appendix

Haplotype frequencies among plots continued

Haplotypes	Duda	Font Lake	Barney's Lake	Slop Town	Airport
1	0.375	0.235	0.308	0.176	0.091
2	0.063	0.235	0.308	0.353	0.455
3	0.188	0.235	0.231	0.059	0.000
4	0.125	0.059	0.000	0.118	0.091
5	0.063	0.059	0.077	0.118	0.000
6	0.063	0.059	0.000	0.000	0.000
7	0.000	0.000	0.000	0.000	0.091
8	0.000	0.000	0.077	0.000	0.000
9	0.063	0.118	0.000	0.000	0.182
10	0.000	0.000	0.000	0.000	0.000
11	0.000	0.000	0.000	0.000	0.000
12	0.000	0.000	0.000	0.118	0.000
13	0.000	0.000	0.000	0.000	0.000
14	0.000	0.000	0.000	0.059	0.000
15	0.000	0.000	0.000	0.000	0.000
16	0.063	0.000	0.000	0.000	0.000
17	0.000	0.000	0.000	0.000	0.091
n=	11	17	13	17	9

Appendix

Haplotype frequencies among plots continued

Haplotypes	Foot Bridge	French Bay	Green's Bay	Wujeck	CMU
1	0.125	0.059	0.333	0.154	0.200
2	0.438	0.176	0.167	0.154	0.133
3	0.063	0.412	0.056	0.385	0.333
4	0.000	0.000	0.167	0.000	0.000
5	0.188	0.059	0.000	0.000	0.200
6	0.000	0.118	0.056	0.000	0.000
7	0.000	0.000	0.000	0.000	0.000
8	0.000	0.059	0.056	0.000	0.000
9	0.063	0.059	0.167	0.154	0.067
10	0.063	0.000	0.000	0.000	0.000
11	0.063	0.000	0.000	0.000	0.000
12	0.000	0.059	0.000	0.000	0.000
13	0.000	0.000	0.000	0.077	0.000
14	0.000	0.000	0.000	0.000	0.000
15	0.000	0.000	0.000	0.077	0.067
16	0.000	0.000	0.000	0.000	0.000
17	0.000	0.000	0.000	0.000	0.000
n=	13	15	16	10	14

Appendix

Haplotype frequencies among plots continued

Haplotypes	High East South	High West	High East South	Power line	Blue Trail
1	0.000	0.133	0.308	0.000	0.263
2	0.462	0.467	0.231	0.333	0.053
3	0.231	0.067	0.154	0.167	0.526
4	0.000	0.000	0.000	0.083	0.053
5	0.000	0.000	0.077	0.167	0.053
6	0.077	0.067	0.000	0.167	0.000
7	0.000	0.000	0.000	0.000	0.053
8	0.077	0.067	0.077	0.083	0.000
9	0.077	0.000	0.077	0.000	0.000
10	0.000	0.000	0.000	0.000	0.000
11	0.000	0.000	0.000	0.000	0.000
12	0.000	0.000	0.000	0.000	0.000
13	0.000	0.000	0.000	0.000	0.000
14	0.077	0.200	0.000	0.000	0.000
15	0.000	0.000	0.077	0.000	0.000
16	0.000	0.000	0.000	0.000	0.000
17	0.000	0.000	0.000	0.000	0.000
n=	13	15	14	11	15

Appendix

Haplotype frequencies among plots

Haplotypes	Garden island	Hog west	Hog East North	Hog East Middle	Hog East South
1	0.245	0.400	0.000	0.636	0.100
2	0.367	0.200	0.667	0.000	0.400
3	0.143	0.200	0.333	0.000	0.300
4	0.061	0.000	0.000	0.000	0.200
5	0.082	0.000	0.000	0.000	0.000
6	0.082	0.200	0.000	0.000	0.000
7	0.020	0.000	0.000	0.000	0.000
8	0.000	0.000	0.000	0.091	0.000
9	0.000	0.000	0.000	0.091	0.000
10	0.000	0.000	0.000	0.091	0.000
11	0.000	0.000	0.000	0.000	0.000
12	0.000	0.000	0.000	0.091	0.000
13	0.000	0.000	0.000	0.000	0.000
14	0.000	0.000	0.000	0.000	0.000
15	0.000	0.000	0.000	0.000	0.000
16	0.000	0.000	0.000	0.000	0.000
17	0.000	0.000	0.000	0.000	0.000
n=	49	10	6	14	10

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